STUDY PROTOCOL

J1522-A RANDOMIZED PHASE 2 STUDY OF SIPULEUCEL-T WITH OR WITHOUT RADIUM-223 IN MEN WITH ASYMPTOMATIC OR MINIMALLY SYMPTOMATIC BONE-METASTATIC CASTRATE-RESISTANT PROSTATE CANCER

Coordinating Center: The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

Principal Investigator and IND Sponsor:
Emmanuel Antonarakis, MD
The Sidney Kimmel Comprehensive Cancer
201 N. Broadway, RM 9129
Baltimore, MD 21287
Phone: 443-287-0553
Fax: 410-367-2668
Email: eantona1@jhmi.edu

Co-PI:
A. Oliver Sartor, MD
Tulane Cancer Center
1430 Tulane Ave., SL-42
New Orleans, LA 70112
Phone: 504-988-7869
Email: osartor@tulane.edu

Statistician:
Brandon Luber, ScM
550 North Broadway
550 Bldg. 1103
Baltimore, MD 21205
Phone: 443-287-2908
Email: bluber1@jhmi.edu

Lead Site Study Coordinator:
Serina King
201North Broadway, RM 9132

Baltimore, MD 21287
Phone: 410-614-6139
Email: sking18@jhmi.edu

Lead Site Research Nurse:

Michaella Afful, RN
201 North Broadway, 9th Floor

Baltimore, MD 21287
Phone: 410-502-0017
Email: mafful1@jhmi.edu

Participating Site Specific Co-Investigator

The Sidney Kimmel Comprehensive Cancer Emmanuel Antonarakis, MD 201 N. Broadway, RM 9129 Baltimore, MD 21231 Phone: 443-287-0553 Email: eantona1@jhmi.edu	Tulane Cancer Center A. Oliver Sartor, MD 1430 Tulane Ave., SL-42 New Orleans, LA 70112 Phone: 504-988-7869 Email: osartor@tulane.edu

Statement about Proper Study Conduct

This study will be conducted in compliance with Good Clinical Practices, according to ICH Harmonized Tripartite Guideline.

Confidentiality Statement

The information in this document is provided to you as an investigator, potential investigator, consultant, or contractor, for review by you, your staff, and the appropriate Institutional Review Board or Ethics Committee. By accepting this document, you agree that the information contained herein will not be disclosed to others without written authorization from the lead site/sponsor, except to the extent necessary to initiate the study or conduct study-related activities

PROTOCOL SIGNATURE PAGE

A RANDOMIZED PHASE 2 STUDY OF SIPULEUCEL-T WITH OR WITHOUT RADIUM-223 IN MEN WITH ASYMPTOMATIC OR MINIMALLY SYMPTOMATIC BONE-METASTATIC CASTRATE-RESISTANT PROSTATE CANCER

Investigator Name: _____

Title: _____

Address: _____

Address: _____

Address: _____

Phone: _____

Facsimile: _____

Signature: _____

Date: _____

Amendment Rationale

The quantification of radium-223 radioactivity in Xofigo® is based on the primary standardization performed by the US National Institute of Standards and Technology (NIST). The NIST Standard Reference Material is used to calibrate the instruments in production and quality control of both the drug substance and drug product. Additionally, the calibrated instruments in production at the Institute for Energy Technology (IFE, Norway) are used to prepare the NIST traceable radium-223 reference material, which are then sent to the treatment sites (e.g., nuclear medicine laboratory physicians or technicians) for dial setting of their dose calibrators, to allow verification of the patient dose. A reassessment of the primary standardization was initiated by the NIST. A discrepancy of approximately 10% between the published NIST primary standardization (Cessna, 2010, NIST 2010) and current measurements was confirmed and a revised NIST primary reference standard has been issued (Zimmerman, 2015, NIST update). As a result of the revised NIST primary standardization, an adaption of the numerical description of patient dose and the description of radioactive concentration of the drug product solution becomes necessary. This concerns Xofigo® for commercial use and product used in clinical trials.

After the implementation of the new standard (NIST update) the numerical description of the patient dose will be adjusted from 50 kBq/kg to 55 kBq/kg, and the numerical description of the radioactivity in the vial will be changed from 1,000 kBq/mL to 1,100 kBq/mL

The values in this protocol have been revised as per the United States National Institute of Standards and Technology (NIST) standardization update.

Bayer has submitted a variation application to the FDA. The current standard (NIST 2010), dial setting and dose will remain in effect for this study until Bayer has confirmed the unique implementation date in the 2nd quarter of 2016 as agreed with FDA and notified the **Principal Investigator and IND Sponsor:**

Emmanuel Antonarakis, MD
The Sidney Kimmel Comprehensive Cancer
201 N. Broadway, RM 9129
Baltimore, MD 21287

PROTOCOL SYNOPSIS

Protocol Title: A Randomized Phase 2 Study of Sipuleucel-T With or Without Radium-223 IN Men With Asymptomztic or Minimally Symptomatic Bone-Metastatic Castrate-Resistant Prostate Cancer

Protocol Number:

Phase: II

Study Centers: 4-5 centers in the U.S.

Investigational Product, Dosage Form, Route, and Dose Regimen:

Radium-223 (Xofigo®) is an alpha-emitting radioisotope developed by Algeta ASA. Radium-223 is indicated for treatment of men with castrate-resistant prostate cancer (CRPC) with symptomatic bone metastases and no known visceral metastatic disease. The approved dose and schedule of radium-223 is 50 kBq (or 1.35 microcurie) (55 kBq/kg (or 1.49 microcurie)) after implementation of the NIST update) per kg body weight intravenous (IV) infused at 4 week interval for 6 injections.

Sipuleucel-T (Provenge®) is an autologous cellular immunotherapy product designed to stimulate an immune response against prostate cancer. Sipuleucel-T is indicated for use in patients with asymptomatic or minimally symptomatic metastatic CRPC (mCRPC). A dose of sipuleucel-T is prepared using PBMCs from a single leukapheresis procedure. A minimum of 50 million antigen presenting cells (APCs, the biologically active component of sipuleucel-T) are administered via a single IV infusion. A complete treatment of sipuleucel-T will include 3 doses of autologous cells, infused at approximately 2-week intervals.

Primary Objective

- To determine whether addition of radium-223 to sipuleucel-T enhances immune response to sipuleucel-T measured by peripheral PA2024-specific T-cell proliferation compared with sipuleucel-T alone in men asymptomatic or minimally symptomatic bone-metastatic CRPC

Secondary Immune Objectives

- To evaluate peripheral antigen-specific T-cell proliferation over time
- To evaluate peripheral antigen specific T-cell activation to sipuleucel-T over time

- To evaluate antigen specific antibody response to sipuleucel-T over time
- To evaluate the sipuleucel-T product immune parameters

Secondary Clinical Objectives

- To evaluate safety of combined use of radium-223 and sipuleucel-T.
- To evaluate PSA50 response (at least a 50% decline in PSA)
- To evaluate time to radiographic or clinical progression.

Study Design and Duration:

This is a randomized study designed to assess the antigen-specific immune response of sipuleucel-T when administered with or without radium-223.

Eligible subjects will be registered and randomly assigned in a 1:1 ratio to receive sipuleucel-T and radium-223 or sipuleucel-T alone;

> Arm 1: 6 infusions of radium-223 with 3 infusions of sipuleucel-T starting after second dose of radium-223
>
> Arm 2: 3 infusions of sipuleucel-T alone

Subjects in both arms will undergo a standard 1.5 to 2.0 blood volume leukapheresis, followed approximately 3 days later by an IV infusion of sipuleucel-T. This process will occur a total of 3 times at approximately 2-week intervals. Subjects in Arm 1 will receive a total of 6 infusions of radium-223 at IV dose of 50 kBq/kg (55 kBq/kg after implementation of the NIST update) at 4-week interval

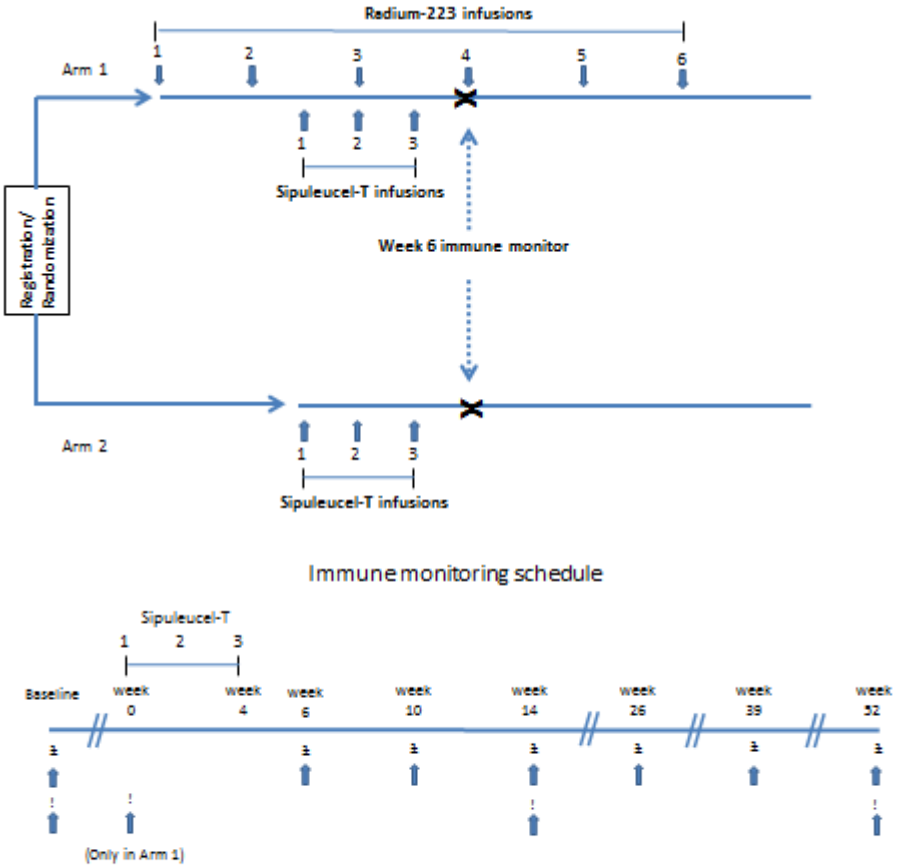
Research visits will be scheduled at screening, at each radium-223 infusion, prior to each leukapheresis (preleukapheresis visit), and week 32, 45 and 58 for Arm 1 (radium-223 and sipuleucel-T combination arm) and at screening, prior to each leukapheresis (preleukapheresis visit), and week 6, 10, 14, 26, 39, and 52 for Arm 2 (sipuleucel-T alone arm) for physical examinations, vital signs, ECOG performance status, adverse event (AE) monitoring, anticancer therapies, first opioid use for cancer-related pain, and laboratory tests. Serum PSA will be assessed at baseline, and week 16, 32, 45, and 58 for Arm 1 and at baseline, and week 10, 26, 39, and 52 for Arm 2. There is a ± 7 day window for each visit to accommodate holidays, vacations, etc.

Imaging studies including bone scintigraphy test (bone scan) or Na-F PET or C11 acetate PET and abdomen/pelvis CT or MRI will be obtained at screening and week 16, 32, 45, and 58 for Arm 1 and at screening and week 10, 26, 39, and 52 for Arm 2. After the last study visit, imaging studies will be obtained according to the participating institution's standard and/or at the investigator's discretion.

Immune monitoring blood samples will be drawn at screening and at 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T in both arms. Additional blood samples will be obtained at baseline, first pre-leukapheresis visit (only in Arm 1), and weeks 14 and 52 post the first infusion of sipuleucel-T for exploratory antigen spread assessment.

After last study research visit, safety, treatment-related AEs, survival status, PSA, first opioid use for cancer-related pain (if applicable), and anticancer therapies will be assessed every 3 months for additional 1 year or until the subject's death.

The study design is illustrated below.



Study Population and Sample Size

This study will enroll 30 evaluable patients with asymptomatic or minimally symptomatic mCRPC, 18 years of age or older. Up to 33 patients are estimated to be required to be enrolled to allow for drop-outs and subjects who may not receive 3 infusions of sipuleucel-T or 2 infusions of radium-223.

Inclusion Criteria

1. Written informed consent provided prior to the initiation of study procedures.

2. Age ≥ 18 years.

3. Histologically documented adenocarcinoma prostate cancer confirmed by a pathology report from prostate biopsy or a radical prostatectomy specimen. If prostatic tumor is of mixed histology, > 50% of the tumor must be adenocarcinoma.

4. Bone metastases as manifested by one or more lesions on a bone scan performed within 2 months of screening

5. Castrate-resistant prostate cancer, in the setting of castrate levels of testosterone (≤ 50 ng/dL), defined as current or historical evidence of disease progression concomitant with surgical castration or androgen deprivation therapy (ADT), as demonstrated by two consecutive rises in PSA OR new lesions on bone scan:

    - PSA progression will be defined as 2 rising PSA values compared to a reference value, measured at least 7 days apart and the second value is ≥ 2 ng/mL [1]. It must be documented within 2 months of screening.

    - Appearance of one or more new areas of abnormal uptake on bone scan when compared to imaging studies acquired during castration therapy or against the precastration studies if there was no response. Increased uptake of pre-existing lesions on bone scan does not constitute progression. It must be documented within 4 months of screening.

6. Serum PSA ≥ 2.0 ng/mL.

7. Screening ECOG performance status ≤ 1

8. Asymptomatic or minimally symptomatic disease (no narcotic analgesic; other analgesics use is allowed).

9. Prior abiraterone and enzalutamide are permitted, but not required.
10. Concurrent osteoclast-inhibitory therapies (zoledronic acid, denosumab) are permitted if patients have been on a stable dose for at least 1 month.

11. Adequate screening hematologic, renal, and liver function as evidenced by laboratory test results within the following ranges ≤ 28 days prior to registration:

    - Absolute neutrophil count (ANC) ≥ 1.5 x10^9/L
    - Platelet count ≥ 100 x10^9/L
    - Hemoglobin ≥ 10.0 g/dL
    - Total bilirubin level ≤ 1.5 x institutional upper limit of normal (ULN)
    - Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x ULN
    - Creatinine ≤ 1.5 x ULN
    - Albumin > 2.5 g/L

Exclusion Criteria

1. The presence of known lung or liver metastases greater than 1.0 cm in the long axis diameter.

2. The presence of lymphadenopathy greater than 3 cm in the short-axis diameter.

3. The presence of known brain metastases.

4. Spinal cord compression, imminent long bone fracture, or any other condition that, in the opinion of the investigator, is likely to require radiation therapy and/or steroids for pain control during the active phase.

5. Previous treatment with chemotherapy for mCRPC, or chemotherapy for any reason within 6 months prior to registration. (Chemotherapy in the adjuvant setting or for hormone-sensitive prostate cancer is permitted, as long as it was completed more than 6 months before registration).

6. Intention to receive chemotherapy within 6 months after enrollment in protocol therapy.

7. History of radiation therapy, either via external beam or brachytherapy within 28 days prior to registration.

8. Systemic radiotherapy with strontium-89, samarium-153, rhenium-186 or rhenium-188 for the treatment of bony metastases within previous 24 weeks

9. Prior history of other cancers (except non-melanoma skin cancers or low-grade low-stage urothelial cancers), or other localized cancers without evidence of recurrence for at least 3 years.

10. Use of prednisone or equivalent systemic corticosteroid within 2 weeks of treatment. Use of inhaled, intranasal, intra-articular, and topical steroids is allowed. Oral or IV steroids to prevent or treat IV contrast reactions are allowed.

11. Use of opioid analgesics for cancer-related pain

12. Use of experimental drug within 4 weeks of treatment.

13. Uncontrolled medical conditions including diabetes, heart failure, COPD, ulcerative colitis, or Crohn's disease.

14. Uncontrolled fecal incontinence.

15. Any medical intervention, any other condition, or any other circumstance which, in the opinion of the investigator, could compromise adherence with study requirements or otherwise compromise the study's objectives.

Study Endpoints

Primary Endpoint

- Peripheral PA2024-specific T-cell proliferation using a tritiated thymidine (^{3}H-thymidine) incorporation assay at week 6 after the first sipuleucel-T infusion in each arm reported as stimulation index (SI), defined as ^{3}H-thymidine incorporation in the presence of PA2024 antigen divided by ^{3}H-thymidine incorporation with media alone.

Secondary Endpoints

Secondary Immune Endpoints

- The mean peripheral PA2024-and PAP-specific T-cell proliferation using a ^{3}H-thymidine incorporation assay reported as SI at baseline and 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T.
- The mean peripheral PA2024-and PAP-specific T-cell activation to sipuleucel-T using interferon gamma (IFNγ) enzyme-linked immunosorbent spot (ELISPOT) at baseline and weeks 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T.

- The mean PA2024-and PAP-specific antibody (IgM and IgG) response to sipuleucel-T using enzyme-linked immunosorbent assay (ELISA) at baseline and weeks 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T.
- Product immune parameters including CD54 + cell counts and upregulation and total nucleated cell (TNC) counts

Secondary Clinical Endpoints

- The frequency of the maximum observed grade of each toxicity. Incidence and severity of adverse events and laboratory abnormalities, graded according to CTCAE v4.0 [2].
- To evaluate the PSA50 response
    - PSA50 response is defined as at least a 50% decline in PSA from baseline value

- Median time to radiographic progression using (RECIST and PCWG2 criteria for soft tissue and bone lesions, respectively, as assessed by the investigator).

Exploratory Objectives:

- Median time to PSA progression based on Prostate Cancer Working Group 2 (PCWG2) criteria [1]:
    - in patients with no PSA decline from baseline as: ≥ 25% increase from the baseline value and an increase in absolute value of ≥ 2 ng/mL, at least 12 weeks from baseline
    - in patients with an initial PSA decline from baseline as: ≥ 25% increase and an absolute increase of ≥ 2 ng/mL above the nadir value, which is confirmed by a second value obtained three or more weeks later
- Median time to ALP progression.
    - in patients with no ALP decline from baseline as: ≥ 25% increase from the baseline value, at least 12 weeks from baseline
    - in patients with an initial ALP decline from baseline as: ≥ 25% increase above the nadir value, which is confirmed by a second value obtained three or more weeks later.
- Median time to pain progression defined as use of opioid analgesics for cancer-related pain.
- Median time to occurrence of first skeletal related event (SRE):
    - the use of external beam radiotherapy to relieve skeletal symptoms or the occurrence of new symptomatic pathological bone fractures (vertebral or non-vertebral) or the occurrence of spinal cord compression or a tumor related orthopedic surgical intervention.
- Median time to occurrence of first start of chemotherapy use.

Statistical Considerations

The study is designed to test the null hypothesis of no difference in immune response to sipuleucel-T evaluated on the basis of PA2024-stimulated T cell proliferation via ^{3}H-thymidine uptake between sipuleucel-T alone and sequential administration of radium-223.

For primary endpoint, PA2024-specific ^{3}H-thymidine uptake was selected because it has demonstrated the highest signal to noise ratio and showed the strongest correlation with OS of all cellular and humoral assays evaluated in prior studies of sipuleucel-T.

With 15 subjects in each arm, we seek to detect a 3.6-fold difference between the arms with 80% power in mean SI of PA2024-specific ^{3}H-thymidine uptake at week 6 after the first infusion of sipuleucel-T

The immune response population will consist of all subjects who receive 3 infusions of sipuleucel-T and at least 4 infusions of radium-223.

The intent-to-treat population is defined as all randomized subjects, regardless of whether they receive treatment. Analysis of non-immune response efficacy endpoints will be performed using this analysis population.

The safety population will include all subjects who undergo at least 1 leukapheresis procedure or receive at least 1 dose of radium-223.

TABLE OF CONTENTS

Contents

The values for radium-223 in this protocol has been revised as per the US NIST standardization update (agreed on 17 Mar 2015)

The values for radium-223 in this protocol has been revised as per the US NIST standardization update (agreed on 17 Mar 2015)

1.0 INTRODUCTION

1.1 Background and Rationale

Prostate cancer is the most common malignancy in men in the United States, with estimated 238, 590 new cases and 29,720 deaths in 2013 [3]. Approximately 80% of prostate cancer cases are diagnosed when the cancer is still confined to the primary site (localized prostate cancer). But 12% prostate cancers are diagnosed as locally advanced stage and 4% as metastatic disease.

Localized prostate cancer can be treated with various local therapies and some selected cases can be followed with active surveillance. However, approximately 20% to 40% of patients who have been treated with a curative intent develop disease recurrence [4]. While some selective patients with PSA-only recurrence with no evidence of disseminated disease can be treated with local salvage therapies, majority of patients with recurrent disease require systemic therapy such as ADT with or without concomitant antiandrogen agents. Despite high rate of initial response to ADT, virtually all patients will progress to metastatic disease, the disease condition called metastatic castrate-resistant prostate cancer (mCRPC). When patients develop mCRPC, the disease is considered noncurable and lethal with a median survival around 2 years.

There has been tremendous advance in the treatment of mCRPC over the last decade since the approval of docetaxel in 2003. Currently 6 therapeutic agents with various mechanisms that have shown survival benefit have been approved by Food and Drug Administration (FDA) for the treatment of mCRPC. While the approval of multiple agents have resulted in increased overall survival and improved quality of life and provided wider therapeutic options for the patients, it has created challenges of establishing the optimal use of these therapeutics including the optimal sequence and possible combination of those agents.

Sipuleucel-T is an autologous cellular immunotherapy product designed to stimulate an immune response against prostate cancer. Sipuleucel-T has improved survival in patients with asymptomatic or minimally symptomatic mCRPC in 3 phase III trials [5-7]. Despite the survival benefit, majority of patients treated with sipuleucel-T do not experience radiographic or PSA response, making the assessment of treatment response difficult. Furthermore, there is no established clinical or molecular marker to predict the response to sipuleucel-T immune therapy. However recently published analysis of immune responses from these 3 trials showed that antigen-specific immune responses to sipuleucel-T, both humoral and T-cell responses, correlate with survival benefit [8], confirming the immune-based mechanism of action of sipuleucel-T and also suggesting the possibility that producing a stronger immune response may translate to the better clinical outcome.

It has been suggested that immune modulation can be enhanced by radiation therapy through a variety of mechanisms. Radiation-induced cell death has been shown to stimulate tumor-specific

immune responses by enhanced display of tumor associated antigens and upregulation of tumor suppressive proteins and inflammatory cytokines [9]. This synergistic antitumor effect of combined immune therapy and radiation therapy has been evaluated in various cancer types. In prostate cancer, a phase I/II study of ipilimumab in combination with radiotherapy in mCRPC showed a promising antitumor effect including PSA decline and radiographic tumor reduction.

Similar to external radiotherapy, radiopharmaceutical agent also has shown to upregulate tumor antigens in prostate cancer models. In a study by Chakraborty et, al., the exposure to beta particle emitting radiopharmaceutical, samarium-153-ethylenediaminetetrame-thylenephosphonate (^{153}Sm-EDTMP), resulted in upregulation of the prostate cancer antigens, such as PSMA and PAP [10]. Such ^{153}Sm-EDTMP-induced phenotypic changes in tumor cells rendered these tumor cells more susceptible to T cell–mediated killing.

Radium-223 is an alpha-emitting radioisotope which is a bone-seeking calcium mimetic. It selectively targets the area with increased bone turnover, especially within the microenvironment of osteoblastic or sclerotic metastases [11]. It has been approved by FDA for the treatment of mCRPC with symptomatic bone metastases and without known visceral disease based on its ability to extend overall survival as shown its pivotal phase III trial [12]. Unlike beta particle emitting radiopharmaceuticals, radium-223 emits high-energy alpha particles of short range (<100 μm) thus saving the surrounding healthy tissues and significantly reducing unwanted myelotoxicity [13].

Based on the immune-modulatory effects of radiotherapy and radiopharmaceutical shown in the previous studies, we hypothesized that combined use of radium-223 and sipuleucel-T can enhance the sipuleucel-T-induced immune response, which translates to better clinical outcome. Since more than 90% of patients with mCRPC have bone metastases [14], this approach can be widely applied to early stage of mCRPC when patients are asymptomatic or minimally symptomatic from the bone lesions when the greatest clinical benefit can be obtained with immune-based therapies [15].

1.2 Study Agents

1.2.1 Sipuleucel-T

Sipuleucel-T is an autologous cellular immunotherapy product designed to stimulate an immune response against prostate cancer. It consists of autologous peripheral blood mononuclear cells (PBMCs), including antigen presenting cells (APCs, defined as large CD54-positive PBMCs) that have been activated in vitro with prostate antigen PA2024, a recombinant fusion protein composed of prostatic acid phosphatase (PAP) linked to granulocyte-macrophage colony-stimulating factor (GM-CSF).

Sipuleucel-T was approved in the United States in 2013 for the treatment of men with asymptomatic or minimally symptomatic mCRPC. In the pivotal trial D9902B (IMPACT), 512 patients were randomized 2:1 to receive sipuleucel-T (N=341) or control (N=171) [7]. Overall survival was longer in the sipuleucel-T group (median of 25.8 months versus 21.7 months; hazard ratio (HR) =0.78; 95% confidence interval (CI): 0.62, 0.98; P=0.03). More than 3,339 infusions of sipuleucel-T and control (a product manufactured from PBMCs without activation with the PA2024 antigen), have been administered to men with prostate cancer in clinical trials.

Clinical data to support the safety of sipuleucel-T are provided from 904 subjects (sipuleucel-T, N=601; control, N=303) who participated in 4 multicenter, randomized, double-blind, controlled Phase 3 studies. Three of these studies were conducted in men with mCRPC (studies D9902B, D9901, and D9902A), and 1 study was conducted in men with androgen dependent prostate cancer (ADPC) [5-7;16]. In the 4 randomized Phase 3 studies, AEs reported in ≥ 20% of all subjects were chills, fatigue, pyrexia, and back pain. The most common AEs (observed in ≥ 5% of sipuleucel-T subjects as well as at least twice the rate of that in control subjects) included chills, pyrexia, headache, myalgia, influenza-like illness, and hyperhidrosis. The majority of these events occurred within 1 day of infusion; were grade 1 or 2 in severity; and were generally of short duration (i.e., resolved in ≤ 2 days). Grade 3 or 4 events were reported in 27.6% of subjects in the sipuleucel-T group, compared with 28.4% in the control group.

1.2.2 Radium-223

Radium-223 (Bayer Pharmaceuticals, NJ) is a calcium mimetic which localizes to bone at areas with increased bone turnover. Radium-223 decays via a chain of short-lived daughter nuclides to lead, producing four alpha-particles [13]. The high energy of alpha particle induces effective double stranded DNA breaks with short range of less than 100 μm, minimizing myelosuppression and causing limited effects on normal tissue.

Radium-223 was approved by the US FDA on May 15, 2013 based on the interim results from a Phase III randomized trial, the ALSYMPCA study (Alpharadin in the treatment of patients with symptomatic bone metastases in castration-resistant prostate cancer). In this trial, 921 patients who had received, were not eligible to receive, or declined docetaxel were randomly assigned in a 2:1 ratio to receive six injections of radium-223 (at a dose of 50 kBq per kilogram of body weight intravenously (55 kBq/kg after implementation of NIST standard update) every 4 weeks or matching placebo. At the interim analysis, which involved 809 patients, radium-223, as compared with placebo, significantly improved overall survival (median, 14.0 months vs. 11.2 months; HR=0.70; 95% CI, 0.55 to 0.88; two-sided P=0.002). The updated analysis involving 921 patients confirmed the radium-223 survival benefit (median, 14.9 months vs. 11.3 months; HR=0.70; 95% CI, 0.58 to 0.83; P<0.001). The rate of AEs was consistently lower in the radium-223 group than in the placebo group for all AEs (93% vs. 96%), grade 3 or 4 AEs (56% vs.

62%), and serious AEs (47% vs. 60%). There were no clinically meaningful differences in the frequency of grade 3 and 4 hematologic AEs. Only one
patient developed grade 3 febrile neutropenia in the radium-223 group. For serious AEs that occurred in at least 5% of patients in the radium-223 group or the placebo group, the respective frequencies were as follows: disease progression (11% and 12%), bone pain (10% and 16%), anemia (8% and 9%), and spinal cord compression (4% and 5%). Also a significantly higher percentage of patients who received radium-223 had a meaningful improvement in the quality of life according to the Functional Assessment of Cancer Therapy-Prostate (FACT-P) total score during the period of study-drug administration (25% vs. 16%, P=0.02).

2.0 OBJECTIVES

2.1 Primary Objective

- To determine whether radium-223 in combination with sipuleucel-T enhances immune response to sipuleucel-T measured by peripheral PA2024-specific T-cell proliferation compared with sipuleucel-T alone in men asymptomatic or minimally symptomatic bone-metastatic CRPC

2.2 Secondary Objectives

2.2.1 Secondary Immune Objectives

- To evaluate peripheral antigen-specific T-cell proliferation over time.
- To evaluate peripheral antigen specific T-cell activation to sipuleucel-T over time.
- To evaluate antigen specific antibody response to sipuleucel-T over time.
- To evaluate the sipuleucel-T product immune parameters.

2.2.2 Secondary Clinical Objectives

- To investigate safety of combined use of radium-223 and sipuleucel-T.
- To evaluate PSA50 response (at least a 50% decline in PSA)
- To evaluate time to radiographic or clinical progression.

3.0 STUDY DESIGN

This is a randomized study designed to assess the antigen-specific immune response of sipuleucel-T with or without radium-223.

Eligible subjects will be registered and randomly assigned in a 1:1 ratio to receive sipuleucel-T and radium-223 or sipuleucel-T alone;

Arm 1: 6 infusions of radium-223 with 3 infusions of sipuleucel-T starting after second dose of radium-223

Arm 2: 3 infusions of sipuleucel-T alone

Subjects in both arms will undergo a standard 1.5 to 2.0 blood volume leukapheresis, followed approximately 3 days later by an IV infusion of sipuleucel-T. This process will occur a total of 3 times at approximately 2-week intervals. Subjects in Arm 1 will receive a total of 6 infusions of radium-223 at IV dose of 50 kBq/kg (55 kBq/kg after implementation of the NIST update) at 4-week interval.

All participants are allowed to receive the best supportive care which includes secondary hormonal manipulation (including flutamide, bicalutamide or nilutamide-but not abiraterone or enzalutamide) as required. No chemotherapy, external-beam radiation, or other radionuclides are allowed while on active treatment but are permitted after completion of active treatment. Glucocorticoid-containing treatments should be minimized to less than the equivalent dose of prednisone 10mg daily if feasible for the 3 months following sipuleucel-T therapy. All patients continue medical or surgical castration during treatment.

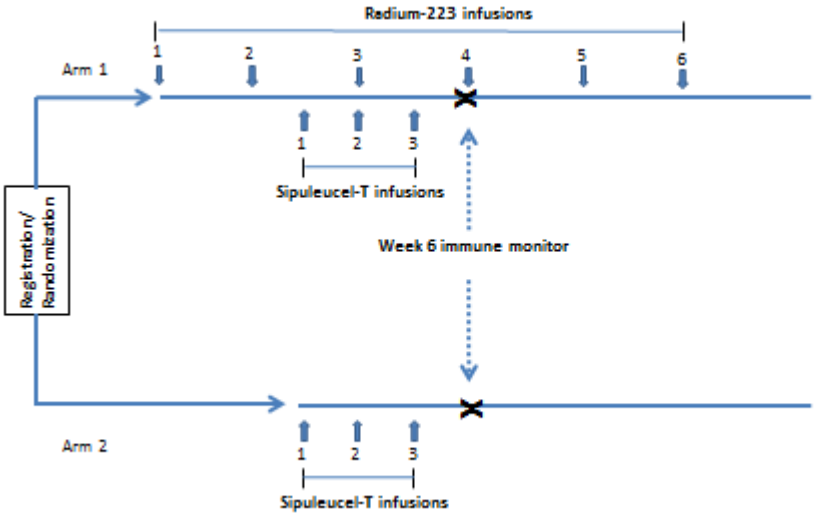
Research visits will be scheduled at screening, at each radium-223 infusion, prior to each leukapheresis (preleukapheresis visit), and week 32, 45 and 58 for Arm 1 (radium-223 and sipuleucel-T combination arm) and at screening, prior to each leukapheresis (preleukapheresis visit), and week 6, 10, 14, 26, 39, and 52 for Arm 2 (sipuleucel-T alone arm) for physical examinations, vital signs, ECOG performance status, adverse event (AE) monitoring, anticancer therapies, first opioid use for cancer-related pain, and laboratory tests. Serum PSA will be assessed at baseline, and week 16, 32, 45, and 58 for Arm 1 and at baseline, and week 10, 26, 39, and 52 for Arm 2. There is a ±7 day window for each visit to accommodate holidays, vacations, etc.

Imaging studies including bone scintigraphy test (bone scan) or Na-F PET or C11 acetate PET and abdomen/pelvis CT or MRI will be obtained at screening and week 16, 32, 45, and 58 for Arm 1 and at screening and week 10, 26, 39, and 52 for Arm 2. After the last study visit, imaging studies will be obtained according to the participating institution's standard and/or at the investigator's discretion.

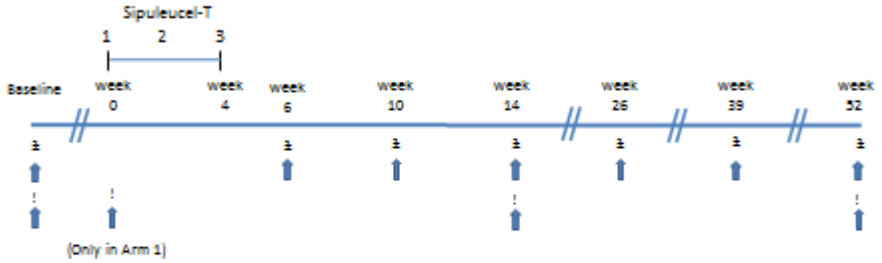
Immune monitoring blood samples will be drawn at screening and at 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T in both arms. Additional blood samples will be obtained at baseline, first pre-leukapheresis visit (only in Arm 1), and weeks 14 and 52 post the first infusion of sipuleucel-T for exploratory antigen spread assessment.

After last study research visit at week 58, safety, treatment-related AEs, survival status, PSA, first opioid use for cancer-related pain (if applicable), and anticancer therapies will be assessed every 3 months for additional 1 year or until the subject's death.

The study design is illustrated in Figure 1.



Immune monitoring schedule



*Week 0 = the first dose of sipuleucel-T in both arms

± **Immune response blood test**: at baseline, weeks 6, 10, 14, 26, 39, 52 after first sipuleucel-T infusion

! **Antigen spread blood test**:
arm 1: at baseline, 1st pre-leukapheresis visit, and weeks 14 and 52 after first sipuleucel-T infusion
arm 2: at baseline and weeks 14 and 52 after first sipuleucel-T infusion

4.0 STUDY POPULATION

To participate in this study, subjects must meet all inclusion criteria and none of the exclusion criteria.

4.1 Inclusion Criteria

4.1.1 Written informed consent provided prior to the initiation of study procedures.

4.1.2 Age ≥ 18 years.

4.1.3 Histologically documented adenocarcinoma prostate cancer confirmed by a pathology report from prostate biopsy or a radical prostatectomy specimen. If prostatic tumor is of mixed histology, > 50% of the tumor must be adenocarcinoma.

4.1.4 Bone metastases as manifested by one or more lesions on a bone scan performed within 2 months of screening

4.1.5 Castrate-resistant prostate cancer, in the setting of castrate levels of testosterone (≤ 50 ng/dL), defined as current or historical evidence of disease progression concomitant with surgical castration or ADT, as demonstrated by two consecutive rises in PSA OR new lesions on bone scan:

PSA progression will be defined as 2 rising PSA values compared to a reference value, measured at least 7 days apart and the second value is ≥ 2 ng/mL [1]. It must be documented within 2 months of screening.

Appearance of one or more new areas of abnormal uptake on bone scan when compared to imaging studies acquired during castration therapy or against the precastration studies if there was no response. Increased uptake of pre-existing lesions on bone scan does not constitute progression. It must be documented within 4 months of screening.

4.1.6 Serum PSA ≥ 2.0 ng/mL.

4.1.7 Screening ECOG performance status ≤ 1.

4.1.8 Asymptomatic or minimally symptomatic disease (no narcotic analgesic; other analgesics use is allowed)

4.1.9 Prior abiraterone and enzalutamide are permitted, but not required.

4.1.10 Concurrent osteoclast-inhibitory therapies (zoledronic acid, denosumab) are permitted if patients have been on a stable dose for at least 1 month

4.1.11 Adequate screening hematologic, renal, and liver function as evidenced by laboratory test results within the following ranges ≤ 28 days prior to registration:

- Absolute neutrophil count (ANC) ≥ 1.5 x10^9/L
- Platelet count ≥ 100 x10^9/L
- Hemoglobin ≥ 10.0 g/dL
- Total bilirubin level ≤ 1.5 x institutional upper limit of normal (ULN)
- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x ULN
- Creatinine ≤ 1.5 x ULN
- Albumin > 25 g/L

4.2 Exclusion Criteria

4.2.1 The presence of known lung or liver metastases greater than 1.0 cm in long-axis diameter.

4.2.2 The presence of lymphadenopathy greater than 3 cm in the short-axis diameter

4.2.3 The presence of known brain metastases.

4.2.4 Spinal cord compression, imminent long bone fracture, or any other condition that, in the opinion of the investigator, is likely to require radiation therapy and/or steroids for pain control during the active phase.

4.2.5 Previous treatment with chemotherapy for mCRPC or chemotherapy for any reason within 6 months prior to registration. (Chemotherapy in the adjuvant setting or for hormone-sensitive prostate cancer is permitted, as long as it was completed more than 6 months before registration).

4.2.6 Intention to receive chemotherapy within 6 months after enrollment in protocol therapy.

4.2.7 History of radiation therapy, either via external beam or brachytherapy within 28 days prior to registration.

4.2.8 Systemic radiotherapy with strontium-89, samarium-153, rhenium-186 or rhenium-188 for the treatment of bony metastases within previous 24 weeks

4.2.9 Prior history of other cancers (except non-melanoma skin cancers or low-grade low-stage urothelial cancers), or other localized cancers without evidence of recurrence for at least 3 years.

4.2.10 Use of prednisone or equivalent systemic corticosteroid within 2 weeks of sipuleucel-T. Use of inhaled, intranasal, intra-articular, and topical steroids is allowed. Oral or IV steroids to prevent or treat IV contrast reactions are allowed.

4.2.11 Use of opioid analgesics for cancer-related pain

4.2.12 Use of experimental drug within 4 weeks of treatment.

4.2.13 Uncontrolled medical conditions including diabetes, heart failure, COPD, ulcerative colitis, or Crohn's disease.

4.2.14 Uncontrolled fecal incontinence.

4.2.15 Any medical intervention, any other condition, or any other circumstance which, in the opinion of the investigator, could compromise adherence with study requirements or otherwise compromise the study's objectives.

5.0 STUDY TREATMENTS

5.1 Radium-223

5.1.1 Dosage Forms and Strength

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

5.1.2 Preparation

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

5.1.3 Dosage

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

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

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

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

$$\text{Volume to be administered (mL)} = \frac{\text{Body weight (kg) x dose (50 kBq/kg b.w)}^{a}}{\text{DK factor x 1,000 kBq (0.027 mCi)/mL}^{b}}$$

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

Decay Correction Factor Table

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

The Decay Correction Factor Table is corrected to 12 noon Central Standard Time (CST). To determine the decay correction factor, count the number of days before or after the reference date. The Decay Correction Factor Table includes a correction to account for the 7 hour time

difference between 12 noon Central European Time (CET) at the site of manufacture and 12 noon US CST, which is 7 hours earlier than CET.

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

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

All sites will be notified by Bayer when FINAL regulatory approval from the FDA is in place and the updated NIST standardization is to be implemented.

5.1.4 NIST Standardization Update

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

In 2014, NIST performed a re-assessment of the primary standardization based on preliminary information suggesting a potential discrepancy of approximately 8-10% between the published NIST primary standardization [20] and results obtained by other national metrology institutes (United Kingdom, Germany, Japan). After completion of the re-assessment, NIST reported their findings [21] and had issued a revised NIST SRM in 2015. The discrepancy in the NIST

standardization was determined to be -9.5% between activity values obtained using the old reference standard relative to the new primary standardization. Consequently, the current numerical values need to be corrected by approximately +10.5%

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

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

5.1.5 Administration

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

5.1.6 Handling and Patient Protection

Radium-223 is primarily an alpha emitter, with a 95.3% fraction of energy emitted as alpha-particles. The fraction emitted as beta-particles is 3.6%, and the fraction emitted as gamma-radiation is 1.1%. The external radiation exposure associated with handling of patient doses is expected to be low, because the typical treatment activity will be below 8,000 kBq (216 microcurie). (8,800 kBq (238 microcurie) after implementation of the NIST update) In keeping with the **A**s **L**ow **A**s **R**easonably **A**chievable (ALARA) principle for minimization of radiation exposure, it is recommended to minimize the time spent in radiation areas, to maximize the distance to radiation sources, and to use adequate shielding. Any unused product or materials

used in connection with the preparation or administration are to be treated as radioactive waste and should be disposed of in accordance with local regulations. The gamma radiation associated with the decay of radium-223 and its daughters allows for the radioactivity measurement of radium-223 and the detection of contamination with standard instruments.

General warning

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

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

Radiation protection

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

For drug handling

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

For patient care

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

5.2 Sipuleucel-T

The patients included in the study are scheduled for standard treatment with commercially available sipuleucel-T according to institutional practice and the approved product information (available at; http://www.dendreon.com/prescribing-information.pdf).

5.2.1 Leukapheresis and Infusion Scheduling

Leukapheresis appointments will be scheduled at approximately 2-week intervals. The clinical trial site will schedule the subject's infusion appointments based on this schedule. If the subject is subsequently deemed ineligible, the clinical trial site will be informed and the leukapheresis and infusion appointments will be canceled.

5.2.2 Leukapheresis

The collection of blood cells to manufacture sipuleucel-T is analogous to that for autologous blood transfusions. Subjects undergo a standard 1.5 to 2.0 blood volume leukapheresis to harvest PBMCs (primarily lymphocytes and monocytes).

5.2.3 Product Quality assessment

Prior to infusion, small samples (3% to 4%) of cellular components from pre and post-culture (PA2024) cells will be used to assess product potency as part of the normal sipuleucel-T manufacturing process. Sipuleucel-T quality assessments include total TNC count, CD54+ cell count and CD54 upregulation.

5.2.4 Sipuleucel-T Infusion

Each sipuleucel-T dose is released for infusion approximately 3 days following the leukapheresis procedure. Subjects will be infused according to the infusion guidelines (available at; http://www.dendreon.com/prescribing-information.pdf).

5.2.5 Sipuleucel-T Product Failures and Leukapheresis Rescheduling

In the event that a subject's leukapheresis procedure fails, sipuleucel-T does not meet quality release specifications, or infusion is not possible for any other reason, the subject may be scheduled to undergo a repeat leukapheresis procedure. A minimum of 2 days must have elapsed since the last leukapheresis procedure. Scheduling will depend upon capacity of the leukapheresis and Dendreon manufacturing facilities. If the second or third leukapheresis appointment is delayed or rescheduled for any reason, a physical examination and all other pre-leukapheresis procedures must be repeated within the 5 days prior to the next leukapheresis procedure.

5.3 Treatment Compliance

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

5.4 Drug Accountability

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

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

5.5 Concomitant Medications

Subjects should receive full supportive care, including transfusions of blood and blood products, antibiotics, antiemetics, or other therapies, in accordance with standard practice at the clinical trial site. Subjects receiving ADT, bisphosphonate therapy such as zoledronic acid (Zometa®), or denosumab (Xgeva®) at the time of registration should continue the therapy at a stable dose during radium-223 treatment.

All concomitant medications administered from screening through the last research visit at week 58, including indication, dose, route, frequency and treatment dates, will be recorded in the subject's medical record and on the CRF. After the last research visit at week 58, only anticancer therapies will be recorded. The first opioid taken for cancer-related pain will be recorded, regardless of when it is started.

5.6 Prohibited and/or Restricted Treatments

Subjects on ADT must continue this therapy until the post-treatment visit or disease progression, whichever occurs first.

The following medications and interventions must not be initiated until the completion of radium-223 treatment or disease progression, whichever occurs first:

- Investigational vaccines or other investigational products.
- Ipilimumab or nivolumab.
- Hemibody radiation

Glucocorticoid-containing treatments should be minimized to the equivalent dose of prednisone 10mg daily or less if feasible for the 3 months following sipuleucel-T. Use of inhaled, intranasal, intra-articular, and topical steroids is acceptable, as well as a short course (i.e., ≤ 1 day) of prophylactic steroid therapy prior to administration of IV contrast for CT imaging.

5.7 Discontinuation from Study Treatments or Assessments

5.7.1 Patient withdrawal

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

- Consent withdrawal by the subject for any reason.
- Occurrence of AEs, if discontinuation of study medication is considered necessary by the Investigator and/or patient.
- The subject develops any condition requiring a prohibited treatment (Section 5.6).
- Lack of patient compliance
- Protocol violation.
- Study termination.
- The subject is lost to follow-up.
- Progressive disease (patients will only come off study after meeting PCWG2 and/or RECIST criteria for radiographic progression and not for PSA)

Subjects who withdraw consent, or lose the ability to freely provide consent, will be followed by the investigator for survival every 3 months using the Social Security Death Index (SSDI) and National Death Index (NDI). Date of death, where available, will be recorded in the subject's medical record and on the CRF.

Whenever possible, subjects discontinuing from the study will undergo a post-treatment visit. All AEs considered by the investigator to be related to study treatment(s) that are ongoing at the time of discontinuation will be followed by the investigator until resolution or until the subject returns to baseline. The investigator should make every reasonable attempt to ensure that the subject is

contacted (via telephone or during a post-treatment visit) to obtain the final status of any AEs and concomitant medications.

5.7.2 Discontinuation from Sipuleucel-T Treatment

Subjects who are unable to, or refuse to, undergo leukapheresis or receive at least 1 partial sipuleucel-T infusion should discontinue sipuleucel-T treatment.

Subjects who have received at least 1 partial (> 0 mL) infusion of sipuleucel-T may discontinue sipuleucel-T without the requirement to discontinue radium-223. These subjects will continue all protocol-specified assessments in the active phase.

Any toxicity requiring sipuleucel-T discontinuation will be recorded as an AE in the subject's medical record and on the CRF.

5.7.3 Discontinuation from Radium-223 Treatment

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

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

5.7.4 Study Termination

The whole study may be discontinued at the discretion of the investigator in the event of any of the following:

- Occurrence of AEs not seen previously which by virtue of their nature, severity and duration are considered to necessitate study termination.
- Hematologic adverse events criteria for study termination
  - >2 events of grade ≥ 3 thrombocytopenia or neutropenia in the first 10 subjects
  - >3 events of grade ≥ 3 anemia in the first 10 subjects
- Medical or ethical reasons affecting the continued performance of the study.
- Bayer is unable to provide radium-223 for the subject.
- Dendreon is unable to manufacture siptuleucel-T in > 2 of first 10 patients.

- Difficulties in the recruitment of patients.

5.8. Lost to Follow-Up

If the subject fails to respond to requests for follow-up, the clinical trial site will send a registered letter, at a minimum, to the subject requesting contact with the clinic. All attempts to resume contact (including copies of written correspondence) will be included in the source documentation. Subjects who do not respond to requests for follow-up after all reasonable attempts to establish contact will be considered "lost to follow-up". Subjects who are lost to follow-up will be followed by the investigator for survival every 3 months using the SSDI and the NDI. Date of death, where available, will be recorded in the subject's medical record and on the CRF.

5.9 Disease Progression

Disease progression is determined by the investigator per the definition below. The earliest date of disease progression will be recorded in the subject's medical record and on the CRF. Following disease progression, all study treatments will cease, the subject will undergo a post-treatment visit and will be treated at the investigator's discretion.

5.9.1 PSA Progression

Any 1 or more of the following events constitutes PSA progression:

- PSA Progression based on Prostate Cancer Working Group 2 (PCWG2) criteria [1]:
  - Decline from baseline: the first PSA increase that is ≥ 25% and ≥ 2 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later (i.e., a confirmed rising trend).
  - No decline from baseline: PSA progression ≥ 25% and ≥ 2 ng/mL after 12 weeks from baseline.

PSA measurements obtained during the treatment should not be used as the sole criterion for determining progression. Study treatments may be continued in equivocal cases where there is PSA progression but no clear evidence of disease progression or clinical deterioration, and where the investigator feels the subject is deriving clinical benefit from the study treatments and subject safety is not compromised.

5.9.2 Clinical and Radiographic Progression

5.9.2.1 Clinical Progression

- New spinal cord or nerve root compression.
- New pathologic fracture.
- Use of opioid analgesics for cancer-related pain

5.9.2.2 Radiographic Progression

- Disease progression based on radiographic imaging based on RECIST and PCWG2 criteria for soft tissue and bone, respectively.

6.0 STUDY ASSESSMENTS AND PROCEDURES

6.1. Schedule of Assessments

a. Arm 1: sipuleucel-T and radium-223

	Screen[a] Im-1	Rad-1[b]	Rad-2	PreL-1[c]	Sip-1	Rad-3	PreL-2[c]	Sip-2	PreL-3[c]	Sip-3	Rad-4	Im-2[d]	Rad-5	Im-3[d]	Rad-6	Im-4[d]	Im-5[d]	Im-6[d]	Im-7[d]	F/U[e]
	Week	0	4	6[c]	6	8	8[c]	8	10[c]	10	12	12[d]	16	16[d]	20	20[d]	32[d]	45[d]	58[d]	
Informed consent	X																			
Registration/	X																			
Pathology	X																			
Rad-223 infusion		X	X			X					X		X		X					
Sip-T infusion					X			X		X										
Clinical Assessments																				
Medical History	X																			
P/E	X	X	X	X		X	X		X		X		X		X		X	X	X	
Vital Signs	X	X	X	X	X[f]	X	X	X[f]	X	X[f]	X	X	X	X	X	X	X	X	X	
ECOG PS	X	X	X	X		X	X		X		X	X	X	X	X	X	X	X	X	X
Imaging[g]	X												X				X	X	X	X[h]
Safety Assessment																				
AEs/SAEs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[i]
Medication																				
Anticancer Tx	X	X	X	X		X	X		X		X		X		X		X	X	X	X
1st Opioid use	X	X	X	X		X	X		X		X		X		X	X	X	X	X	X
Survival Status																				
Lab Assessments																				
Hematology	X	X	X	X		X	X		X		X		X		X		X	X	X	
Chemistry/ LDH/ magnesium/phosphorus	X	X	X	X		X	X		X		X		X		X		X	X	X	
Testosterone	X																			
PSA	X												X				X	X	X	
Coagulation[L]	X																			
Immune Assessments																				
Dendreon[j]	X											X		X		X	X	X	X	
JH Lab[k]	X			X												X			X	

The values for radium-223 in this protocol has been revised as per the US NIST standardization update (agreed on 17 Mar 2015)

Abbreviations: AE = adverse event; A/P = abdomen/pelvis; CT = computed tomography; ECOG = Eastern Cooperative Oncology Group; F/U = follow up visit; Im = Immune assessment visit; JH = Johns Hopkins; MRI = magnetic resonance imaging; PAP = prostatic acid phosphatase; P/E = physical exam; PET = positron emission tomography; Sip = sipuleucel-T infusion visit; PS = performance status; PreL = preleukapheresis visit; PSA = prostatic specific antigen; Rad = radium-223 infusion visit; SAE = serious adverse event; Tx = treatment

a. All screening procedures must occur within 28 calendar days prior to registration, with the exception of written informed consent, histological documentation of prostate cancer with Gleason grading, and imaging for confirmation of metastatic disease
b. Radium-223 visits will occur 6 times at weeks 0, 4, 8, 12, 16, and 20
c. Applies to all leukapheresis; pre-leukapheresis visits will occur ≤ 5 days prior to each leukapheresis. First sipuleucel-T infusion may occur any time between the second and third radium-223 infusion.
d. At baseline, week 6, 10, 14, 26, 39, and 52 post-first sipuleucel-T infusion
e. Follow-up visits will occur at every 3 months after week 58 visit for additional 1 year or until the subject's death
f. Vital signs will be measured 1 to 30 minutes prior to, and at 30 minutes (± 10 min) following each infusion
g. Bone scan and CT or MRI abdomen/pelvis
h. Per institution's standard procedure/at the investigator's discretion
i. Only treatment-related events will be collected
j. Baseline sample can be drawn at subject's "day one visit" (prior to dosing). See Appendix 1 for sample preparation instructions.
k. Blood samples for antigen spread analysis will be collected at baseline (can be drawn at subject's "day one visit" (prior to dosing) , 1st pre-leukapheresis visit, and weeks 14 and 52 post-first sipuleucel-T infusion.
l. PTT, PT/INR

b. Arm 2: sipuleucel-T alone

	Screen[a] Im-1	PreL-1[b]	Sip-1	PreL-2[b]	Sip-2	PreL-3[b]	Sip-3	Im-2[c]	Im-3[c]	Im-4[c]	Im-5[c]	Im-6[c]	Im-7[c]	F/U[d]
	Week	0	0	2	2	4	4	6[c]	10[c]	14[c]	26[c]	39[c]	52[c]	
Week														
Informed consent	X													
Registration/	X													
Pathology	X													
Sip-T infusion			X		X		X							
Clinical Assessments														
Medical History	X													
P/E	X	X		X		X								
Vital Signs	X	X	X[e]	X	X[e]	X	X[e]	X	X	X	X	X	X	
ECOG PS	X	X		X		X		X	X	X	X	X	X	
Imaging[f]	X									X	X	X	X	X[g]
Safety Assessment														
AEs/SAEs		X	X	X	X	X	X	X	X	X	X	X	X	X[h]
Medication														
Anticancer Tx	X	X		X		X								X
1st Opioid use	X	X		X		X								X
Survival Status														
Lab Assessments														
Hematology	X	X		X		X								
Chemistry/ LDH/ magnesium / phosphorus	X	X		X		X								
Testosterone	X													
PSA	X								X		X	X	X	
Coagulation[K]	X													
Immune Assessments														
Dendreon[i]	X							X	X	X	X	X	X	
JH Lab[j]	X									X			X	

The values for radium-223 in this protocol has been revised as per the US NIST standardization update (agreed on 17 Mar 2015)

Abbreviations: AE = adverse event; A/P = abdomen/pelvis; CT = computed tomography; ECOG = Eastern Cooperative Oncology Group; F/U = follow up visit; Im = Immune assessment visit; JH = Johns Hopkins; MRI = magnetic resonance imaging; PAP = prostatic acid phosphatase; P/E = physical exam; PET = positron emission tomography; Sip = sipuleucel-T infusion visit; PS = performance status; PreL = preleukapheresis visit; PSA = prostatic specific antigen; SAE = serious adverse event; Tx = treatment

a. All screening procedures must occur within 28 calendar days prior to registration, with the exception of written informed consent, histological documentation of prostate cancer with Gleason grading, and imaging for confirmation of metastatic disease
b. Applies to all leukapheresis; pre-leukapheresis visits will occur ≤ 5 days prior to each leukapheresis
c. At baseline, week 6, 10, 14, 26, 39, and 52 post-first sipuleucel-T infusion
d. Follow-up visits will occur at every 3 months after week 58 visit for additional 1 year or until the subject's death
e. Vital signs will be measured 1 to 30 minutes prior to, and at 30 minutes (± 10 min) following each infusion
f. Bone scan and CT or MRI abdomen/pelvis
g. Per institution's standard procedure/at the investigator's discretion
h. Only treatment-related events will be collected
i. Baseline sample can be drawn at subject's "day one visit" (prior to dosing). See Appendix 1 for sample preparation instructions.
j. Blood samples for antigen spread analysis will be collected at baseline (can be drawn at subject's "day one visit" (prior to dosing), and weeks 14 and 52 post-first sipuleucel-T infusion.
k. PTT, PT/INR

6.2 Registration

After eligibility screening, patients who are selected to participate will be registered with the study site/institution. A record of patients who fail to meet entry criteria (ie, screen failures) will be maintained by each individual site. Patient registration must be complete before beginning any treatment or study activities.

Prior to protocol enrollment and initiation of treatment, subjects must sign and date an IRB-approved consent form. To register a patient, the following documents must be completed and faxed or emailed to the Lead Center Program Coordinator:

- Signed patient consent form
- Registration Form
- Copies of the prostate cancer pathology report, baseline laboratory studies including CBC with differential, liver and kidney function tests, testosterone, PSA, and CT/bone scan. Other materials may also be sent if considered pertinent for confirming patient eligibility.

The Lead Center will review the documents to confirm eligibility. To complete the registration process the Lead Center will:

- Assign a patient study number
- Register the patient on the study with SKCCC's Clinical Research Office
- Fax or e-mail the patient study number to the participating site

6.3 Randomization

Eligible subjects will be registered and randomly assigned in a 1:1 ratio to receive 6 infusions of radium-223 and 3 infusions of sipuleucel-T starting after second dose of radium-223 (Arm 1) or 3 infusions of sipuleucel-T alone (Arm 2).

Arm 1: 6 infusions of radium-223 with 3 infusions of sipuleucel-T starting after second dose of radium-223

Arm 2: 3 infusions of sipuleucel-T

6.4 Demographics

Demographic information, including birth date, race and ethnicity will be recorded in the subject's medical record and on the CRF.

6.5 Clinical Assessments

Clinical assessments will be performed at the times noted in Table 1.

6.5.1 Medical History

Significant historic and current medical conditions or illness, allergies to medications, and prior surgical interventions will be recorded in the subject's medical record and on the CRF. Information regarding the subject's history of smoking and other risk factors for CVEs will also be recorded.

Symptoms that are ongoing at the time of, or that develop after the subject provides informed consent and before registration, will also be considered medical history.

Medical history will include the subject's 6 most recent PSA values, vaccinations in the two years prior to registration, and all prior anticancer therapies.

6.5.2 Physical Examination

Physical examinations will be performed at the times noted in Table 1 and must be conducted by an appropriately qualified investigator listed on the Form FDA 1572.

The physical examinations conducted at screening and at pre-leukapheresis visits will include a review of the skin, pulmonary, cardiovascular, and neurologic systems, abdomen, and extremities. A review of additional body systems will be at the discretion of the investigator.

For all other visits that call for a physical examination, the body systems reviewed will be at the discretion of the investigator.

Each physical examination will include weight measurement. Height will be measured at screening only.

Abnormal physical examination findings at screening will be considered part of the medical history and will be recorded on the Medical History CRF. Any new or worsening physical examination findings identified after the subject's registration will be considered AEs and will be recorded in the subject's medical record and on the CRF.

6.5.3 Vital Signs

Vital signs (respiration rate, temperature, heart rate, and blood pressure) will be measured at the times noted in Table 1. Vital signs will be recorded in the subject's medical record and on the CRF.

6.5.4 ECOG Performance Status

Eastern Cooperative Oncology Group performance status will be assessed at the times noted in Table 1. The assessment must be conducted by an appropriately qualified investigator listed on the Form FDA 1572. The ECOG performance status will be recorded in the subject's medical record and on the CRF.

6.5.5 Imaging

Subjects must have a bone scintigraphy test (bone scan) or Na-F PET or C11-acetate PET and CT or MRI scan of the abdomen and pelvis within 56 days prior to registration. If a bone scan is used, solitary lesions or lesions which could be attributed to causes other than metastatic prostate cancer must be confirmed with a second modality (i.e., CT or MRI).

When available, standard of care scans will be used to demonstrate metastatic disease at screening. If scans meeting the eligibility criteria are not available, scans may be obtained during the screening phase. If post-screening radiographic imaging is performed per standard of care to assess disease progression, the same modalities used at screening should be used.

6.6 Safety Assessments

Safety assessments will be performed in conjunction with all visits as indicated in Table 1.

6.6.1 Adverse Events and Serious Adverse Events

Adverse events, including serious AEs (SAEs) will be assessed at all visits and as needed during the course of the study.

All non-serious AEs and SAEs, regardless of relationship to study treatment, will be collected from registration through the week 20 and will be recorded in the subject's medical record and on the CRF. Following week 20, only new treatment-related AEs and SAEs will be recorded.

Only treatment-related AEs and SAEs ongoing at week 20 visit will be followed by the investigator until resolution, return to baseline, or a determination by the investigator that no further improvement is expected.

See Section 7.0 for information regarding AE and SAE reporting.

6.7 Concomitant Medications

The subject's current prescription and nonprescription medications will be reviewed at the screening visit and recorded in the subject's medical record and on the CRF. Medications taken from registration through the week 20 will also be recorded. Any medications associated with a possible or probable treatment-related AE will be recorded on the CRF, regardless of when they are taken. The concomitant medication administered, indication, dose, route, frequency, and start and stop dates will be recorded in the subject's medical record and on the CRF.

6.7.1 Anticancer Therapies

All anticancer therapies received prior to screening and through the end of the follow-up will be recorded in the subject's medical record and on the CRF. Anticancer therapies include, but are not limited to, radiation, chemotherapy, hormone therapy, investigational cancer therapies, all other systemic therapies, and surgery. The start/stop date and dose of anticancer therapies will be recorded.

6.7.2 First Cancer-Related Opioid Use

Each subject's first cancer-related opioid use, as determined by the investigator, will be recorded in the subject's medical record and on the CRF. Cancer-related opioid use is defined as use for cancer-related pain that is of at least 2 consecutive days in duration.

Opioid analgesic use for treatment or prevention of infusion reactions, such as chills or rigors, post-procedural pain, or for indications clearly unrelated to cancer pain (e.g., cough, pain due to an injury or accident) should NOT be considered cancer related.

6.8 Survival Status

Confirmation of survival status will be obtained by speaking directly with the subject on the telephone or in person. Death certificates will be obtained as a source document for the date and cause of death recorded on the CRF.

If a death certificate cannot be obtained, one or more of the following documents (in preferential order) will be the source document for the date and cause of death:

1. SSDI/NDI (date of death only).
2. Hospital report.
3. Hospice report.
4. Clinical chart note.
5. Other medical records.

6.9 Laboratory Test Assessments

At the times noted in Table 1, blood samples will be obtained for assessment of the parameters presented in Table 2.

Table 2: Clinical Laboratory Tests

Hematology	**Chemistry**
hemoglobin	sodium
hematocrit	potassium
platelets	bicarbonate
mean corpuscular volume	chloride
mean corpuscular hemoglobin	lactate dehydrogenase
mean corpuscular hemoglobin concentration	blood urea nitrogen
leukocytes	creatinine
neutrophils	phosphorus
lymphocytes	calcium
monocytes	magnesium
eosinophils	glucose
basophils	albumin
	total protein
	total bilirubin
	alkaline phosphatase
	alanine aminotransferase
	aspartate aminotransferase
Other Assessments	
total testosterone	
prostatic specific antigen	
Coagulation	
prothrombin time	
Partial thromboplastin time	
international normalized ratio	

6.10 Immune Assessments

6.10.1 Peripheral Immune Response Assessments

Cellular and humoral immune responses will be assessed from 100 mL blood samples (9x10 mL heparin whole blood tubes and 1x10 mL serum tube) collected at the times noted in the Schedule of Assessments (Table 1).

Immune response assessments will include T-cell activation to PA2024 and PAP using IFN-γ ELISPOT assay, T-cell proliferation response to PA2024 and PAP by ^{3}H-thymidine uptake, and humoral (IgG and IgM) response to PA2024 and PAP by ELISA.

The following research blood samples should be collected and processed as instructed in Immune Monitoring Requisition Form (Appendix 1):

Ship the specimen as instructed in Immune Monitoring Shipping Instructions (Appendix 2)

6.10.2 Antigen Spread Assessment

Antigen spread induced by sipuleucel-T treatment will be evaluated by measuring humoral (IgG) response to 10 off-target antigens which were previously identified from subjects in the sipuleucel-T arm of IMPACT study using protein microarrays (ProtoArrays) [17]. Serum IgG level against each antigen will be measured using Luminex xMAP.

Exploration of new secondary antigens will also be performed by detecting significant IgG responses using the protein microarray.

At each time points (baseline, 1st pre-leukapheresis visit (only in Arm 1), and weeks 14 and 52 post-first sipuleucel-T infusion), the following research blood samples should be collected and processed as outlined below:

- Draw approximately 10 mL of peripheral blood into 2 plain (red top) or SST (gold top) tubes, each containing ≥ 5 mL of blood per vacutainer.
- Allow blood to coagulate for 20 minutes, then centrifuge at 25^0C, 1500 x g (2700-3000 rpm), for 15 minutes.
- Pipette the serum into 10 cryotubes (about 0.5 mL/tubs).
- Store cryotubes frozen, at - 80 ^{0}C , until the time of analysis

Antigen Spread analysis will be performed in the Laboratory of Charles Drake MD/PhD at Johns Hopkins. Plasma pharmacokinetic samples collected at outside institutions should be shipped on dry ice (a minimum of 5 kg) to laboratory at Johns Hopkins, at the address below. Overnight shipments should occur on Monday–Thursday, except when the following day is a holiday. A fax or call should be placed to the laboratory prior to shipment, providing the tracking information.

Charles Drake, MD/PhD
Johns Hopkins Hospital
Attn: Thomas Nirschl and Brian Francica
CRB1, 4th floor, Room 416.
1650 Orleans Street
Baltimore, MD 21231
Phone number:
Lab phone: 410-502-9778

Cell phone: 703-635-9449 or 256-975-1661
Email: tnirsch1@jhmi.edu or bfranci3@jhmi.edu

6.10.3 Product Immune Parameter Assessments

Prior to infusion, small samples (3% to 4%) of cellular components from pre and post-culture (PA2024) cells will be used to assess product potency as part of the normal sipuleucel-T manufacturing process. The data of the product immune parameters will be obtained for analysis for correlation with clinical outcome.

6.11 Study Materials

The clinical trial site will have a calibrated scale for recording body weight and a calibrated sphygmomanometer and thermometer for vital signs assessments. A current and fully stocked advanced cardiac life support cart will be immediately available on the premises. The clinical trial site will also have a refrigerated centrifuge, a monitored and alarmed refrigerator, and a freezer (-20°C or below) as well as containers and dry ice for shipment of blood samples.

The clinical trial site will provide all materials required for accurate documentation of subject visits and study activities.

Materials needed for the collection of laboratory samples, including immune monitoring samples, will be provided by Dendreon.

7.0 ADVERSE EVENTS

An AE is defined as any untoward medical occurrence in a subject while on study that does not necessarily have a causal relationship with the treatment. An AE can, therefore, be any unfavorable and unintended sign (e.g., abnormal laboratory finding), symptom, or disease temporally associated with the treatment, whether or not considered related to the treatment. Adverse events include exacerbation of a pre-existing illness, an increase in frequency or intensity of a pre-existing episodic event or condition, a condition detected or diagnosed after product administration (even though it may have been present prior to the start of the study) or a continuous persistent disease or symptoms present at screening that worsen following the start of the study.

7.1 Categories for Ranking Severity of Adverse Events

The NCI CTCAE [2] will be used to score AE severity. In general, the following general severity definitions apply:

Mild (Grade 1):	The AE results in mild, easily tolerated symptoms, or is asymptomatic with clinical or diagnostic observations only. Intervention is not usually indicated.
Moderate (Grade 2):	The AE produces moderate symptoms with discomfort sufficient to interfere with some aspect of the subject's normal daily activity. Minimal, local, or noninvasive intervention is required.
Severe (Grade 3):	The AE is medically significant but not immediately life threatening, results in discomfort or disability which is incapacitating and prevents most normal daily activities, clearly damaging to the health, requiring hospitalization, prolongation of existing hospitalization or complicated treatment.
Life Threatening (Grade 4):	The AE could reasonably result in death unless immediate medical intervention is undertaken.
Fatal (Grade 5):	The AE results in death.

7.2 Relationship of Adverse Events to Study Treatments

The following categories will be used to determine relatedness of AEs to study treatments:

None:	The AE is clearly related to other factors, such as the subject's clinical state, environmental factors, or other modes of therapy or concomitant medications administered to the subject.
Possible:	The AE follows a reasonable temporal sequence from administration of study treatment, but could readily have been produced by the subject's clinical state, environmental factors, or other modes of therapy or concomitant medications administered to the subject.
Probable:	The AE follows a reasonable temporal sequence from administration of study treatment and cannot readily have been produced by the subject's clinical state, environmental factors, or other modes of therapy or concomitant medications administered to the subject.

7.3 Adverse Events Reporting

The principal investigator will notify the appropriate regulatory agencies of any SAEs occurring during the study period, regardless of causality. These agencies include the Sidney Kimmel Comprehensive Cancer Center (SKCCC) Data and Safety Monitoring Committee (DSMC), and the Institutional Review Board (IRB) and the Institutional Biosafety Committee (IBC) of the Johns Hopkins Medical Institutions (JHMI). The representatives of Dendreon will also be notified. Expedited reporting to the PI within 24 hours is required for all SAEs (see Section 7.4.1). For SAEs that are fatal, life-threatening, or treatment-related but non-life threatening: IRB/IBC reporting by the PI is required within 3 days. For unrelated SAEs, IRB/IBC reporting

by the PI is required within 15 days. All other AEs should be documented on CRFs and submitted according to the standard data management guidelines.

Adverse event information will be collected continuously throughout the duration of the study. Participants will be instructed to notify their treating provider of any new signs or symptoms, and providers will actively assess patients for adverse events at each visit (including by evaluation of laboratory studies). The investigator will assess each AE for its severity and for its relationship to the study drug, and all events Grade 1 or higher will be documented on CRFs and then reported as described above within the required time frame. Any AE occurring while a patient is on study (*i.e.* after informed consent has been signed) or within 30 days of study termination requires reporting. AEs occurring later than this must still be reported if a causal relationship with the study drug is suspected.

For all AEs, the investigator must pursue and obtain information to adequately determine the causality and outcome of the event, and to assess whether it meets criteria for a SAE. In addition, follow-up of an AE is required until the event either resolves or stabilizes.

The principal investigator must keep copies of all CRFs and other AE information, including correspondence with the IRB and/or FDA, for as long as required to comply with national and international regulations.

7.4 Serious Adverse Events

An SAE is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life threatening adverse drug experience, subject hospitalization or prolongation of an existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

7.4.1 Serious Adverse Event Collection and Reporting

All SAEs and unknown/unexpected reactions that occur from the signing of the study-specific consent through the duration of the post-therapy adverse event collection should be reported to the Study Coordinator, Dendreon Safety Manager, and Bayer Global Pharmacovigilance within 24 hours of being made aware of the SAE. If the Study Coordinator cannot be reached within 24

hours, the PI (Emmanuel Antonarakis MD) should be contacted at 443-287-0553 or at eantona1@jhmi.edu. Notification can be made via phone or telefacsimile or email.

Study Coordinator
Attn: Serina King
Facsimile: 410-614-7287
Phone: 410-614-6139

Dendreon Corporation
Attn: Safety Manager
Facsimile: 206-829-1647
Phone: 206-219-7899
After Hours: 206-274-6774

Bayer
Attn: Global Pharmacovigilance - USA
Email DrugSafery.GPV.US@bayer.com
Facsimile: 973-709-2185
Address:
Mail only: Bayer HealthCare
P.O. Box 915Whippany, NJ 07981-0915
Address: 100 Bayer Blvd., Whippany, NJ 07981
FDX or UPS only 67 Whippany Road, Whippany, NJ 07981 for UPS

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

The initial report for each SAE or death to the Study Coordinator should include the following information:

- name and contact information of the reporter
- protocol # and title
- patient identified by one or more of the following:
  - patient initial
  - patient identification number
  - knowledge that a patient who experienced the adverse event exists
  - age
  - sex
- date the event occurred
- description of the SAE

- investigational agent(s) received (and dose level) at the time the SAE occurred
- time from administration of the investigational agent(s) to start of the event
- description of the patient's condition

Follow-up information including severity, causality, action taken, concomitant medications, and outcome should be communicated to the principal investigator as soon as possible with an indication whether an amendment will need to be made to the protocol, the consent form, or both, as a result of this event.

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

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

OR

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

The report of SAE to Dendreon should be made using an SAE Report Form provided by Dendreon (Appendix 3). Significant new information regarding an ongoing SAE and the resolution must be sent to Dendreon within 3 business days of awareness of the new information to Dendreon on the SAE Report Form.

Sponsor investigator shall notify institution, co-investigators and IRB immediately during the conduct of the study and/or after the study is completed should it become aware of information related to the study that would impact participant safety or clinical care. Institution shall promptly disclose such information to study participants.

7.5 Death Reports

Death is an expected outcome of this study. Deaths that meet any of the following criteria must be reported as an SAE:

- Deaths that occur within 30 days of receiving the last study treatment.
- Deaths that are the outcome of a SAE that occurs within 30 days from the last study treatment and are not due to disease recurrence.
- Deaths that occur at any time during the study and are considered by the investigator to be possibly or probably related to the study treatment.

Deaths that do not meet these criteria will not be reported as an SAE, but will be recorded on the CRF.

7.6 Laboratory Test Result Abnormalities

The following laboratory abnormalities should be captured on the AE CRF or SAE Report Form as appropriate:

- Any clinically significant result that is not part of another reported clinical diagnosis.
- Any result that meets the definition of an SAE.
- Any result leading to study drug discontinuation or interruption.
- Any result that required therapeutic intervention or a change in subject management.

Laboratory abnormalities not meeting the above conditions will not be reported on the AE CRF or SAE report form.

8.0 STATISTICAL CONSIDERATIONS

8.1 General Considerations

This randomized phase 2 study is designed to test the null hypothesis of no difference in immune response to sipuleucel-T based on PA2024-stimulated T cell proliferation via tritiated-thymidine uptake between sipuleucel-T alone and sequential administration of radium-223

8.2 Sample Size Determination

The study is designed to test the null hypothesis of no difference in the stimulation index (SI) 6 weeks after the first vaccination with sipuleucel-T between sipuleucel-T alone and sequential administration of radium-223 and sipuleucel-T using PA2024-stimulated T cell proliferation via ^{3}H-thymidine uptake as the assay. With 15 patients per treatment group, there is 80% power to detect a 3.6-fold increase in mean SI between the sequential radium-223 and sipuleucel-T and sipuleucel-T alone (data from OpenACT trial) at 6 weeks after the first sipuleucel-T infusion [18]. These power calculations are based on one-sided tests at the 0.05 level. The calculations assume that SI values are lognormally distributed with the same SD in each group, namely 1.4. The value of 1.4 comes from the P09-1 (OpenACT) trial.

To allow for drop-outs and subjects who may not receive 3 infusions of sipuleucel-T or 4 infusions of radium-223, 34 subjects will be enrolled.

8.3 Populations for Analyses

The immune response population will be defined as subjects who receive 3 infusions of sipuleucel-T and 2 infusions of radium-223. Analysis of immune response endpoints, including

the primary endpoint, will be performed using this population. This population will also be used to perform supplementary analyses of non-immune response efficacy endpoints.

The safety population will include all subjects who receive at least 1 leukapheresis procedure or receive at least 1 dose of radium-223. All safety variables (e.g., AEs, laboratory data, and vital sign data) will be analyzed based on the safety population.

8.4 Study Termination

The whole study may be discontinued at the discretion of the investigator in the event of any of the following:

- Occurrence of AEs not seen previously which by virtue of their nature, severity and duration are considered to necessitate study termination.
- Hematologic adverse events criteria for study termination
  - >2 events of grade ≥ 3 thrombocytopenia or neutropenia in the first 10 subjects
  - >3 events of grade ≥ 3 anemia in the first 10 subjects

  Medical or ethical reasons affecting the continued performance of the study.
- Bayer is unable to provide radium-223 for the subject.
- Dendreon is unable to manufacture siptuleucel-T in >2 of first 10 patients.
- Difficulties in the recruitment of patients.

8.5 Endpoints

8.5.1 Primary Endpoints

- Peripheral PA2024-specific T-cell proliferation using a tritiated thymidine (^{3}H-thymidine) incorporation assay at week 6 after the first sipuleucel-T infusion in each arm reported as stimulation index (SI), defined as ^{3}H-thymidine incorporation in the presence of PA2024 antigen divided by ^{3}H-thymidine incorporation with media alone.

8.5.2 Secondary Endpoint(s)

8.5.2.1 Secondary Immune Endpoint(s)

- The mean peripheral PA2024-and PAP-specific T-cell proliferation using a ^{3}H-thymidine incorporation assay reported as SI at baseline and 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T.
- The mean peripheral PA2024-and PAP-specific T-cell activation to sipuleucel-T using interferon gamma (IFNγ) enzyme-linked immunosorbent spot (ELISPOT) at baseline and weeks 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T.

- The mean PA2024-and PAP-specific antibody (IgM and IgG) response to sipuleucel-T using enzyme-linked immunosorbent assay (ELISA) at baseline and weeks 6, 10, 14, 26, 39, and 52 weeks after the first infusion of sipuleucel-T.
- Product immune parameters including CD54 + cell counts and upregulation and total nucleated cell (TNC) counts.

8.5.2.1 Secondary Clinical Endpoint(s)

- The frequency of the maximum observed grade of each toxicity. Incidence and severity of adverse events and laboratory abnormalities, graded according to CTCAE v4.0 [2].
- To evaluate the PSA50 response
    - PSA50 response is defined as at least a 50% decline in PSA from baseline value
- Median time to radiographic progression using (RECIST and PCWG2 criteria for soft tissue and bone lesions, respectively, as assessed by the investigator).

Exploratory Objectives:

- Median time to PSA progression based on Prostate Cancer Working Group 2 (PCWG2) criteria [1]:
    - in patients with no PSA decline from baseline as: ≥ 25% increase from the baseline value and an increase in absolute value of ≥ 2 ng/mL, at least 12 weeks from baseline
    - in patients with an initial PSA decline from baseline as: ≥ 25% increase and an absolute increase of ≥ 2 ng/mL above the nadir value, which is confirmed by a second value obtained three or more weeks later
- Median time to ALP progression.
    - in patients with no ALP decline from baseline as: ≥ 25% increase from the baseline value, at least 12 weeks from baseline
    - in patients with an initial ALP decline from baseline as: ≥ 25% increase above the nadir value, which is confirmed by a second value obtained three or more weeks later.
- Median time to pain progression defined as use of opioid analgesics for cancer-related pain.
- Median time to occurrence of first skeletal related event (SRE):
    - the use of external beam radiotherapy to relieve skeletal symptoms or the occurrence of new symptomatic pathological bone fractures (vertebral or non-vertebral) or the occurrence of spinal cord compression or a tumor related orthopedic surgical intervention.
- Median time to occurrence of first start of chemotherapy use.

8.6 Analyses

8.6.1 Subject Disposition

Subject disposition will include, but will not be limited to, the following:

- Number of subjects registered.
- Number of subjects infused.
- Number of subjects who prematurely discontinued the study (i.e., refused further study assessments [with the possible exception of survival status]).
- Reason(s) for premature discontinuation from the study.
- Number of subjects who died.
- Cause of death summary.
- Number of subjects in the immune response and safety analysis sets.

In addition, the number of subjects screened for the trial will be summarized for the overall study population.

8.6.2 Demographics and Baseline Characteristics

Demographic information and baseline disease information and characteristics will be summarized with descriptive statistics for all analysis populations.

8.6.3 Efficacy Analyses

The primary efficacy analysis will compare the two randomized groups in terms of SI measured 6 weeks after the first infusion ofsipuleucel-T. We will compute the logarithms of each patient's SI and use these data in a t test to compare the two groups.

A secondary analysis will look at the trends in SI over time. This analysis will consist of a repeated measurement analysis of immune response over time evaluated using PA2024-stimulated T cell proliferation via ^{3}H-thymidine uptake based on the immune response population. The logarithm of response will be used for analysis. Fixed-effect terms in the model will include treatment arm, visit as a class effect, and a treatment-by-visit interaction. The correlation of responses across time within a subject will be modeled using several candidate variance-covariance structures including unstructured, compound symmetry, heterogeneous compound symmetry, autoregressive, and heterogeneous autoregressive. The variance-covariance structure providing the smallest Bayesian information criteria will be selected for the final model. Fixed-effect terms in the model will be tested for significance using type III sums of squares. The formal test for difference between the studies will be based on the p-value for the treatment effect unless we find a significant treatment-by-visit interaction ($p < 0.05$), in which case by-visit treatment comparisons will be made using appropriate contrast statements.

Estimates of treatment effect and corresponding 95% CIs will be provided on the ratio scale obtained by exponentiation of the model derived treatment effect and CI on the logarithm scale.

Immune responses over time for other assays will be analyzed using identical methods described for the primary efficacy analysis, except for the IFNγ ELISPOT which we will analyze using ranks instead of on the logarithm scale.

Because PSA and imaging scans are conducted at different time points across the two groups, the time-to-event secondary clinical endpoints will be summarized using generalized Kaplan-Meier methods for interval censored data and displayed graphically where appropriate

8.6.4 Safety Analyses

Safety data will be summarized descriptively in aggregate within the safety population. No formal statistical testing is planned for the safety data.

8.6.4.1 Adverse Events

Adverse events will be summarized and listed by the Medical Dictionary for Regulatory Activities (MedDRA) terms, by preferred term within each system organ class. Summary tables will include all AEs reported from the first radium-223 infusion.

Summaries to be produced include the following:

- Incidence within system organ class (MedDRA).
- Incidence by decreasing frequency.
- Incidence by NCI CTCAE severity grade, by decreasing frequency.
- Incidence of Grade ≥ 3 AEs, by decreasing frequency.
- Incidence of SAEs.
- Incidence of AEs within 1 day of infusion.
- Incidence of AEs that resulted in premature discontinuation of study treatments.

Adverse events that occur multiple times for a subject will be counted only once per subject in incidence summary tables. In tables that enumerate AEs by severity, only the greatest severity for an AE occurring multiple times for a subject will be counted.

8.6.4.2 Laboratory Data

Summaries of laboratory data collected from baseline until study completion will include:

- Incidence of clinically significant laboratory abnormalities by NCI CTCAE v4 [2]. A clinically significant laboratory toxicity is defined as a post-baseline grade 3 or higher toxicity where the baseline value was grade 2 or lower.
- Summary statistics (mean, median, standard deviation, minimum, and maximum) for laboratory values and their change from baseline by time point.

Baseline results will be defined as the most recent nonmissing value obtained on or prior to the randomization date.

8.6.4.3 Vital Signs

Vital sign data (blood pressure, respiration rate, heart rate, and body temperature) will be summarized descriptively by time point.

8.6.5 Study Treatment Administration

Leukapheresis and sipuleucel-T infusion information will be summarized.

This will include the following:

- Number of subjects who received a total of 0, 1, 2, or 3 infusions.
- Reason(s) for not completing 3 infusions.
- Number of subjects who receive a total of 0, 1, 2, 3, 4, or 5 or more leukaphereses.
- Number of subjects who receive a total of 3 leukaphereses and 3 infusions of sipuleucel-T

The duration of treatment with radium-223 will be summarized.

This will include the following:

- Number of subjects who received a total of 0, 1, 2, 3, 4, 5, or 6 infusions.
- Reason(s) for not completing 6 infusions.

8.6.6 Concomitant Medications and Procedures

Concomitant medications and procedures will be presented in data listings and will be coded using the World Health Organization Drug Dictionary. Separate summaries will be provided for medications that were started prior to registration and for medications started after registration.

8.7 Interim Analyses

We will carry out interim safety assessments with statistical monitoring. We will monitor patients in groups of 10 (5 per treatment arm). The formal monitoring rule relates to the risk of hematologic adverse events. Specifically, we will monitor thrombocytopenia, neutropenia, and anemia.

>2 events of grade ≥ 3 thrombocytopenia or neutropenia in the first 10 subjects
>3 events of grade ≥ 3 anemia in the first 10 subjects

9.0 REGULATORY REQUIREMENTS

9.1 Investigator Responsibilities

The Principal Investigator is responsible for performing the following tasks:

- Coordinating, developing, writing, submitting, and obtaining IRB-approval for the protocol as well as its subsequent amendments.
- Assuring that all study personnel are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study and for monitoring the progress of the study.
- Reviewing and ensuring reporting of serious adverse events (SAEs).
- Reviewing data from all patients.

9.2. Study Coordinator Responsibilities

The study coordinator is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained prior to patient registration, and maintaining copies of IRB approvals (including approval of amendments).
- Managing patient registration.
- Collecting and compiling data from each patient.
- Establishing procedures for documentation, reporting, and submission of AEs/ SAEs to the principal investigator (Emmanuel Antonarakis, MD) and other applicable parties.
- Data entry and query answering.
- Facilitating audits by securing selected source documents and research records from participating patients for audit.

9.3 Study Personnel Responsibilities

Study personnel (co-investigators, research nurses) are responsible for these tasks:

- Following the protocol as written, and Good Clinical Practice (GCP) guidelines.

- Submitting data to the project manager.
- Registering all patients by submitting the patient registration form and signed informed consent form promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct the trial according to the protocol.
- Maintaining regulatory binders and providing copies of all required documents to the project manager.
- Collecting/submitting data according to the schedule specified by the protocol.

9.4 Participating Sites Responsibilities

Participating sites are responsible for performing the following tasks:

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

9.5 Patient Information and Informed Consent

Before obtaining consent, members of the study team will review the rationale for the treatment program with the patient. The discussion will review the alternatives available (including hormonal therapy, chemotherapy, or supportive care as appropriate), the potential benefits of this program, the risks and the probability of their occurrence, and the procedures to minimize these risks. Should an adverse event occur, the provisions available to ensure medical intervention will also be reviewed. Why the risks are reasonable in relation to the anticipated benefits, incentives, or costs that will or may be incurred as a result of participating in the study, as well as the efforts to maintain confidentiality, will also be discussed with the patient.
Patients will be required to sign and date (in triplicate) a statement of informed consent that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the IRB. The medical record will include a statement that written informed consent was obtained (and document the date that it was obtained) before the patient is enrolled in the study. The original signed document will become part of the patient's medical record and a copy will be sent home with each patient.
The consent form will include the following:

- the nature and objectives, potential toxicities, and benefits of the intended study
- the length of therapy and likely follow-up required
- alternatives to the proposed therapy (standard and investigational therapies)
- the name of the investigator(s) responsible for the protocol
- the right of the patient to accept or refuse treatment and to withdraw from the study

Subjects who are on-treatment at the time the new NIST reference standard goes into effect should be notified of this change and should be required to sign a Patient Information Sheet to acknowledge that they have received information on the updated NIST standard calibration. All patients randomized after the new reference standard is in effect should sign a revised Informed Consent Form that contains the updated NIST standardization.

9.6 Subject Confidentiality

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. After this discussion, they will be asked to sign a Notice of Privacy Practice research authorization/HIPAA form. The original signed documents will become part of the patient's medical records, and each patient will receive a copy of the signed documents. The use and disclosure of protected health information will be limited to the individuals described in the research authorization form. The research authorization form must be completed by the Principal Investigator and approved by the IRB.

9.7 Good Clinical Practice

The study will be conducted in accordance with the ICH for GCP and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to appropriate regulations.

9.8 Ethical Considerations

This study will be conducted in compliance with the protocol, GCP guidelines established by the International Conference on Harmonization, and the ethical standards set forth in the Declaration of Helsinki 2004 (available at: www.laakariliitto.fi/e/ethics/helsinki.html).

10.0 STUDY MANAGEMENT

10.1 Clinical Trial Agreement

This trial is being conducted under one or more clinical trial agreements that contain, among other terms, the publication policy, indemnity agreements, and financial arrangements for the study.

10.2 Lead research program coordinators

A Lead Research Program Coordinator at the SKCCC coordinating center will be assigned to the study. A Lead Research Program Coordinator will manage the study activities at each of the participating sites. The responsibilities of the Lead Research Program Coordinator include project compliance, data collection, data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol team.

10.3 Case report forms

Case report forms will be generated by Staff at the Lead Site Coordinating Center at SKCCC for the collection of all study data. The data should be entered in a timely manner, (within 2 weeks of the visit). Investigators will be responsible for ensuring that the CRFs are kept up-to-date.

10.4 Source documents

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

10.5 Study Monitoring and Quality Assurance

This is a DSMP Level I study under the SKCCC Data Safety Monitoring Plan (12/6/2012). *The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring.*
The Clinical Research Office will perform an audit after the first subject has been treated and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee.

The PI is responsible for internally monitoring the study. Data must be reviewed to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial, review safety reports, and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study."

Contact the CRO QA Office (croqaoffice@jhmi.edu) with questions.

10.6 Protocol Amendments

Before starting the study, the protocol must be approved by each institution's IRB or Independent Ethics Committee (IEC). Any changes to the protocol or discontinuation of the trial require a written protocol amendment or statement, respectively. The Investigators and IEC/IRB must approve the protocol amendment or statement. The Principal Investigator(s) will sign the protocol amendment.

10.7 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the CRFs.

Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 7 years after the completion of all study activities.

10.8 Publication policy

Bayer recognizes the right of the investigator to publish results upon completion of the study. All Investigator Initiated studies are the property of the investigator and publications generated from such studies are at the discretion of the investigator. Bayer strongly encourages investigators to publish the results of all studies supported through the Investigator Initiated Research (IIR) Program.
As an important milestone to the IIR, we encourage all investigators to submit a draft manuscript of the supporting publication(s) or abstract to submission for a courtesy review prior to submission of the final version for publication or congress presentation. This will be reviewed promptly and will not be withheld unreasonably. All relevant aspects regarding data reporting and publication will be part of the contract between Bayer and the investigator/institution
The Principal Investigator should ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

10.8.1 Publications and NIST

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

11.0 LIST OF ABBREVIATIONS

Abbreviation	Definition
^{153}Sm-EDTMP	samarium-153-ethylenediaminetetrame-thylenephosphonate
^{3}H-thymidine	tritiated thymidine
AE	adverse event
ADT	androgen deprivation therapy
ALT	alanine aminotransferase
APC	antigen presenting cell
AST	aspartate aminotransferase
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRF	case report form
CRPC	castrate-resistant prostate cancer
CT	computed tomography
DK	decay correction factor
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
ECOG	Eastern Cooperative Oncology Group
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot assay
FACT-P	Functional Assessment of Cancer Therapy-Prostate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GM-CSF	granulocyte-macrophage colony-stimulating factor
HR	hazard ratio
IB	investigator's brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
IFNγ	Interferon γ
IRB	Institutional Review Board
IV	intravenous
LDH	lactate dehydrogenase
mCRPC	metastatic castrate-resistant prostate cancer
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events

Abbreviation	Definition
NDI	National Death Index
NIST	National Institute of Standards and Technology
PAP	prostatic acid phosphatase
PBMC	peripheral blood mononuclear cell
PET	positron emission tomography
PSA	prostate specific antigen
QC	quality control
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	ribonucleic acid
SAE	serious adverse event
SKCCC	Sidney Kimmel Comprehensive Cancer Center
SRE	skeletal related event
SRM	Standard Reference Material
SSDI	Social Security Death Index
TNC	total nucleated cells
ULN	upper limit of normal

Reference List

1. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol 2008; 26(7):1148-1159.
2. NCI. Common Terminology Criteria for Adverse Events (CTCAE). Version 4.03. National Cancer Institute; 14 Jun 2010. 2010.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63(1):11-30.

4. Ward JF, Moul JW. Rising prostate-specific antigen after primary prostate cancer therapy. Nat Clin Pract Urol 2005; 2(4):174-182.
5. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol 2006; 24(19):3089-3094.
6. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer 2009; 115(16):3670-3679.
7. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010; 363(5):411-422.
8. Sheikh NA, Petrylak D, Kantoff PW, Dela RC, Stewart FP, Kuan LY et al. Sipuleucel-T immune parameters correlate with survival: an analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer. Cancer Immunol Immunother 2013; 62(1):137-147.
9. Shore ND, Mantz CA, Dosoretz DE, Fernandez E, Myslicki FA, McCoy C et al. Building on sipuleucel-T for immunologic treatment of castration-resistant prostate cancer. Cancer Control 2013; 20(1):7-16.
10. Chakraborty M, Wansley EK, Carrasquillo JA, Yu S, Paik CH, Camphausen K et al. The use of chelated radionuclide (samarium-153-ethylenediaminetetramethylenephosphonate) to modulate phenotype of tumor cells and enhance T cell-mediated killing. Clin Cancer Res 2008; 14(13):4241-4249.
11. Henriksen G, Breistol K, Bruland OS, Fodstad O, Larsen RH. Significant antitumor effect from bone-seeking, alpha-particle-emitting (223)Ra demonstrated in an experimental skeletal metastases model. Cancer Res 2002; 62(11):3120-3125.
12. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fossa SD et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med 2013; 369(3):213-223.
13. Bruland OS, Nilsson S, Fisher DR, Larsen RH. High-linear energy transfer irradiation targeted to skeletal metastases by the alpha-emitter 223Ra: adjuvant or alternative to conventional modalities? Clin Cancer Res 2006; 12(20 Pt 2):6250s-6257s.
14. Roodman GD. Mechanisms of bone metastasis. N Engl J Med 2004; 350(16):1655-1664.
15. Gulley JL, Madan RA, Schlom J. Impact of tumour volume on the potential efficacy of therapeutic vaccines. Curr Oncol 2011; 18(3):e150-e157.
16. Beer TM, Bernstein GT, Corman JM, Glode LM, Hall SJ, Poll WL et al. Randomized trial of autologous cellular immunotherapy with sipuleucel-T in androgen-dependent prostate cancer. Clin Cancer Res 2011; 17(13):4558-4567.
17. Debraj GuhaThakurta. Induction of antigen spread after sipuleucel-T treatment and its association with improved clinical outcome. 1(Suppl 1):P101[Journal for ImmunoTherapy of Cancer]. 2013.

18. van Belle G, Martin DC. Sample-Size as a Function of Coefficient of Variation and Ratio of Means. American Statistician 1993; 47:165-167.
19. Pocock SJ. The combination of randomized and historical controls in clinical trials. J Chronic Dis 1976; 29(3):175-188.
20. Cessna JT, Zimmerman BE. Standardization of radium-223 by liquid scintillation counting. Appl. Radiat. Isot. 2010; 68(7-8):1523-1528.
21. Zimmerman BE, Bergeron DE, Cessna JT, Fitzgerald R. Revision of the NIST standard for 223Ra: New measurements and review of 2008 data. J Res Natl InstStand Technol. 2015; 120:37-57.

APPENDIX 1: Investigator Initiated Trial Immune Monitoring Requisition Form

Sample Draw Instructions

1. There is an expiry limit on samples. Schedule blood draws in the afternoon if possible.

2. Prepare 10 immune monitoring tubes from the Dendreon immune monitoring kit:
   - 1 red-top/no additive tube and 9 green-tops/sodium heparin vacutainer tubes
   - Complete subject initials, date, and time-point on the labels affixed to each vacutainer tube

3. Draw blood using a 21G needle into the immune monitoring tubes:
   - Draw the red-top first, followed by the green-top tubes.
   - Make sure to completely fill the vacutainer tubes (10mL per tube). Mix the tubes at least 5 times by gentle inversion.
   - Do NOT centrifuge the immune monitoring samples.
   - Keep the immune monitoring samples at **ambient temperature**. No gel packs are required.

4. Fill out form completely, then photocopy and retain a copy with the appropriate patient records.

5. Notify Clinical Immunology at clinicalimmunology@dendreon.com when samples are shipped.

Site and Subject Information

Sponsor Investigator Name:

Institution Name:

Patient Identification:

Subject Number:

Subject Initial: ☐☐☐ First Middle Last

Subject Date of Birth: ☐☐/☐☐/☐☐☐☐ M M/ D D / Y Y Y Y

For Dendreon Use Only

Affix barcode label here

Sample Collection Information

Immune Monitoring Time-point (check the corresponding box):

☐ Baseline ☐ Imm-1 ☐ Imm-2 ☐ Imm-3 ☐ Imm-4 ☐ Imm-5 ☐ Imm-6

Time Samples Drawn: ☐☐:☐☐ 24 hour clock

Date Samples Drawn: ☐☐/☐☐/☐☐☐☐ M M/ D D / Y Y Y Y

Time Zone (Circle One): ET CT MT PT

Sample Collected By:_____ _____

Signature Full Name (Capitals)

APPENDIX 2: Dendreon Specimen Shipping Instructions

Dendreon

Targeting Cancer, Transforming Lives®

Immune Monitoring Shipping Instructions for Sponsor Investigators

Notifying Dendreon of specimen collection:

1. Dendreon requires a minimum of 24 hours notice before the specimens arrive at Dendreon.
2. At least 24 hours prior to Dendreon receiving the specimens:
    a. Contact Dendreon by e-mail at: clinicalimmunology@dendreon.com.
    b. Provide the following information:
        i. Complete subject number
        ii. Date of specimen collection
        iii. FedEx air bill number
3. If the specimen is not collected as planned, contact Dendreon right away by email.

Shipping the specimen:

1. After the subject's blood draw is complete, wrap specimen tubes in the absorbent cloth provided and place into the main pouch of the 95 kPA flexible envelope.
2. Remove the adhesive backing from the flexible envelope and seal by folding along the "FOLD HERE" slit at the opening of the envelope.
3. Press very firmly at the middle of the slit, working outward to achieve a complete seal.
4. Make a photocopy of the completed Investigator Initiated Trial Immune Monitoring Requisition Form and include in the blood shipping box.
5. Keep the original Investigator Initiated Trial Immune Monitoring Requisition Form with the subject's medical record.
6. Wrap one bubble-wrap sheet around the sealed flexible envelope to provide additional protection for the specimen tubes. The bubble-wrap should be secured around the flexible envelope with adhesive tape.
7. Place the bubble-wrapped, sealed flexible envelope containing the specimens inside the shipping carton and tape the carton closed with 2-inch wide packing tape.
8. **Place the sealed carton inside the UN3373 pak orange bag, remove the adhesive backing and completely seal the bag.**
9. Fill out the FedEx billable stamp with your information and attach label to the package. Do not cover the UN3373 orange label.

10. Saturday, Sunday, and holiday deliveries are not acceptable. A list of holidays has been provided.
11. Send the sample(s) **priority overnight** via FedEx Next Day to:

    Dendreon Corporation
    Attention: Clinical Immunology
    1208 Eastlake Avenue E, Seattle, WA 98102
    Phone: 206-829-1639

APPENDIX 3: Dendreon SAE Form

Dendreon

SERIOUS ADVERSE EVENT FORM

FAX TO DENDREON DRUG SAFETY WITHIN 24 HOURS OF BECOMING AWARE OF EVENT: 206-829-1647

Section 1a

PROTOCOL #: _____

SUBJECT #: _____

SUBJECT INITIALS: ☐☐☐

INITIAL REPORT DATE: ____/____/____ (MM DD YY)

SUBJECT DATE OF BIRTH: ____/____/____ (MM DD YY)

FOLLOW-UP REPORTS:

#1 DATE: ____/____/____ (MM DD YY)

#2 DATE: ____/____/____

#3 DATE: ____/____/____ (MM DD YY)

Section 2

Date site became aware of event: ____/____/____ (MM DD YY)

Principal Investigator: _____ (print name)

Institution: _____

City/State: _____

Country: _____

Form completed by: _____ (print name)

Telephone: _____

Fax: _____

Section 3

SIPULEUCEL-T INFUSION DATE(S): ____/____/____ (MM DD YY) ____/____/____ (MM DD YY) ____/____/____ (MM DD YY)

OTHER PRODUCT: _____ ☐ NA **DOSE:** _____ First Dose: ____/____/____ (MM DD YY) Most Recent Dose: ____/____/____ (MM DD YY)

Section 4a

IF SUBJECT WAS HOSPITALIZED

Attach Discharge Summary when available

Check One: ☐ New ☐ Prolonged

Admission date: ____/____/____ (MM DD YY) Discharge date: ____/____/____ (MM DD YY)

Section 4b

IF SUBJECT EXPIRED

Attach Death Certificate when available

Autopsy performed? ☐ Yes ☐ No

Date of Death: ____/____/____ (MM DD YY)

Autopsy results available? ☐ Yes ☐ No

Section 5

SERIOUS ADVERSE EVENT TERM OR DIAGNOSIS NOTE: If more than one event term is required for this report, please list each event term separately. All event terms must be recorded on the Adverse Event CRF.	ONSET DATE MM/DD/YY	RESOLUTION DATE MM/DD/YY	[1]SERIOUSNESS enter all that apply	[2]RELATIONSHIP TO PRODUCT enter only one	[3]OUTCOME enter only one

Section 6

USE THESE CODES IN THE COLUMNS ABOVE:

[1]SERIOUSNESS
1: Death
2: Life Threatening
3: Hospitalization
4: Disability
5: Congenital Anomaly
6: Medically Significant Intervention

[2]RELATIONSHIP TO PRODUCT
1: None
2: Possible
3: Probable

[3]OUTCOME
1: Recovered/Resolved
2: Recovering/Resolving
3: Recovered with Sequelae
4: Not Recovered/Not Resolved
5: Fatal (please fill in section 4b)
6: Unknown

IIT SAE Form; xxNOV 2011

Page 1 of 2

The values for radium-223 in this protocol has been revised as per the US NIST standardization update (agreed on 17 Mar 2015)

Dendreon

SERIOUS ADVERSE EVENT FORM

FAX TO DENDREON DRUG SAFETY WITHIN 24 HOURS OF BECOMING AWARE OF EVENT: 206-829-1647

Section 1b

SUBJECT # : _____

SUBJECT INITIALS: ☐☐☐

Section 7

CASE NARRATIVE:

Describe the SAE. Include presenting signs and symptoms, clinical cause of the event, treatment of the event, and any other assessments which help explain the event. Give duration of the event if it persisted for less than 24 hours. State whether the event is or is not related to the apheresis procedure or investigational product infusion.

Section 8

OTHER RELEVANT HISTORY, TESTS OR LABORATORY DATA, CONCOMITANT MEDICATIONS OR THERAPY:

For example: cancer, allergies or concurrent illnesses. Attach relevant laboratory and imaging results. Attach updated Concomitant Medications CRF.

Section 9 **INITIAL REPORT**

INVESTIGATOR SIGNATURE: _____

DATE: _____/_____/_____ (MM DD YY)

FOLLOW-UP REPORTS

INVESTIGATOR INITIALS: _____ **DATE:** _____/_____/_____

INVESTIGATOR INITIALS: _____ **DATE:** _____/_____/_____

INVESTIGATOR INITIALS: _____ **DATE:** _____/_____/_____ (MM DD YY)

Page 2 of 2

IIT SAE Form; xxNOV 2011