TITLE: A Randomized, Phase II Study Evaluating the Addition of Pembrolizumab (MK-3475) to Radium-223 in Metastatic Castration Resistant Prostate Cancer (mCRPC)

NCT Number: NCT03093428

Protocol Version Date: November 15, 2019

DF/HCC Protocol #: 16-498

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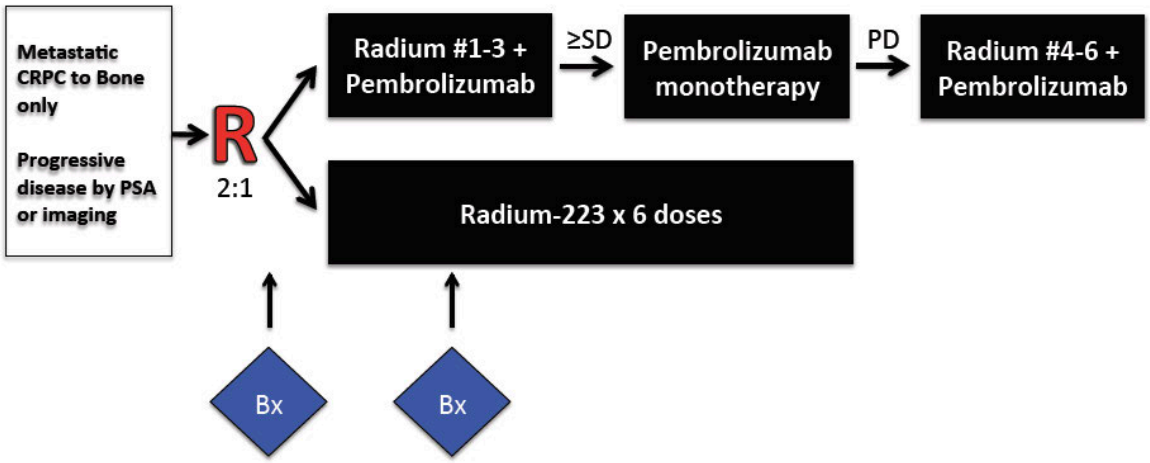
Statistician: [redacted]

Agents: Pembrolizumab: Merck & Co.
Radium-223: Commercial Supply

IND #: 130854
IND Sponsor: [redacted]

SCHEMA



*Stratified by serum total alkaline phosphatase <220 or ≥220 and volume of bone metastases (high: ≥ 4 bone metastases including one beyond the axial skeleton vs. low: <4 bone metastases)
*Treatment is in terms of weeks not cycles given that radium-223 is given every 4 weeks and pembrolizumab is given every 3 weeks.
*Arm A (radium-223 + pembrolizumab) will have a break in radium-223 dosing after 3 doses if at least stable disease (SD). Once radiologic progressive disease is demonstrated in bone and if no new visceral disease, the last 3 doses of radium will be given. For further detail regarding radium-223 dosing, refer to Section 5.4.

Table of Contents

SCHEMA 2

1. OBJECTIVES 5
1.1 Study Design 5
1.2 Primary Objectives 5
1.3 Secondary Objectives 5
1.4 Exploratory Objectives 5

2. BACKGROUND 6
2.1 Castration resistant prostate cancer (CRPC) 6
2.2 Study Agents 6
2.3 Rationale for Study 12

3. PARTICIPANT SELECTION 13
3.1 Eligibility Criteria 13
3.2 Inclusion Criteria 13
3.3 Exclusion Criteria 15
3.4 Inclusion of Women and Minorities 17

4. REGISTRATION PROCEDURES 17
4.1 General Guidelines for DF/HCC and DF/PCC Institutions 17
4.2 Registration Process for DF/HCC and DF/PCC Institutions 18

5. TREATMENT PLAN 18
5.1 Treatment Regimen 18
5.2 Pre-Treatment Criteria 20
5.3 General Concomitant Medication and Supportive Care Guidelines 20
5.4 Criteria for Taking a Participant Off Protocol Therapy 23
5.5 Duration of Follow Up 26
5.6 Criteria for Taking a Participant Off Study 26
5.7 Clinical Criteria for Early Trial Termination 27

6. DOSING DELAYS 27
6.1 Dose Delay Guidelines for Pembrolizumab 28
6.2 Dose Modifications for Radium-223 45

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS 46
7.1 Expected Toxicities 46
7.2 Adverse Event Characteristics 53
7.3 Expedited Adverse Event Reporting 53
7.4 Events of Clinical Interest 55
7.5 Expedited Reporting to the Food and Drug Administration (FDA) 55
7.6 Expedited Reporting to Hospital Risk Management 56
7.7 Routine Adverse Event Reporting 56

8. PHARMACEUTICAL INFORMATION....57
8.1 Pembrolizumab[4]....57
8.2 Radium-223[3] (Xofigo)....59

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES....63
9.1 Immune biomarker studies....63
9.2 Symptomatic Skeletal Events (SSEs)....64
9.3 Quality of Life....64
9.4 Exploratory Bone Response Criteria....65
9.5 Mutations in DNA Repair Genes in Prostate Cancer (to be funded by Bayer)....66

10. STUDY CALENDAR....69

11. MEASUREMENT OF EFFECT....72
11.1 Antitumor Effect – Solid Tumors....72

12. DATA REPORTING / REGULATORY REQUIREMENTS....77
12.1 Data Reporting....77
12.2 Data Safety Monitoring....78

13. STATISTICAL CONSIDERATIONS....78
13.1 Study Design/Endpoints....78
13.2 Accrual Rate and Study Duration....80
13.3 Stratification Factors....81
13.4 Sample size justification....81
13.5 Safety Monitoring Plan....82
13.6 Definition of Analysis Populations....83
13.7 Analysis of Primary Endpoints....83
13.8 Analysis of Secondary Endpoints....83
13.9 Analysis of Exploratory Clinical Endpoints....84
13.10 Analysis of Exploratory Immune Endpoints....84
13.11 Analysis of Exploratory Genomic Endpoints....86
13.12 Reporting and Exclusions....87

14. PUBLICATION PLAN....88
APPENDIX A: Performance Status Criteria....88
APPENDIX B: FACT-P (version 4)....90
APPENDIX C: Brief Pain Inventory (Short Form)....94
APPENDIX D: Narcotic Analgesic Diary....96

1. OBJECTIVES

1.1 Study Design

This is a randomized phase II open label study of pembrolizumab, a humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands PD-L1 and PD-L2, in combination with radium-223. A total of 45 patients with advanced metastatic CRPC will be randomized in a 2:1 fashion to the combination of pembrolizumab plus radium-223 (Arm A) or to radium-223 alone (Arm B).

1.2 Primary Objectives

- To compare differences in immune infiltrating cells (e.g., CD8+, CD4+, T cells) in bone biopsy specimens from baseline to 8 weeks on study therapy between the treatment arms.

1.3 Secondary Objective

- To assess the safety and tolerability of combination therapy with pembrolizumab plus radium-223

- To preliminarily investigate the efficacy of the combination of pembrolizumab plus radium-223 as evaluated by progression-free survival (PFS) and overall survival (OS)

1.4 Exploratory Objectives

- To characterize changes in immune cell response and function elicited by radium-223 with or without pembrolizumab (PD-1 blockade).
- To evaluate the PSA response including change in PSA and time to PSA progression
- To assess the rate of symptomatic skeletal events (SSEs) and time to SSE in patients treated with radium-223 and pembrolizumab.
- To explore the use of quantitative SPECT/CT for evaluating changes in osteoblastic activity on bone scan after radium-223 and pembrolizumab
- To assess the effect of radium-223 and pembrolizumab on quality of life, pain, and analgesic use.
- To investigate the impact of genomic alterations including those in DNA damage repair pathways on clinical outcomes in patients treated with radium-223 with or without pembrolizumab.
- To identify biomarkers of response and to investigate mechanisms of treatment resistance through targeted sequencing of cell free DNA at baseline, on therapy, and at the time of disease progression.

2. BACKGROUND

2.1 Castration resistant prostate cancer (CRPC)

Castration resistant prostate cancer (CRPC) is a fatal disease from which nearly 30,000 men die annually in the United States and over 300,000 worldwide.[1] Significant improvements in the treatment armamentarium for CRPC have occurred in the last several years with the introduction of the next generation androgen biosynthesis inhibitor abiraterone, the androgen antagonist enzalutamide, the chemotherapy agent cabazitaxel, the therapeutic vaccine, sipuleucel-T, and the novel bone metastasis-homing radiotherapeutic, radium-223 dichloride--all of which improve survival. Bone metastases occur in over 90% of patients with metastatic CRPC.[2] They remain the most frequent cause of morbidity and mortality in this disease and incur high treatment-related costs.

2.2 Study Agents

2.2.1 ***Radium-223 (Xofigo)***[3]

Radium-223 is a calcium mimetic that hones in on bone metastases due to their high rate of bone turnover. It binds to new bone stroma and emits alpha particles, which induce double-stranded DNA breaks that result in tumor cell death.[2] The short path length of alpha particles limits toxicity to surrounding healthy tissue. Overall, radium-223 is well tolerated. The most frequently observed adverse events (AEs) of any grade in the phase 3 study were anemia (31%), thrombocytopenia (12%), constipation (18%), diarrhea (25%), nausea (36%), vomiting (18%), fatigue (26%), peripheral edema (13%), anorexia (17%), and bone pain (50%). Compared to placebo, radium-223 increased median overall survival from 11.3 months to 14.9 months and reduced the risk of death by 30% (HR: 0.70; 95%CI: 0.58-0.83; $p<0.001$).[2] Radium-223 has also been shown to significantly increase the median time to the first symptomatic skeletal event (15.6 vs. 9.8 months) and the time to increase in total alkaline phosphatase (7.4 vs. 3.8 mo.) Compared to placebo, it elicited a higher rate of normalization of serum alkaline phosphatase (34 vs. 1%) and PSA declines ≥30% (16% vs. 6%).

Description
Radium Ra 223 dichloride is an alpha particle-emitting radiopharmaceutical.

Radium-223 is supplied as a clear, colorless, isotonic, and sterile solution to be administered intravenously with pH between 6 and 8. Each milliliter of solution contains 1,000 kBq radium-223 dichloride (27 microcurie), corresponding to 0.53 ng radium-223, at the reference date. Radium is present in the solution as a free divalent cation.

Each vial contains 6 mL of solution (6,000 kBq (162 microcurie) radium-223 dichloride at the reference date). The inactive ingredients are 6.3 mg/mL sodium chloride USP (tonicity agent), 7.2 mg/mL sodium citrate USP (for pH adjustment),

0.2 mg/mL hydrochloric acid USP (for pH adjustment), and water for injection USP.

The molecular weight of radium-223 dichloride, $^{223}RaCl_2$, is 293.9 g/mol.

Radium-223 has a half-life of 11.4 days. The specific activity of radium-223 is 1.9 MBq (51.4 microcurie)/ng.

The six-stage-decay of radium-223 to stable lead-207 occurs via short-lived daughters, and is accompanied predominantly by alpha emissions. There are also beta and gamma emissions with different energies and emission probabilities. The fraction of energy emitted from radium-223 and its daughters as alpha-particles is 95.3% (energy range of 5 - 7.5 MeV). The fraction emitted as beta-particles is 3.6% (average energies are 0.445 MeV and 0.492 MeV), and the fraction emitted as gamma-radiation is 1.1% (energy range of 0.01 - 1.27 MeV).

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

Pharmacodynamics
Compared with placebo, there was a significant difference in favor of radium-223 for all five serum biomarkers for bone turnover studied in a phase 2 randomized study (bone formation markers: bone alkaline phosphatase [ALP], total ALP and procollagen I N propeptide [PINP], bone resorption markers: C-terminal crosslinking telopeptide of type I collagen [S-CTX-I] and type I collagen crosslinked C-telopeptide [ICTP]).

Pharmacokinetics: Elimination
The whole body measurements indicated that approximately 63% of the administered radioactivity was excreted from the body within 7 days after injection (after correcting for decay). Fecal excretion is the major route of elimination from the body. At 48 hours after injection, the cumulative fecal excretion was 13% (range 0 - 34%), and the cumulative urine excretion was 2% (range 1 - 5%). There was no evidence of hepato-biliary excretion based on imaging data.

The rate of elimination of radium-223 dichloride from the gastrointestinal tract is influenced by the high variability in intestinal transit rates across the population. Patients with a slower intestinal transit rate could potentially receive a higher intestinal radiation exposure. It is not known whether this will result in increased gastrointestinal toxicity.

Summary of Clinical Experience
The efficacy and safety of radium-223 were evaluated in a double-blind, randomized, placebo-controlled phase 3 clinical trial of patients with castration-resistant prostate cancer with symptomatic bone metastases. Patients with visceral metastases and malignant lymphadenopathy exceeding 3 cm were excluded. The primary efficacy endpoint was overall survival. A key secondary efficacy endpoint was time to first symptomatic skeletal event (SSE) defined as external beam radiation therapy (EBRT) to relieve skeletal symptoms, new symptomatic pathologic bone fracture, occurrence of spinal cord compression, or tumor-related orthopedic surgical intervention. There were no scheduled radiographic assessments performed on study. All patients were to continue androgen deprivation therapy. At the cut-off date of the pre-planned interim analysis, a total of 809 patients had been randomized 2:1 to receive radium-223 50 kBq (1.35 microcurie)/kg intravenously every 4 weeks for 6 cycles (n = 541) plus best standard of care or matching placebo plus best standard of care (n = 268). Best standard of care included local EBRT, corticosteroids, antiandrogens, estrogens, estramustine or ketoconazole. Therapy was continued until unacceptable toxicity or initiation of cytotoxic chemotherapy, other systemic radioisotope, hemi-body EBRT or other investigational drug. Patients with Crohn's disease, ulcerative colitis, prior hemibody radiation or untreated imminent spinal cord compression were excluded from the study. In patients with bone fractures, orthopedic stabilization was performed before starting or resuming treatment with radium-223.

The following patient demographics and baseline disease characteristics were balanced between the arms. The median age was 71 (range 44-94) with a racial distribution of 94% Caucasian, 4% Asian, 2% Black and <1% Other. Patients were enrolled predominantly from Europe (85%) with 4% of patients enrolled from North America. ECOG performance status was 0-1 in 86% of patients. Eighty-five percent of patients had 6 or more bone scan lesions and of those 40% had > 20 lesions or a superscan. Opiate pain medications were used for cancer-related pain in 54% of patients, non-opiate pain medications in 44% of patients and no pain medications in 2% of patients. Patients were stratified by baseline ALP, bisphosphonate use, and prior docetaxel exposure. Prior bisphosphonates were used by 41% of patients and 58% had received prior docetaxel. During the treatment period, 83% of radium-223 patients and 82% of placebo patients received gonadotropin-releasing hormone agonists and 21% of radium-223 patients and 34% of placebo patients received concomitant antiandrogens. Use of systemic steroids (41%) and bisphosphonates (40%) was balanced between the arms.

The pre-specified interim analysis of overall survival revealed a statistically significant improvement in patients receiving radium-223 plus best standard of care compared with patients receiving placebo plus best standard of care. An exploratory updated overall survival analysis performed before patient crossover with an additional 214 events resulted in findings consistent with the interim analysis (Table 1).

Table 1: Analysis Data of Radium-223 (Xofigo) plus best standard of care vs. Placebo plus best standard of care

	Xofigo	Placebo
Interim Analysis		
Subjects randomized	541	268
Number of deaths	191 (35.3%)	123 (45.9%)
Censored	350 (64.7%)	145 (54.1%)
Median survival (months) [a] (95% CI)	14.0 (12.1, 15.8)	11.2 (9.0, 13.2)
p-value[b]	0.00185	
Hazard ratio (95% CI)[c]	0.695 (0.552, 0.875)	
Updated Analysis		
Subjects randomized	614	307
Number of deaths	333 (54.2%)	195 (63.5%)
Censored	281 (45.8%)	112 (36.5%)
Median survival (months) [a] (95% CI)	14.9 (13.9, 16.1)	11.3 (10.4, 12.8)
Hazard ratio (95% CI)[c]	0.695 (0.581, 0.832)	

[a] Survival time is calculated as months from date of randomization to date of death from any cause. Subjects who are not deceased at time of analysis are censored on the last date subject was known to be alive or lost to follow-up.
[b] p-value is from a log-rank test stratified by total ALP, current use of bisphosphonates, and prior use of docetaxel.
[c] Hazard ratio is from a Cox proportional hazards model adjusted for total ALP, current use of bisphosphonates, and prior use of docetaxel. Hazard ratio < 1 favors radium-223 dichloride.

Summary of Adverse Events

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

2.2.2 ***Pembrolizumab (MK-3475, Keytruda)***[4,5]

Despite the confirmed efficacy of radium-223 in CRPC, resistance is universal. Novel tolerable combinations are imperative to improve outcomes in CRPC. The last several years have witnessed a resurgence of interest in mobilizing the immune system to combat cancer. The PD-1 pathway is a promising target. PD-1 is an inhibitory receptor expressed on activated T and B lymphocytes. It regulates T cell antigen-specific signaling and modulates T cell activation, inactivation, and survival.[6] Interaction with its two known ligands, PD-L1 and PD-L2, inhibit T cell activation, apoptosis, and memory responses.[7,8] Tumor cells can express PD-L1 and other immunoinhibitory ligands, which may attenuate the effects of antitumor cytotoxic T cells and stymie production of essential immune stimulating cytokines.[9-11] PD-L1 may be constitutively expressed on tumor cells or can arise as a consequence of adaptive immunity in response to stressors such as the cytotoxic interferon-γ secreted by TILs in the tumor microenvironment and treatment with other anticancer agents.[12-18]

Blocking antibodies, such as pembrolizumab have been developed to counteract this tumor evasion mechanism. Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

In a phase 1 study of multiple solid tumors, pembrolizumab has demonstrated tolerability and preliminary efficacy. The most frequent AEs were fatigue, asthenia, fever, chills, myalgias, diarrhea, rash, and pruritus.[19,20] While PD-L1 has not been shown to be upregulated in the majority of prostate cancer cases sampled,[21] data from work with other PD-1 pathway blocking antibodies such as Genentech's anti-PD-L1 molecule MDPL3280A demonstrate that the PD-L1 expression on the tumor infiltrating immune cells is also of importance and correlates with a higher chance of response to the drug.

Preclinical and Clinical Trial Data:
Refer to the Investigator's Brochure for Preclinical and Clinical data for full details.[5]

Clinical Summary of Results
As of 18-Oct-2013, 1,000 patients have been treated with MK-3475 at several dose-schedules, including 10 mg/kg every 2 weeks. MK-3475 has been generally well tolerated, as expected based on preclinical findings and other anti-PD-1 monoclonal antibodies. As of 18-Oct-2013 no serious infusions reactions have been reported in PN001. Less than 1% of patients thus far assayed had confirmed positive ADA samples and among these, no or no clear impact on exposure has been observed. There is no contraindication to further clinical investigation with MK-3475.

Pharmacokinetics were as expected, based on MK-3475 being an IgG mAb and based on preclinical data, which support dosing once every 2 or 3 weeks.

MK-3475 monotherapy induces an ORR of 25-27% in patients with ipilimumab-exposed melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. MK-3475 monotherapy induces an ORR of 39%/43% in patients with ipilimumab-naive melanoma by central independent RECIST and oncology review/investigator assessed irRC, respectively. These responses are remarkably durable. The preliminary 1-year survival rate for patients, many of whom have had multiple therapies, including ipilimumab, who receive MK-3475 is 81%. MK-3475 monotherapy induces an ORR of 21%/24% in patients with previously-treated NSCLC by central independent RECIST/investigator assessed irRC, respectively, with these responses also remarkably durable.

Preliminary data suggest higher levels of PD-L1 expression in tumors of NSCLC are associated with increased activity (ORR 67% by investigator assessed irRC/57% by central independent RECIST); additional data are required to define the optimal PD-L1 cut point.

The most commonly reported treatment emergent AEs experienced were fatigue (43.8%), nausea (26.7%), cough (25.3%), pruritus (24.6%), diarrhea (22.3%) and rash (21.5%). Immune-related adverse events were reported in 21.4% of melanoma patients; most of these events (15.8%) were considered drug-related by the investigator. The most commonly reported, immune-related adverse events across the dose-schedules were rash (3.2%), pruritus (2.9%), vitiligo (2.9%), hypothyroidism (2.7%), arthralgia (2.2%), diarrhea (2.2%), and pneumonitis (1.9%).

Rationale for Dose Selection:

An open-label Phase I trial (Protocol 001) is evaluating the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No maximally tolerated dose (MTD) has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (see IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the **fixed dose of 200 mg every 3 weeks** will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

2.3 Rationale for Study

We posit that tumor cell killing with radium-223 will release tumor-associated antigens (TAAs). Dendritic cells will take up these antigens, process them, and present them to naïve T cells. These T cells will mature into tumor-specific effector cells that will proliferate and ultimately hone in on the cancer. The addition of PD-1 blockade may reverse any potential anergy of these PD-1+ infiltrating anti-tumor immune cells. Tumor heterogeneity and the dynamic nature of PD-L1 expression on tumor cells and PD-1 and PD-L1 expression on immune cells in the tumor microenvironment provides further rationale for this approach even in the absence of high PD-L1 expression upfront.

Theoretically, if the TAAs released by radium-induced tumor killing stimulate higher numbers of tumor specific T cells, this may induce adaptive upregulation of PD-L1 by the tumor in response to IFN-γ secreted by these cells.

While 6 treatments of radium-223 is the standard regimen that was studied in the registration clinical trial, there is actually no data on what the correct number of doses for treatment is, nor is there any evidence that the schedule must be maintained for efficacy. Indeed, when administering standard of care radium-223, treatment can be held or discontinued because of inadequate blood counts, particularly during later cycles of treatment. Because pembrolizumab is dependent on lymphocytes to mediate anti-tumor responses, we proposed this modified schedule of radium-223. All patients will receive the planned 6 doses if counts allow, but the investigational cohort will have a delay in the final three radium-223 doses, so that any treatment-induced myelosuppression does not impact the efficacy of pembrolizumab. Radiation therapy has been described to influence the T cell repertoire when given with checkpoint blockade. [22] Modulation of circulating PD-1+ CD8 T cells has been seen after a single treatment of radium-223 (Kim et al. GU ASCO 2016). Thus, we assert that an empirically defined 6 doses of radium-223 is not needed to see immune modulation.

While our treatment armamentarium has significantly improved in the last few years with next generation chemotherapeutic and antiandrogen-targeted therapies in CRPC, resistance is universal. Harnessing the patient's immune system to enhance proven effective therapies like radium-223 has great potential to make significant inroads in the fight against CRPC and impact the lives of the more than 300,000 patients that die from CRPC globally.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

All screening assessments must be completed within 42 days prior to the date of treatment initiation.

3.2 Inclusion Criteria

- Histologically confirmed adenocarcinoma of the prostate
- Castration-resistant prostate cancer requires the following 3 criteria:
  - Progression after surgical castration or on GnRH agonist or antagonist
  - A castrate level of testosterone (<50ng/dL)
  - Prostate cancer progression documented by PSA rise or bone progression according to PCWG2[23]
- There is no limit to number of prior therapies
- Metastatic disease by bone scan
- Age ≥18 years

- Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (Appendix A)
- Be willing to undergo a core or excisional biopsy of a bone metastasis prior to study drug initiation for tumor tissue. *Newly-obtained is defined as a specimen obtained up to 12 weeks (84 days) prior to initiation of treatment on Day 1. Bone biopsy can have been done prior to screening; archival specimens of bone metastasis are permitted if done for other purpose and available.*
    - *If biopsy is non-diagnostic, patient must undergo repeat biopsy as proof of tumor tissue by pathology review. Proof of tumor specimen is required for eligibility. If 2nd biopsy is non-diagnostic, the patient may enroll as good faith effort.*
- Be willing to undergo a second core or excisional biopsy of a bone metastasis on therapy (approximately after 8 weeks of study therapy or after 2 doses of radium-223 if delays have occurred).
- Demonstrate adequate organ function as defined in the Table below, all screening labs for eligibility should be performed within 42 days prior to treatment initiation.

Table 2: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L; transfusions permitted
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X institutional upper limit of normal (ULN) **OR** ≥30 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X institutional ULN **OR**
	Direct bilirubin ≤ institutional ULN for subjects with total bilirubin levels > 1.5 institutional ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X institutional ULN
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X institutional ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X institutional ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

- The effects of radium-223 and pembrolizumab on the developing human fetus are unknown. For this reason, men must agree to use adequate contraception. Specifically, they must agree to use a condom (even men with vasectomies) and another effective method of birth control if he is having sex with a woman of childbearing potential or agree to use a condom if he is having sex with a woman who is pregnant while on study drug and for 120 days (4 months) following the

last dose of study drug. They must also agree not to donate sperm during the study and for 4 months after receiving the last dose of study drug.

- Ability to understand and the willingness to sign a written informed consent document.

3.3 Exclusion Criteria

- Pathology consistent with majority of specimen having small cell carcinoma of the prostate (prostate cancer with other neuroendocrine features is acceptable).
- Prior treatment with radium-223
- Prior treatment with a PD-1, PD-L1, or PD-L2 blocking therapy
- Evidence of nodal disease greater than or equal to 15 mm in short axis as these findings are concerning for metastases that would not be targeted with radium-223 alone (Arm B). However, lymph nodes with short axis measurements between 1.5-3cm that have not enlarged more than 5mm (to account for reader variability) over the last 6 months and which are not inducing symptoms, causing obstruction, or in the opinion of the investigator pose a risk of impending obstruction of any structures, will be allowed.
- Pulmonary nodules > 10 mm
    - Pulmonary nodules > 10mm that have been stable for >6 months and are not clearly metastatic disease per the treating investigator are permitted
- Soft tissue components of bone metastases ≥ 1.0 cm in longest axis
    - Soft tissue components of bone metastases < 1.0 cm that have been stable for >6 months (must not have enlarged > 5mm) are permitted
- Soft tissue lesions ≥ 1.0 cm in longest axis
    - Soft tissue lesions < 1.0 cm that have been stable for > 6 months (must not have enlarged > 5mm) are permitted.
- If present, primary disease in the prostate must be stable for > 6 months (defined as no growth > 5mm)
- Evidence of local recurrence in the prostate bed
- Evidence of liver metastases or visceral disease
- Has a diagnosis of immunodeficiency
- Patients requiring systemic steroid therapy with >10mg prednisone or its equivalent daily. Patients receiving systemic steroid therapy for non-autoimmune processes (e.g., COPD, adrenal replacement therapy) are permitted if on stable dose and asymptomatic.
- Has a known history of active TB (Bacillus Tuberculosis)
- Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
- Has had prior systemic therapy (exception: GnRH agonist or antagonist) or radiation therapy for prostate cancer within 2 weeks prior to study Day 1. There must be at least a 2 week washout period from last dose of any prior systemic or radiation therapy for prostate cancer prior to Day 1 of study treatment (including

nonsteroidal antiandrogens). Screening may commence during this washout window.

- Any patient who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events or complications due to a previously administered systemic agent, radiation therapy, or major surgery.
    - Exceptions:
        - Subjects with ≤ Grade 2 neuropathy, hot flashes, or hypertension may qualify for the study if all other eligibility criteria met.
        - Other toxicity or complications that are deemed by the treating investigator as not clinically significant (e.g., urinary incontinence from past prostatectomy, peripheral edema from past taxane therapy)
- Diethylstilbestrol, estrogens, saw palmetto, or other preparations that are known to have possible endocrine effects on prostate cancer that have been started within the past 8 weeks, as they may affect PSA levels or response. These are allowed if the patient has been on a stable dose for at least 8 weeks prior to C1D1.
- Receiving other investigational agents
- History of allergic reactions to compounds with similar biologic or chemical composition to pembrolizumab or radium-223
- Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that is planned for curative therapy.
- Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability.
- Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment and is allowed.
- Has known history of, or any evidence of active, non-infectious pneumonitis. A history of radiation pneumonitis which is asymptomatic with no signs of active process is allowed.
- Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
- Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
- Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies). HIV-positive participants on combination antiretroviral therapy are

ineligible because of the potential for pharmacokinetic interactions with pembrolizumab. In addition, these participants are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

- Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA is detected).
- Has received a live vaccine within 30 days of planned start of study therapy.

  Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, severe or unstable angina, myocardial infarction, symptomatic congestive heart failure (defined as New York Heart Association Grade II or greater), arterial or venous thromboembolic events (e.g., pulmonary embolism, cerebrovascular accident including transient ischemic attacks) or clinically significant ventricular arrhythmias within 6 months prior to randomization; or significant vascular disease (e.g., aortic aneurysm, aortic dissection), symptomatic peripheral vascular disease
- Evidence of QTc prolongation as defined as QTcF > 470 ms (per Fridericia's formula)
- Inability to comply with study and/or follow-up procedures

3.4 Inclusion of Women and Minorities

Men of all races and ethnic groups are eligible for this trial.

Women are not eligible for this trial as they do not have prostates and as such do not get prostate cancer.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

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

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's

registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

Following registration, participants will be randomized in a 2:1 ratio to either Arm A: a combination of Radium-223 and pembrolizumab or Arm B: Radium-223 alone.

Patients will be stratified based on:

- Baseline alkaline phosphatase <220 or ≥220
- Volume of bone metastases (high: ≥ 4 bone metastases including one beyond the axial skeleton vs. low: <4 bone metastases)
  -Based on CHAARTED (ECOG 3805) criteria[24]

Given the varied dosing of the two agents, we will refer to weeks on study instead of cycles.

Radium-223 55kBq/kg (1.49μCi)/kg will be administered intravenously (IV) slowly over 1 minute every 4 weeks for a maximum of 6 doses. Weight used for dosing should be based on institutional standards. Radium-223 will be administered in the nuclear medicine suite at DFCI and at the corresponding department for participating investigational sites.

Pembrolizumab will be given only to patients randomized to the investigational arm (Arm A). Pembrolizumab will be given at a dose of 200mg intravenously every 3 weeks. It will be administered in the Yawkey Infusion Center at DFCI and at the corresponding department for participating investigational sites.

Treatment with both agents will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's prostate cancer.

No dose modifications will be made for either agent. If study agents are held for more than 12 weeks (for pembrolizumab) or 8 weeks (for Radium-223) due to study drug-related toxicity, the patient should be discontinued from the offending agent. Patients may continue on either agent alone if tolerating.

Table 3: ARM A - Combination Investigational Arm

Agent	***Premedication***	***Dose***	***Route***	***Schedule***
Pembrolizumab	*None unless prior reaction; (see Table 6)*	*200mg (diluted per institutional guidelines /package insert)*	*IV 30 minutes -5 min/+ 10 min*	*Every 3 weeks (21 +/-3 days)* **Should be given prior to radium on days when they are given together.*
Radium-223	*None*	*55kBq/kg (1.49µCi)/kg*	*IV over 1 minute or longer per institutional standards*	*Every 4 weeks (28 +/-7 days) for 3 doses followed by a break until radiologic disease progression. Then the last 3 doses will be given every 4 weeks (28 +/-7 days)*

*Lupron/GnRH agonist or antagonist should be continued unless intolerable or discontinued for other safety reasons.
**Adhering to the schedule should be encouraged, and the 7 day window should not be utilized to give pembrolizumab and radium-223 on the same day for scheduling convenience.

Order of Administration – In the combination arm, pembrolizumab should be administered prior to radium-223 on days when both are given on the same day. No minimum time is required between the administration of drugs.

Table 4: ARM B - Control Arm

Agent	***Premedication***	***Dose***	***Route***	***Schedule***
Radium-223	*None*	*55kBq/kg (1.49µCi)/kg*	*IV over 1 minute or longer per institutional standards*	*Every 4 weeks (28 +/- 7 days) for 6 doses*

*Lupron/GnRH agonist or antagonist should be continued unless intolerable or discontinued for other safety reasons.

Trial treatment should begin on the day of randomization or within 7 days after randomization.

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution (Section 8.1).

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

Pre-Treatment Criteria for C1D1. D1 dosing criteria of subsequent cycles (2 and beyond) should follow Section 6 guidelines:

C1D1 labs must meet the following criteria prior to treatment but do not need to re-meet eligibility criteria:

- Absolute neutrophil count ≥1,500/mcL
- Hemoglobin ≥10g/dL
  *transfusions and/or erythropoietin supplementation permitted
- Platelets ≥100,000/mcL
- Total bilirubin ≤1.5x institution's upper limit of normal (unless known Gilbert's syndrome)
- AST(SGOT)/ALT(SGPT) ≤2.5 × institutional upper limit of normal ULN
- Creatinine clearance ≥30 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN. *(Note: creatinine clearance should be calculated per institutional standard)*
- If screening assessments have been done within 72 hrs prior to C1D1, they may be used in lieu of repeat C1D1 assessments (e.g., labs, ECOG, exam).

5.2.2 Subsequent Cycles

- Day 1 complete metabolic panel and complete blood count (CBC) with differential must be reviewed by study staff prior to treatment to ensure they meet criteria as detailed in Section 6
  - Other labs such as thyroid function tests that may take longer to result do not have to be reviewed prior to the dose
- CBC with differential should be obtained prior to each dose of radium-223 or pembrolizumab and doses given only if eligible per guidelines in Section 6

- Day 1 assessments may be performed within 72 hours prior to study drug administration.

5.3 General Concomitant Medication and Supportive Care Guidelines

If there is a clinical indication for medications or vaccinations specifically prohibited in the exclusion criteria, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding the use of these agents with the PI who will discuss with the Merck Clinical team as needed. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary treating physician.

5.3.1 **Acceptable Concomitant Medications**

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

Treatment with non-conventional therapies and vitamin/mineral supplements is acceptable provided that, in the opinion of the investigator, such treatment will not interfere with study treatment and the trial endpoints. If they do, the subject may need to be discontinued from the trial and this should be discussed with the PI.

Subjects may receive standard of care for any underlying illness provided therapy does not interfere with study treatment and the trial endpoints.

In the event of neutropenia, anemia, or thrombocytopenia, subjects may receive appropriate supportive care (such as transfusion, biologic response modifiers such as G-CSF or GM-CSF, prophylactic antibiotics, antifungals and/or antivirals, hematopoietic growth factors).

If surgery is required during study drug treatment, the surgeon needs to be notified that the patient has been treated with a radioactive product and adequate precautions for radioactive protection should be applied during the surgical procedure. The patient should continue with study treatment if considered safe in the treating investigator's opinion.

Radium-223 can be administered with concurrent osteoclast-targeting agents including bisphosphonates and denosumab if the patient is already on them for > 28 days prior to Day 1 of study treatment. These agents should not be started while the patient is on the study unless needed for the management of hypercalcemia or a skeletal-related event. If a patient has already been receiving an osteoclast-targeting agent prior to study entry, it may be continued or suspended during concurrent administration of radium-223 at the discretion of the treating investigator.

Consideration should be given to using concomitant bone health agents such as bisphosphonates on either arm of the study. Patients receiving bisphosphonates, denosumab, zoledronic acid or similar therapy prior to randomization may be maintained on this therapy during the study. Bisphosphonates denosumab, zoledronic acid or similar therapy can be stopped or started at the discretion of the investigator throughout the study.

5.3.2 **Prohibited Concomitant Medications**

Subjects are prohibited from receiving the following therapies during the Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy other than radium-223
    - Note: Patients may undergo palliative radiation to combat clinical (e.g., symptomatic) progression while on study if (1) the patient was achieving overall clinical benefit according to the treating investigator; and (2) the Principal Investigator has determined it acceptable to do so. Study drugs should be continued during radiation unless discussed with the PI that holding is in the best interest of the patient (e.g., concurrent toxicity concerns).
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: intranasal influenza, measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids are prohibited, with the following exceptions:
    - To modulate symptoms from an event of clinical interest (ECI) of suspected immunologic etiology while on study.
    - Baseline systemic steroids ≤10mg of prednisone or its equivalent for non-autoimmune processes (e.g., COPD, physiologic replacement doses) if on stable dose and asymptomatic.
- Diethylstilbestrol, estrogens, saw palmetto or other preparations thought to have endocrine effects on prostate cancer are allowed if the patient has been on a stable dose for at least 8 weeks prior to C1D1.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describe other medications, which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.3.3 **Supportive Care Medications**

- Drugs needed for supportive care such as anti-emetics are permitted

- Steroids for nausea: **dexamethasone, prednisone and other systemic steroids should be avoided, if possible, due to their immunosuppressive properties. If clinically indicated, steroid dose should not exceed 10mg of prednisone per day or equivalent.**

- Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. These treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab. If not thought related, care is per investigator discretion.

  It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

5.4 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s) or the scheduled break after the first 3 doses of radium-223 in arm A, treatment may continue for up to a total of *6 doses of radium-223 and up to 35 doses (24 months) of pembrolizumab if achieving clinical benefit and tolerating* or until one of the following criteria applies:

- Disease progression

  - **Arm A**:

    - After 3 months on study therapy (generally week 12 re-staging scans)

      - If the patient has stable disease or better, they will start on a radium-223 break, but continue on pembrolizumab.
        - If there have been treatment delays, the break should not commence until at least 3 doses of radium are given unless the radium-223 is deemed intolerable/toxic.
          If pembrolizumab has been discontinued due to toxicity despite stable disease or better, radium-223 will be continued without a break, and study assessments will be conducted on the same schedule as Arm B.

- Due to the intricacies of assessing response/progression in bone only disease, in cases where the treating investigator feels the patient is clinically benefiting from the radium-223 and that a break would be harmful to disease control, omission of the break will be permitted on a case-by-case basis after discussion with the PI. These patients will be continued on the radium-223 every month without break as is standard of care. Study assessments during combination therapy will continue per the required data table in Arm A. Scans will continue to adhere to the study schedule (every 12 weeks). Alternatively, the patient or clinician may choose to stop study therapy and proceed with alternative systemic therapies at their discretion.

- If 2 or more new bone lesions ("unconfirmed progression") are present on initial scans and the patient has received all 3 initial doses, radium-223 should be continued until reassessment with confirmatory scans ≥ 6 weeks.

- If "unconfirmed progression" of nodal disease occurs, but no new visceral disease, the patient may continue on pembrolizumab and radium-223 until reassessment with confirmatory scans ≥ 6 weeks or be discontinued per treating investigator's discretion.

- For patients with unconfirmed bone or nodal progression who continue on study therapy after initial restaging scans (~12 weeks), if subsequent scans (at least ≥ 6 weeks from initial scans) show:
  - The disease has stabilized or improved: they may continue on study therapy until disease progression or until intolerability/toxicity precludes further administration.
  - Continued radiologic progression per RECIST v1.1 or PCWG2: the patient should be discontinued from study therapy. If the treating investigator believes the patient is clinically benefiting, continuation of one or both study drugs may be permitted upon discussion with PI.

- If there is evidence of new visceral disease at any point, the patient should be discontinued from both agents.

- For all subsequent scans of patient who commence on radium-223 break:

  - If the patient continues to have stable disease or better, they will continue on pembrolizumab alone.

  - If the patient went on radium-223 break, it may be restarted:
    - Whenever the patient meets progressive disease criteria per

protocol as long as there is no new visceral disease. No confirmatory scans are required unless the treating investigator prefers to wait to confirm before restarting. The rationale to allow restarting of radium-223 in patients who are progressing in the nodes is the possible role of radium-223 as an immune modulator that may help stimulate the immune response to pembrolizumab.

- In cases where treating investigator feels that patient would clinically benefit from restarting radium-223, even though not technically progressing by RECIST v 1.1 or PCWG2, will be discussed and permitted only on a case-by-case basis with the PI.
- If the patient meets protocol criteria for disease progression on the next scheduled scans after restarting radium-223 (and has received at least 2 more doses of radium-223), they should be discontinued from study therapy. If they do not meet these criteria or treating investigator believes they are clinically benefiting, they may be permitted to continue on a case-by-case basis after discussion with the PI. Similarly, if treating physician feels the patient is clinically progressing prior to 2 more doses of radium-223, the patient can be discontinued from study therapy.

- If patient has signs of new visceral disease at any time meeting RECIST v1.1 criteria, they will be discontinued from study therapy.

- For subsequent scans of patients who do not commence on radium-223 break:

  - If the patient continues to have stable disease or better, they will continue on combination study therapy.

  - If scans show progressive disease per protocol criteria, they should discontinue therapy. In cases where treating investigator feels that the patient would clinically benefit from continuing study therapy even though they are technically progressing by RECIST v1.1 or PCWG2, continuation will be discussed on a case-by-case basis with the PI.

- **<u>Arm B</u>**

  - Continue on radium-223 until the patient meets protocol criteria for disease progression, up to a maximum of 6 doses.

- Intercurrent illness that prevents further administration of treatment

- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later. *Note: 24 months of study medication is calculated from the date of first dose.*

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

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

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, [redacted]

5.5 Duration of Follow Up

Participants will have a follow up visit within 30 days of removal from protocol therapy (End of Treatment visit). Adverse event monitoring will continue through End of Treatment visit. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. Follow up scans are not required for patients who do not progress, but will be captured if possible. After documented disease progression each subject will be followed by telephone, chart review, or clinic visit for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first. Regardless of reason for discontinuation, patients will be followed every 6 months until death or 2 years after treatment discontinuation.

5.6 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission

- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

An ODQ Treatment Ended/Off Study Form will be filled out when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

5.7 Clinical Criteria for Early Trial Termination

Early trial termination may occur if the criteria specified below are met:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction, including those determined by DSMC or study team from earlier safety monitoring in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6. DOSING DELAYS

Dose delays will be made as indicated in the following table(s). **No dose lowering/modifications will occur; only dose holds.** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

There will be no dose modifications for either pembrolizumab or Radium-223. Doses may be held for a maximum of 12 weeks for pembrolizumab and a maximum of 8 weeks for Radium-223 for resolution of toxicity. For delays longer than these intervals that are not due to toxicity of the agent and for which the patient was receiving clinical benefit, decisions to restart can be discussed with the PI and will be made on a case-by-case basis. The patient may be continued on either agent if one is discontinued and the other is not thought to be contributing to the toxicity in question.

Doses of either agent will not be omitted but can be given when the patient meets protocol specified criteria to treat. Interim reassessments between protocol specified dosing days are at the discretion of the treating physician.

Scans will stay on the same every 12 week (+/- 7 days) schedule even if doses are delayed.

6.1 Dose Delay Guidelines for Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per the Table below.

If an irAE is suspected, a thorough evaluation should be conducted in an effort to possibly rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to diagnosing an irAE. Serological, immunological, and histological (biopsy) data should be considered to support the diagnosis of an immune-related toxicity.

Table 5: Summary of Dosing Guidelines for Drug-Related Adverse Events (see more detailed recommendations for each event below the table)

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia	Resume pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3	Hold until toxicity resolves to Grade 0-1 or permanently discontinue per treating investigator's discretion	Hold until toxicity resolves to Grade 0-1 or permanently discontinue per treating investigator's discretion
	4 or Recurrent Grade 2 or 3	Permanently discontinue	Permanently discontinue
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Myocarditis	1-2	Hold until toxicity resolves to Grade 0	Per physician discretion, treat with corticosteroids. If pembrolizumab held and corticosteroid required, manage as per Grade 3.
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4 or Recurrent Grade 2	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[1]	Intolerable/ Recurrent Grade 2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3 or Severe	Toxicity resolves to Grade 0-1	
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[1] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held and that do not recover to Grade 0-1 within 12 weeks of the last dose.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). **Subjects should be placed back on pembrolizumab within 12 weeks of the last dose, unless otherwise discussed with the Overall PI and Merck contact.** The reason for interruption should be documented in the patient's study record.

The following are considered events that should be reported to the Sponsor-Investigator within 24 hours of the site responsible investigator becoming aware of the event. The treatments are recommendations and the agents utilized should be determined by the treating clinician as deemed medically necessary.

Pneumonitis
The following AE terms, if considered ≥ Grade 2, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Pneumonitis
– Interstitial lung disease
– Acute interstitial pneumonitis

If symptoms indicate possible new or worsening cardiac abnormalities additional testing and/or a cardiology consultation should be considered. All attempts should be made to rule out other causes such as metastatic disease, bacterial or viral infection**. It is important that patients with a suspected diagnosis of pneumonitis be managed as per the guidance below until treatment-related pneumonitis is excluded. Treatment of both a potential infectious etiology and pneumonitis in parallel may be warranted. Management of the treatment of suspected pneumonitis with steroid treatment should not be delayed for a therapeutic trial of antibiotics.** If an alternative diagnosis is established, the patient does not require management as below; however, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action
Grade 2 events:
– Report to Sponsor-Investigator
– Hold pembrolizumab.
– Consider pulmonary consultation with bronchoscopy and biopsy/BAL.
– Consider ID consult
– Conduct an in person evaluation approximately weekly
– Consider frequent Chest X-ray as part of monitoring
– Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.
– Second episode of pneumonitis – discontinue pembrolizumab if upon re-challenge the patient develops a second episode of Grade 2 or higher pneumonitis.

Grade 3 and 4 events:
– Report to Sponsor-Investigator
– Discontinue pembrolizumab.
– Hospitalize patient
– Bronchoscopy with biopsy and/or BAL is recommended.
– Immediately treat with intravenous steroids (e.g., methylprednisolone 125 mg IV). When symptoms improve to Grade 1 or less, a high dose oral steroid (e.g., prednisone 1 to 2 mg/kg once per day or dexamethasone 4 mg every 4 hours) taper should be started and continued over no less than 4 weeks.
– If IV steroids followed by high dose oral steroids does not reduce initial symptoms within 48 to 72 hours, consider treatment with additional anti-inflammatory measures. Discontinue additional anti-inflammatory measures upon symptom relief and initiate a prolonged steroid taper over 45 to 60 days. If symptoms worsen during steroid reduction, initiate a re-tapering of steroids starting at a higher dose (e.g., prednisone 80 or 100 mg) followed by a more prolonged taper and administer additional anti-inflammatory measures, as needed
– Prophylactic antibiotics against opportunistic infections are recommended.

Diarrhea/Colitis
The following AE terms, if considered ≥ Grade 2 or resulting in dose modification or use of systemic steroids to treat the AE, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Colitis
– Colitis microscopic
– Enterocolitis
– Enterocolitis hemorrhagic
– Gastrointestinal perforation
– Intestinal obstruction
– Necrotizing colitis
– Diarrhea
All attempts should be made to rule out other causes such as metastatic disease, bacterial or parasitic infection, viral gastroenteritis, or the first manifestation of an inflammatory bowel disease by examination for stool leukocytes, stool cultures, a Clostridium difficile titer and endoscopy. However, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action
Grade 2 Diarrhea/Colitis (4-6 stools/day over baseline, dehydration requiring IV fluids < 24 hours, abdominal pain, mucus or blood in stool):
– Report to Sponsor-Investigator
– Hold pembrolizumab.
– Symptomatic Treatment
– For Grade 2 diarrhea that persists for greater than 3 days, and for diarrhea with blood and/or mucus,

- Consider GI consultation and endoscopy to confirm or rule out colitis

- Administer oral corticosteroids (prednisone 1-2 mg/kg QD or equivalent)

– When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.
– If symptoms worsen or persist > 3 days despite the above interventions, treat as Grade 3

Grade 3 Diarrhea/Colitis (or Grade 2 diarrhea that persists for > 1 week):
– Report to Sponsor-Investigator
– Hold pembrolizumab.
– Rule out bowel perforation. Imaging with plain films or CT can be useful.
– Recommend consultation with Gastroenterologist and confirmation biopsy with endoscopy.
– Treat with intravenous steroids (e.g., methylprednisolone 125 mg) followed by high dose oral steroids (e.g., prednisone 1 to 2 mg/kg once per day or dexamethasone 4 mg every 4 hours) When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Taper over 6 to 8 weeks in patients with diffuse and severe ulceration and/or bleeding.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.
– If IV steroids followed by high dose oral steroids does not reduce initial symptoms within 48 to 72 hours, consider treatment with additional anti-inflammatory measures (e.g., infliximab).
-Discontinue additional anti-inflammatory measures upon symptom relief and initiate a prolonged steroid taper over 45 to 60 days. If symptoms worsen during steroid reduction, initiate a re-tapering of steroids starting at a higher dose of 80 or 100 mg followed by a more prolonged taper and administer additional anti-inflammatory measures as needed.

Grade 4 events:
– Report to Sponsor-Investigator
– Permanently discontinue pembrolizumab.
– Manage as per Grade 3.

Endocrine
The following AE terms, if considered ≥ Grade 3 or if ≥ Grade 2 and require holding/discontinuation/modification of pembrolizumab dosing, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Adrenal insufficiency
– Hyperthyroidism
– Hypophysitis
– Hypopituitarism
– Hypothyroidism
– Thyroid disorder
– Thyroiditis

All attempts should be made to rule out other causes such as brain metastases, sepsis and/or infection. However, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Hypophysitis or other symptomatic endocrinopathy other than hypo- or hyperthyroidism
Grade 2-4 events:
– Report to Sponsor-Investigator if appropriate
– Hold pembrolizumab
– Rule out infection and sepsis with appropriate cultures and imaging.
– Monitor thyroid function or other hormonal level tests and serum chemistries more frequently until returned to baseline values.
– Pituitary gland imaging should be considered (MRIs with gadolinium and selective cuts of the pituitary can show enlargement or heterogeneity and confirm the diagnosis).
– Treat with prednisone 40 mg p.o. or equivalent per day. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.
– Hypophysitis with clinically significant adrenal insufficiency and hypotension, dehydration, and electrolyte abnormalities (such as hyponatremia and hyperkalemia) constitutes adrenal crisis.
– Consultation with an endocrinologist may be considered.

Hyperthyroidism and Hypothyroidism
Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

Grade 2 hyperthyroidism, Grade 2-4 hypothyroidism events:
– Report to Sponsor-Investigator if appropriate (see Table 5)
– Monitor thyroid function or other hormonal level tests and serum chemistries more frequently until returned to baseline values.
– Thyroid hormone and/or steroid replacement therapy to manage adrenal insufficiency.
– Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
– In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
– In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
– Consultation with an endocrinologist may be considered.

Grade 3 hyperthyroidism events:
– Report to Sponsor-Investigator
– Hold pembrolizumab.

– Rule out infection and sepsis with appropriate cultures and imaging.
– Treat with an initial dose of methylprednisolone 1 to 2 mg/kg intravenously followed by oral prednisone 1 to 2 mg/kg per day. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 4 hyperthyroidism events:
– Report to Sponsor-Investigator
– Discontinue pembrolizumab.
– Manage as per Grade 3

Type 1 diabetes mellitus (if new onset) and ≥ Grade 3 Hyperglycemia
The following AE terms should be reported to the Sponsor-Investigator within 24 hours of the event:
– Type I diabetes mellitus (T1DM), if new onset, including diabetic ketoacidosis (DKA)
– Grade 3 or higher hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA).
-Immune-mediated diabetes may present as new onset of Type 1 diabetes or an abrupt worsening of preexisting diabetes associated with laboratorial evidence of beta cell failure. All attempts should be made to rule out other causes such as type 2 diabetes mellitus (T2DM), T2DM decompensation, steroid-induced diabetes, physiologic stress-induced diabetes, or poorly controlled pre-existing diabetes (either T1DM or T2DM), but events meeting the above criteria should be reported to the Sponsor-Investigator regardless of etiology. The patients may present with hyperglycemia (abrupt onset or abrupt decompensation) with clinical evidence of diabetic ketoacidosis or laboratory evidence of insulin deficiency, such as ketonuria, laboratory evidence of metabolic acidosis, or low or undetected c-peptide.

Course of Action
T1DM or Grade 3-4 Hyperglycemia events:
- Report to Sponsor-Investigator if appropriate (see Table 5)
- Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure, and resume pembrolizumab when patients are clinically and metabolically stable.
- **Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.**
- Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- Consultation with an Endocrinologist is recommended.
- Consider local testing for islet cell antibodies and antibodies to GAD, IA-2, ZnT8, and insulin may be obtained.

<u>Hepatic</u>

The following AE terms, if considered ≥ Grade 2 or greater (or any grade with dose modification or use of systemic steroids to treat the AE), are considered Events of Clinical Interest (ECIs) and should be reported to the Sponsor-Investigator within 24 hours of the event:
– Autoimmune hepatitis
– Hepatitis
– Transaminase elevations
All attempts should be made to rule out other causes such as metastatic disease, infection or other hepatic diseases. However, the AE should be reported regardless of etiology. See section 7.4 for ECI reporting details.

Drug Induced Liver Injury (DILI)
In addition, the event must be reported as a Drug Induced Liver Injury (DILI) ECI, if the patient meets the laboratory criteria for potential DILI defined as:
– An elevated alanine transaminase (ALT) or aspartate transaminase (AST) lab value that is greater than or equal to three times (3X) the institutional upper limit of normal (ULN) and
– An elevated total bilirubin lab value that is greater than or equal to two times (2X) institutional ULN and
– At the same time, an alkaline phosphatase (ALP) lab value that is less than 2X institutional ULN, as a result of within-protocol-specific testing or unscheduled testing.

Note that any hepatic immune ECI meeting DILI criteria should only be reported once as a DILI event.

Course of Action
Grade 2 events:
– Report as ECI and to Sponsor-Investigator
– Hold pembrolizumab when AST or ALT >3.0 to 5.0 times institutional ULN and/or total bilirubin >1.5 to 3.0 times institutional ULN.
– Monitor liver function tests more frequently until returned to baseline values (consider weekly).
–Treat with 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume pembrolizumab per protocol
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 3 events:
– Report as ECI and to Sponsor-Investigator
– Discontinue pembrolizumab when AST or ALT >5.0 times institutional ULN and/or total bilirubin >3.0 times institutional ULN.
– Consider appropriate consultation and liver biopsy to establish etiology of hepatic injury, if necessary
– Treat with high-dose intravenous glucocorticosteroids for 24 to 48 hours. When symptoms improve to Grade 1 or less, a steroid taper with dexamethasone 4 mg every 4

hours or prednisone at 1 to 2 mg/kg should be started and continued over no less than 4 weeks.
– If serum transaminase levels do not decrease 48 hours after initiation of systemic steroids, oral mycophenolate mofetil 500 mg every 12 hours may be given. Infliximab is not recommended due to its potential for hepatotoxicity.
– Several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 4 events:
– Report as ECI and to Sponsor-Investigator
– Permanently discontinue pembrolizumab
– Manage patient as per Grade 3 above

Neurologic
The following AE terms, regardless of grade, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Autoimmune neuropathy
– Demyelinating polyneuropathy
– Guillain-Barre syndrome
– Myasthenic syndrome
– Encephalitis

All attempts should be made to rule out other causes such as metastatic disease, other medications or infectious causes. However, the AE should be reported to the Sponsor-Investigator regardless of etiology

Course of Action
Grade 2 events:
– Report to Sponsor-Investigator
– Moderate (Grade 2) – consider withholding pembrolizumab.
– Consider treatment with prednisone 1-2 mg/kg p.o. daily as appropriate
– Consider Neurology consultation. Consider biopsy for confirmation of diagnosis.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 3 and 4 events:
– Report to Sponsor-Investigator
– Discontinue pembrolizumab
– Obtain neurology consultation. Consider biopsy for confirmation of diagnosis
– Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg prednisone or equivalent once per day. If condition worsens, consider IVIG or other immunosuppressive therapies as per local guidelines. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Hematologic

The following AE term, if considered Grade ≥3 or requiring dose modification or use of systemic steroids to treat the AE, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Autoimmune hemolytic anemia
– Aplastic anemia
– Disseminated Intravascular Coagulation (DIC)
– Haemolytic Uraemic Syndrome (HUS)
– Idiopathic (or immune) Thrombocytopenia Purpura (ITP)
– Thrombotic Thrombocytopenic Purpura (TTP)
– Any Grade 4 anemia regardless of underlying mechanism

All attempts should be made to rule out other causes such as metastases, sepsis and/or infection.
Relevant diagnostic studies such as peripheral blood smear, reticulocyte count, LDH, haptoglobin, bone marrow biopsy or Coomb's test, etc., should be considered to confirm the diagnosis. However, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action

Grade 2 events:
– Report to Sponsor-Investigator if proven to have autoimmune etiology
– Okay to continue pembrolizumab if no autoimmune signs or pending workup
– If signs of autoimmune effects consider hematology consultation and prednisone 1-2 mg/kg daily
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 3 events:
– Report to Sponsor-Investigator if proven to have autoimmune etiology
– Hematology consultation to rule out autoimmune etiology
– Hold pembrolizumab. Discontinuation should be considered as per specific protocol guidance.
– Treat with methylprednisolone 125 mg iv or prednisone 1-2 mg/kg p.o. (or equivalent) as appropriate
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 4 events:
– Report to Sponsor-Investigator
– Hematology consultation
– Discontinue pembrolizumab for all solid tumor indications
– Treat with methylprednisolone 125 mg iv or prednisone 1-2 mg/kg p.o. (or equivalent) as appropriate

Ocular

The following AE terms, if considered Grade ≥2 or requiring dose modification or use of systemic steroids to treat the AE, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Uveitis
– Iritis
All attempts should be made to rule out other causes such as metastatic disease, infection or other ocular disease (e.g. glaucoma or cataracts). However, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action
Grade 2 events:
– Evaluation by an ophthalmologist is strongly recommended.
– Treat with topical steroids such as 1% prednisolone acetate suspension and iridocyclitics.
– Discontinue pembrolizumab as per protocol if symptoms persist despite treatment with topical immunosuppressive therapy.

Grade 3 events:
– Evaluation by an ophthalmologist is strongly recommended
– Hold pembrolizumab and consider permanent discontinuation as per specific protocol guidance.
– Treat with systemic corticosteroids such as prednisone at a dose of 1 to 2 mg/kg per day. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 4 events:
– Evaluation by an ophthalmologist is strongly recommended
– Permanently discontinue pembrolizumab.
– Treat with corticosteroids as per Grade 3 above

Renal
The following AEs if ≥ Grade 2 should be reported to the Sponsor-Investigator within 24 hours of the event:
– Nephritis
– Nephritis autoimmune
– Renal failure
– Renal failure acute

Creatinine elevations ≥ Grade 3 or any grade with dose modification or use of systemic steroids to treat the AE.
All attempts should be made to rule out other causes such as obstructive uropathy, progression of disease, or injury due to other chemotherapy agents. A renal consultation is recommended. However, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action
Grade 2 events:
– Hold pembrolizumab
– Treatment with prednisone 1-2 mg/kg p.o. daily.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 3-4 events:
– Discontinue pembrolizumab
– Renal consultation with consideration of ultrasound and/or biopsy as appropriate
– Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg prednisone IV or equivalent once per day.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Skin
Rash and Pruritus
The following AEs if ≥ Grade 3 should be reported to the Sponsor-Investigator within 24 hours of the event:
– Pruritus
– Rash
– Rash generalized
– Rash maculo-papular
– In addition to CTCAE Grade 3 rash, any rash that is considered clinically significant, in the physician's judgment, should be treated as an ECI. Clinical significance is left to the physician to determine, and could possibly include rashes such as the following:
  - rash with a duration >2 weeks; OR
  - rash that is >10% body surface area; OR
  - rash that causes significant discomfort not relieved by topical medication or temporary cessation of study drug.

Other Skin events
The following AEs, regardless of grade, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Dermatitis exfoliative
– Erythema multiforme
– Stevens-Johnson syndrome
– Toxic epidermal necrolysis
Please note, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action
Grade 2 events:
– Symptomatic treatment should be given such as topical glucocorticosteroids (e.g., betamethasone 0.1% cream or hydrocortisone 1%) or urea-containing creams in combination with oral anti-pruritics (e.g., diphenhydramine HCl or hydroxyzine HCl).
– Treatment with oral steroids is at physician's discretion for Grade 2 events.

Grade 3 events:
– Hold pembrolizumab.
– Consider Dermatology Consultation and biopsy for confirmation of diagnosis.
– Treatment with oral steroids is recommended, starting with 1 mg/kg prednisone or equivalent once per day or dexamethasone 4 mg four times orally daily. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Grade 4 events:
– Permanently discontinue pembrolizumab.
– Dermatology consultation and consideration of biopsy and clinical dermatology photograph.
– Initiate steroids at 1 to 2 mg/kg prednisone or equivalent. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Immediate Evaluation for Potential Skin events (<u>Recommended but not required</u>)

A. Photographs:
Every attempt should be made to get a photograph of the actual skin lesion or rash as soon as possible**.**
Obtain appropriate consent for subject photographs and document in the patient notes.
– Take digital photographs of:
- the head (to assess mucosal or eye involvement),
- the trunk and extremities, and
- a close-up of the skin lesion/rash.

– If possible, a ruler should be placed alongside the site of a skin occurrence as a fixed marker of distance.
– The time/date stamp should be set in the 'ON' position for documentation purposes.
– Photographs should be stored with the subject's study records.
– The Sponsor-Investigator may request copies of photographs. The local study contact (e.g., CRA) will provide guidance to the site, if needed.

B. Past Medical History:
Collect past medical history relevant to the event. Any preexisting conditions not previously reported (e.g., drug allergy) should be entered into the Medical History eCRF.

C. Presentation of the Event:
Collect information on clinical presentation and potential contributing factors. This information should be summarized and entered in narrative format in the AE eCRF. Please use the available free-text fields, such as Signs and Symptoms. Note pertinent negatives where applicable to reflect that the information was collected. Any treatments administered should be entered on the Concomitant Medication eCRF.

D. Vitals Signs and Standard Laboratory Tests:
Measure vital signs (pulse, sitting BP, oral temperature, and respiratory rate) and record on the Vital Signs eCRF. Perform standard laboratory tests (CBC with manual differential and serum chemistry panel, including LFTs).

E. Focused Skin Examination:
Perform a focused skin examination. Information should be summarized and entered on the Adverse Experience eCRF as part of the narrative.

F. Dermatology Consult
Refer the subject to a dermatologist as soon as possible.
– For a **"severe rash"**, the subject must be seen within **1-2 days** of reporting the event.
– For **clinically significant rash**, the subject should be seen within **3-5 days**.
The dermatologist should submit a biopsy sample to a certified dermatopathology laboratory or to a pathologist experienced in reviewing skin specimens. The site should provide the dermatologist with all relevant case history, including copies of clinical photographs and laboratory test results.

Other/Miscellaneous Immune Related Toxicities
The following AEs, regardless of grade, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Myocarditis
– Pericarditis
– Pancreatitis
– Any additional Grade 3 or higher event, which the physician considers to be immune related
All attempts should be made to rule out other causes. Therapeutic specialists should be consulted as appropriate. However, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action
Grade 2 events or Grade 1 events that do not improve with symptomatic treatment:
– Withhold pembrolizumab.
– Systemic corticosteroids may be indicated.
– Consider biopsy for confirmation of diagnosis.
– If pembrolizumab held and corticosteroid required, manage as per grade 3 below.

Grade 3 events:
– Hold pembrolizumab

– Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg prednisone or equivalent once per day.
– When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
– Permanently discontinue if any grade 3 or higher cardiac toxicity including myocarditis and pericarditis even if improves/resolves
– Permanently discontinue for inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks. Otherwise, pembrolizumab treatment may be restarted as specified in the protocol

Grade 4 events:
– Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg prednisone or equivalent once per day.
– Discontinue pembrolizumab

Infusion Reactions
The following AE terms, regardless of grade, should be reported to the Sponsor-Investigator within 24 hours of the event:
– Allergic reaction
– Anaphylaxis
– Cytokine release syndrome
– Serum sickness
– Infusion reactions
– Infusion-like reactions

Please note, the AE should be reported to the Sponsor-Investigator regardless of etiology.

Course of Action
Refer to infusion reaction table below.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Table below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 6: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who re-develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

Table 7: General Approach to Handling irAEs not otherwise specified

irECI	Withhold/Discontinue pembrolizumab?	Supportive Care
Grade 1	No action	Provide symptomatic treatment
Grade 2	May withhold pembrolizumab	Consider systemic corticosteroids in addition to appropriate symptomatic treatment
Grade 3 and Grade 4	Withhold pembrolizumab Discontinue at investigator discretion for safety/tolerability or if unable to reduce corticosteroid dose to < 10 mg per day prednisone equivalent within 12 weeks of toxicity	Systemic corticosteroids are indicated in addition to appropriate symptomatic treatment. May utilize 1 to 2 mg/kg prednisone or equivalent per day. Steroid taper should be considered once symptoms improve to Grade 1 or less and tapered over at least 4 weeks.

All AEs are to be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0 (http://ctep.cancer.gov).

If an irAE does not resolve or improve to ≤ Grade 1 within 12 weeks after last administration of pembrolizumab, study therapy discontinuation should be considered after discussion with the PI.

Table 8: Dose Modifications and Management of Nausea/Vomiting Thought Related to Pembrolizumab*

Nausea/Vomiting	Management	Next Dose
≤ Grade 1	Supportive care	No change in dose
Grade 2	Hold until ≤ Grade 1.	Hold until ≤ Grade 1. Resume at same dose level.**
Grade 3	Hold until < Grade 2.	Hold until < Grade 2. Resume at same dose level.**
Grade 4	Off protocol therapy	Off protocol therapy if attributed to study drugs
*If nausea/vomiting thought unrelated, dosing of study drugs is per investigator discretion **Participants requiring a delay of >12 weeks should go off pembrolizumab if nausea/ vomiting thought related to pembrolizumab.		
Recommended management for all grades: antiemetics.		

6.2 Dose Modifications for Radium-223

Every effort should be made to administer the full dosing regimen of radium-223. **Adjustment of dose level is not permitted.** Study visits during the treatment period should occur at 4 week intervals (within a window of +/- 7 days). Dosing delays may be instituted under the following circumstances:

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

- If a patient experiences CTCAE version 4.0 grade 3 or 4 neutropenia, thrombocytopenia, or anemia the administration of study drug should be delayed until recovery to grade 2 or better.
- If a patient experiences CTCAE version 4.0 grade 3 or 4 neutropenia, thrombocytopenia, or anemia **lasting 8 weeks**, further Radium-223 administrations must be discontinued.
- Blood transfusion is acceptable between Radium-223 administrations.

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

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

Non-pathological fractures:
For traumatic fractures in weight-bearing bones during treatment phase, Radium-223 administration should be delayed for at least 2-4 weeks from the time of fracture.

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

Other Radium-223 related toxicities:

- If a patient experiences CTCAE version 4.0 grade 3 or 4 toxicity related to radium-223, the administration of radium-223 should be delayed until recovery to grade 2 or better.
- If a patient experiences CTCAE version 4.0 grade 3 or 4 toxicity related to radium-223 lasting 8 weeks, further study drug administrations must be discontinued.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets.

7.1 Expected Toxicities

7.1.1 Adverse Events

7.1.1.1 Adverse Event Expected with Pembrolizumab (MK-3475, Keytruda) [4]

Clinical Trial Experience (selected portions of US Prescribing Information using the melanoma population as the disease with the greatest clinical experience).

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The data described in the WARNINGS and PRECAUTIONS section and below reflect exposure to KEYTRUDA in an uncontrolled, open-label, multiple cohort trial (Trial 1). In Trial 1, the safety data are available from 411 patients with unresectable or metastatic melanoma and 550 patients with metastatic NSCLC who received KEYTRUDA at either 2 mg/kg every 3 weeks (n=61) or 10 mg/kg every 2 or 3 weeks (n=489).

Among the 411 patients with metastatic melanoma enrolled in Trial 1, the median duration of exposure to KEYTRUDA was 6.2 months (range 1 day to 24.6 months) with a median of 10 doses (range 1 to 51). The study population characteristics were: median age of 61 years (range 18 to 94), 39% age 65 years or older, 60% male, 97% white, 73% with M1c disease, 8% with brain metastases, 35% with elevated LDH, 54% with prior exposure to ipilimumab, and 47% with two or more prior systemic therapies for advanced or metastatic disease.

KEYTRUDA was discontinued for adverse reactions in 9% of the 411 patients. Adverse reactions, reported in at least two patients, that led to discontinuation of KEYTRUDA were: pneumonitis, renal failure, and pain. Serious adverse reactions occurred in 36% of patients receiving KEYTRUDA. The most frequent serious adverse drug reactions reported in 2% or more of patients in Trial 1 were renal failure, dyspnea, pneumonia, and cellulitis.

Table 9 presents adverse reactions identified from analyses of 89 patients with unresectable or metastatic melanoma who received KEYTRUDA 2 mg/kg every three weeks in one cohort of Trial 1. Patients had documented disease progression following treatment with ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. This cohort of Trial 1 excluded patients with severe immune-related toxicity related to ipilimumab, defined as any Grade 4 toxicity requiring treatment with corticosteroids or Grade 3 toxicity requiring corticosteroid treatment (greater than 10 mg/day prednisone or equivalent dose) for greater than 12 weeks; a medical condition that required systemic corticosteroids or other immunosuppressive medication; a history of pneumonitis or interstitial lung disease; or any active infection requiring therapy, including HIV or hepatitis B or C. Of the 89 patients in this cohort, the median age was 59 years (range 18 to 88), 33% were age 65 years or older, 53% were male, 98% were white, 44% had an elevated LDH, 84% had Stage M1c disease, 8% had brain metastases, and 70% receive two or more prior therapies for advanced or metastatic disease. The median duration of exposure to KEYTRUDA was 6.2 months (range 1 day to 15.3 months) with a median of nine doses (range 1 to 23). Fifty-one percent of patients were exposed to KEYTRUDA for greater than 6 months and 21% for greater than 1 year.

KEYTRUDA was discontinued for adverse reactions in 6% of the 89 patients. The most common adverse reactions (reported in at least 20% of patients) were fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea.

Table 9: Adverse Reactions in ≥10% of Patients with Unresectable or Metastatic Melanoma

	KEYTRUDA 2 mg/kg every 3 weeks N=89	
Adverse Reaction	All Grades (%)	Grade 3* (%)
General Disorders and Administration Site Conditions		
Fatigue	47	7
Peripheral Edema	17	1
Chills	14	0
Pyrexia	11	0
Gastrointestinal Disorders		
Nausea	30	0
Constipation	21	0
Diarrhea	20	0
Vomiting	16	0
Abdominal pain	12	0
Respiratory, Thoracic and Mediastinal Disorders		
Cough	30	1
Dyspnea	18	2
Skin and Subcutaneous Tissue Disorders		
Pruritus	30	0
Rash	29	0
Vitiligo	11	0
Metabolism and Nutrition Disorders		
Decreased appetite	26	0
Musculoskeletal and Connective Tissue Disorders		
Arthralgia	20	0
Pain in extremity	18	1
Myalgia	14	1
Back pain	12	1
Nervous System Disorders		
Headache	16	0
Dizziness	11	0
Blood and Lymphatic System Disorders		
Anemia	14	5
Psychiatric Disorders		
Insomnia	14	0
Infections and Infestations		
Upper respiratory tract infection	11	1

* There were no Grade 5 adverse reactions reported. Of the ≥10% adverse reactions, none was reported as Grade 4.

Other clinically important adverse reactions observed in up to 10% of patients treated with KEYTRUDA were:
Infections and infestations: sepsis

Table 10: Laboratory Abnormalities Worsened from Baseline in ≥20% of Patients with Unresectable or Metastatic Melanoma

	KEYTRUDA 2 mg/kg every 3 weeks N=89	
Laboratory Test	**All Grades %**	**Grades 3-4 %**
Chemistry		
Hyperglycemia	40	2*
Hyponatremia	35	9
Hypoalbuminemia	34	0
Hypertriglyceridemia	25	0
Increased Aspartate Aminotransferase	24	2*
Hypocalcemia	24	1
Hematology		
Anemia	55	8*

* Grade 4 abnormalities in this table limited to hyperglycemia, increased aspartate aminotransferase, and anemia (one patient each)

Immune-Related Adverse Events

An irAE is defined as a clinically significant AE of any organ that is associated with study drug exposure, is of unknown etiology, and is consistent with an immune-related mechanism. The most commonly reported immune-related adverse events across the dose-schedules in patients with melanoma were rash, pruritus, vitiligo, hypothyroidism, arthralgia, diarrhea, and pneumonitis. All occurred in less than 5% of patients. The organ most frequently affected by irAEs with pembrolizumab is the skin. Less frequently affected tissues include thyroid gland, colon, lung, kidney, and liver.

These events can occur after the first dose to several months after the last dose of treatment. Mild irAEs are usually treated symptomatically and do not require dosing delays or discontinuation. Higher grade and persistent lower grade irAEs typically necessitate withholding or discontinuing treatment and administration of systemic steroids or other immunosuppressive agents (such as tumor necrosis factor blockers), when systemic steroids are not effective. Early recognition of irAEs and initiation of treatment are critical to reduce the risk of complications, since the majority of irAEs are reversible with the use of steroids and other immune suppressants.

Immune-mediated adverse reactions

- Pneumonitis
- Colitis
- Hepatitis
- Nephritis and renal dysfunction
- Endocrinopathies:
    - Hypophysitis
    - Type 1 diabetes mellitus including diabetic ketoacidosis
    - Thyroid disorders: hyperthyroidism, hypothyroidism

- Pancreatitis
- Guillain-Barre Syndrome
- Myositis
- Arthritis
- Uveitis (<1%)
- Severe skin reactions: exfoliative dermatitis (<1%)
- Infusion reactions (severe: <0.1%)
- Hemolytic anemia
- Vasculitis
- Partial seizures related to inflammatory foci in brain parenchyma
- Serum sickness
- Myasthenia gravis

Select potential immune-mediated adverse reactions

Table below presents select treatment-related, potential immune-mediated adverse reactions that occurred in patients receiving KEYTRUDA. In addition, across clinical studies with KEYTRUDA in approximately 5000 patients, induction of type 1 diabetes mellitus has been reported in 0.1% of patients.

Table 11: Select Treatment-related, Potential Immune-mediated Adverse Reactions

	KEYTRUDA 2 mg/kg every three weeks n=162			KEYTRUDA 2 mg/kg every 3 weeks or 10 mg/kg every 2 or 3 weeks n=411		
Adverse Reaction	All Grades (%)	Grade 3 (%)	Grade 4* (%)	All Grades (%)	Grade 3 (%)	Grade 4* (%)
Colitis†	1.2	1.2	0	1.0	0.5	0
Hepatitis‡	1.2	0	0.6	0.5	0	0.2
Hyperthyroidism	0.6	0	0	1.0	0.2	0
Hypophysitis	1.2	0	0.6	0.5	0	0.2
Hypothyroidism	9.3	0	0	8.3	0.2	0
Nephritis§	0	0	0	0.7	0.2	0.2
Pneumonitis	1.2	0.6	0	2.7	0.2	0

* There were no Grade 5 treatment-related potential immune-mediated adverse reactions reported with KEYTRUDA.
† Includes colitis microscopic
‡ Includes autoimmune hepatitis
§ Includes autoimmune nephritis and renal failure with evidence of interstitital nephritis

7.1.1.2 Adverse Events Expected with Radium-223[3]

Bone Marrow Suppression
In the randomized phase 3 trial, 2% of patients on the radium-223 arm experienced bone marrow failure or ongoing pancytopenia compared to no patients treated with placebo. There were two deaths due to bone marrow failure and for 7 of 13 patients treated with radium-223, bone marrow failure was ongoing at the time of death. Among the 13 patients who experienced bone marrow failure, 54% required blood transfusions. Four percent (4%) of patients on the radium-223 arm and 2% on the placebo arm permanently discontinued therapy due to bone marrow suppression.

In the randomized trial, deaths related to vascular hemorrhage in association with myelosuppression were observed in 1% of radium-223-treated patients compared to 0.3% of patients treated with placebo. The incidence of infection-related deaths (2%), serious infections (10%), and febrile neutropenia (<1%) were similar for patients treated with radium-223 and placebo. Myelosuppression; notably thrombocytopenia, neutropenia, pancytopenia, and leukopenia; has been reported in patients treated with radium-223. In the randomized trial, complete blood counts (CBCs) were obtained every 4 weeks prior to each dose and the nadir CBCs and times of recovery were not well characterized. In a separate single-dose phase 1 study of radium-223, neutrophil and platelet count nadirs occurred 2 to 3 weeks after radium-223 administration at doses that were up to 1 to 5 times the recommended dose, and most patients recovered approximately 6 to 8 weeks after administration.

Clinical Trials Experience

In the randomized clinical trial in patients with metastatic castration-resistant prostate cancer with bone metastases, 600 patients received intravenous injections of 50 kBq/kg (1.35 microcurie/kg) of radium-223 and best standard of care and 301 patients received placebo and best standard of care once every 4 weeks for up to 6 injections.[2] Prior to randomization, 58% and 57% of patients had received docetaxel in the radium-223 and placebo arms, respectively. The median duration of treatment was 20 weeks (6 cycles) for radium-223 and 18 weeks (5 cycles) for placebo.

The most common adverse reactions (≥ 10%) in patients receiving radium-223 were nausea, diarrhea, vomiting, and peripheral edema. Grade 3 and 4 adverse events were reported among 57% of radium-223-treated patients and 63% of placebo-treated patients. **The most common hematologic laboratory abnormalities in radium-223-treated patients (≥ 10%) were anemia, lymphocytopenia, leukopenia, thrombocytopenia, and neutropenia**.

Treatment discontinuations due to adverse events occurred in 17% of patients who received radium-223 and 21% of patients who received placebo. The most common hematologic laboratory abnormalities leading to discontinuation for radium-223 were anemia (2%) and thrombocytopenia (2%).

The Table below shows adverse reactions occurring in ≥ 2% of patients and for which the incidence for radium-223 exceeds the incidence for placebo.

Table 12: Adverse Reactions in the Randomized Trial of Radium and best standard of care vs. placebo and best standard of care

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

Laboratory Abnormalities

The Table below shows the hematologic laboratory abnormalities occurring in $\geq$ 10% of patients and for which the incidence for radium-223 exceeds the incidence for placebo.

Table 13: Hematologic Laboratory Abnormalities

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

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

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

Fluid Status

Dehydration occurred in 3% of patients on radium-223 and 1% of patients on placebo. Radium-223 increases adverse reactions such as diarrhea, nausea, and vomiting which may result in dehydration.

Injection Site Reactions

Erythema, pain, and edema at the injection site were reported in 1% of patients on radium-223.

Secondary Malignant Neoplasms

Radium-223 contributes to a patient's overall long-term cumulative radiation exposure. Long-term cumulative radiation exposure may be associated with an increased risk of cancer and hereditary defects. Due to its mechanism of action and neoplastic changes, including osteosarcomas, in rats following administration of radium-223 dichloride, radium-223 may increase the risk of osteosarcoma or other secondary malignant neoplasms. However, the overall incidence of new malignancies in the randomized trial was lower on the radium-223 arm compared to placebo (<1% vs. 2%; respectively), but the expected latency period for the development of secondary malignancies exceeds the duration of follow up for patients on the trial.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information.

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

7.3 Expedited Adverse Event Reporting

A serious adverse event is any adverse event occurring at any dose or during any use of the study drugs that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event in the opinion of the treating physician or PI

- All bone fractures need to be reported as either AE(s) or SAEs if the criteria of SAE were met, regardless of the investigator's causality assessment for a minimum of 1 year after the last dose of radium-223. Taking into consideration applicable guidelines, bone health agents (BHA) such as bisphosphonates or denosumab are recommended.

From the time of consent up to study drug initiation, only serious adverse events deemed related to study procedures are required to be reported to the Overall PI. Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 90 days of the last dose of treatment on the local institutional SAE form.

Any serious adverse event, or follow up to a serious adverse event, deemed related to study procedures from the time of consent up to study drug initiation, must be reported within 24 hours to the Sponsor (DFCI) and within 2 working days to Merck Global Safety. Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study, that occurs to any subject from the time of study drug initiation through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the Sponsor (DFCI) and within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: 215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; [redacted] at the time of submission to FDA.

All subjects with serious adverse events must be followed up for outcome.

For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, all grade 4

toxicities (except those that are expected AND listed as not reportable in this protocol), and grade 5 (death) regardless of study phase or attribution.

7.3.1 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.4 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; [redacted]

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any participant must be reported within 2 working days to Merck Global Safety if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.7.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.5 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the

FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.6 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.7 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

7.7.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; [redacted]

7.7.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if

the subject initiates new anticancer therapy, whichever is earlier. All female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; [redacted]

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

8.1 Pembrolizumab[4]

8.1.1 Description

Pembrolizumab (MK-3475) is a humanized anti-PD-1 mAb of the IgG4/kappa isotype with a stabilizing S228P sequence alteration in the fragment crystallizable (Fc) region. Pembrolizumab binds to human PD-1 and blocks the interaction between PD-1 and its ligands.

The theoretical molecular weight of the polypeptide is 146,288 Da and its theoretical pI is 7.5. The parental murine anti-human PD-1 antibody (hPD-1.09A) was produced by immunizing mice with hPD-1 DNA. The pembrolizumab antibody was generated by humanization of the parental antibody by the Medical Research Council (Cambridge, UK) using complementarily-determining region (CDR) grafting technology (U.S. Patent No. 5,225,539). The gene segments encoding the variable heavy and light chains of pembrolizumab, as well as human IgG4, were codon-optimized, synthesized, and ligated into a vector.

A single expression plasmid, pAPD11V1-GA was constructed for the expression of both the heavy and light antibody chains of pembrolizumab. The nucleotide sequences encoding the heavy and light chains, along with their respective promoters and poly A signal sequences have been confirmed by DNA sequence analysis. The pAPD11V1-GA expression vector was subsequently used to transfect CHO-DXB-11 cells for the development of the pembrolizumab-producing cell line.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (see IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days).

8.1.2 **Form**

Table 14: Form of pembrolizumab

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection

Two drug product (DP) dosage forms are available for pembrolizumab: a white to off-white lyophilized powder, 50 mg/vial, and a liquid, drug product 100 mg/vial, both in Type I glass vials intended for single use only. The pembrolizumab Solution for Infusion 100 mg/vial will be used in this study. It is a liquid drug product (manufactured using the fully formulated DS), and has the identical formulation as that of the reconstituted lyophilized vial.

8.1.3 **Storage and Stability**

Drug should be stored under refrigerated conditions (2°C - 8°C).

8.1.4 **Handling**

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.5 **Availability**

Merck will provide the investigational supply of pembrolizumab to the study site pharmacies at no charge to the site or patient.

8.1.6 **Preparation**

The product after reconstitution with sterile water for injection and the liquid drugproduct are a clear to opalescent solution which may contain proteinaceous and extraneous particulates. The reconstituted lyophilized product and the liquid product are extraneous particulates. The reconstituted lyophilized product and the liquid product are intended for IV administration. The reconstituted DP solution or the liquid DP can be further diluted with normal saline in IV containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be immediately used to prepare the infusion solution in the IV bag and the infusion solution should be immediately administered. If not used immediately, vials and/or IV bags may be stored

at 2-8 °C for up to a cumulative time of 20 hours. If refrigerated, the vials and/or IV bags should be allowed to equilibrate to room temperature prior to subsequent use. Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of reconstituted or liquid DP solution in vials, room temperature storage of infusion solution in the IV bag and the duration of infusion. See Pharmacy Manual for additional information on dose preparation and administration.

8.1.7 **Administration**

Pembrolizumab will be given by intravenous infusion over 30 minutes (-5min, +10 min)

See Pharmacy Manual for additional information on dose preparation and administration.

8.1.8 **Ordering**

The study site will order the product from Merck.

8.1.9 **Accountability**

The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.10 **Destruction and Return**

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

8.2 **Radium-223[3] (Xofigo)**

8.2.1 **Description**

The alpha-pharmaceutical radium-223 is a ready-to-use, sterile, non-pyrogenic, clear and colorless aqueous solution of radium-223 dichloride ($^{223}RaCl_2$) for IV administration. It should not be diluted or mixed with any solutions. Each vial for a single use only.

Radium-223 is an alpha particle emitter with a physical half-life of 11.4 days. The product is isotonic and has a pH of 6.0-8.0. The radioactive concentration at the reference date is 1000 kBq/mL. The product has a pre-calibration of 14 days. When administered

on a day other than the reference day, the volume should be corrected according to the physical decay table.

8.2.2 **Form**

The volume per vial is 6 mL, corresponding to 6 MBq at the calibration day.

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

8.2.3 **Storage and Stability**

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

Do not store above 40°C (104°F). If the syringes have been stored in a refrigerator, they should be left at room temperature for 1 hour prior to use, since cold material should not be injected in a patient. Store radium-223 in the original container or equivalent radiation shielding. This preparation is approved for use by persons under license by the Nuclear Regulatory Commission or the relevant regulatory authority of an Agreement State.

8.2.4 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

General warning
Radium-223 (an alpha particle-emitting pharmaceutical) should be received, used and administered only by authorized persons in designated clinical settings. The receipt, storage, use, transfer and disposal 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-diamine-tetraacetic 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.

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). 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.

8.2.5 **Availability**

Radium-223 is commercially available.

Xofigo (radium Ra 223 dichloride injection) is available in single-use vials containing 6 mL of solution at a concentration of 1,000 kBq/mL (27 microcurie/mL) at the reference date with a total radioactivity of 6,000 kBq/vial (162 microcurie/vial) at the reference date.

8.2.6 **Preparation**

Personnel should use appropriate protective clothing and equipment during syringe handling to prevent contamination with the radioactive solution (medical gloves / protective glasses).

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

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.

The dose will be delivered in a ready-to-use prefilled syringe with a certified activity. The activity in the syringe will be assayed in the dose calibrator according to standard institutional practice and guidelines for administration of therapeutic radiopharmaceuticals to patients.

8.2.7 **Administration**

Administer radium-223 by slow intravenous injection over 1 minute (or longer depending on local institutional standards).
Flush the intravenous access line or cannula with isotonic saline before and after injection of radium-223.

Before administration of radium-223, the participant must be well hydrated; the participant should be instructed to drink ad libitum. Aseptic technique should be used in the administration of radium-223. The study medication will be administered as a bolus IV injection (up to 1 minute or longer depending on local institutional standards). After administration, the equipment used in connection with the preparation and administration of drug is to be treated as radioactive waste and should be disposed in accordance with local procedure for the handling of radioactive material.

8.2.8 **Dosimetry**

The absorbed radiation doses in major organs were calculated based on clinical biodistribution data in five patients with castration-resistant prostate cancer. Calculations of absorbed radiation doses were performed using OLINDA/EXM (**O**rgan **L**evel **IN**ternal **D**ose **A**ssessment/**EX**ponential **M**odeling), a software program based on the Medical Internal Radiation Dose (MIRD) algorithm, which is widely used for established beta and gamma emitting radionuclides. For radium-223, which is primarily an alpha particle-emitter, assumptions were made for intestine, red marrow and bone/osteogenic cells to provide the best possible absorbed radiation dose calculations for radium-223, considering its observed biodistribution and specific characteristics.

The calculated absorbed radiation doses to different organs are listed in the PI. The organs with highest absorbed radiation doses were bone (osteogenic cells), red marrow,

upper large intestine wall, and lower large intestine wall. The calculated absorbed doses to other organs are lower.

8.2.9 **Ordering**

Each center's pharmacy will order radium-223 as per standard institutional guidelines. The product will be delivered as a Patient Ready Dose in a prefilled syringe, ready-to-use with a certified activity. Radium-223 is shipped in a lead container and Type A radioactive package according to international transportation guidelines for radioactive materials.

8.2.10 **Accountability**

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.2.11 **Destruction and Return**

At the end of the study, unused supplies of radium-223 should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Immune biomarker studies

See statistical section for further details on endpoints and laboratory manual for details on collection, processing, and shipping.

9.1.1 Bone biopsies

The bone biopsies will be core needle or excisional biopsies. These biopsies will be performed per standardized clinical guidelines at Brigham and Women's Hospital and at the corresponding department for participating investigational sites. Tissue will be embedded into paraffin. Sites that have been previously irradiated are not permitted. The second biopsy should be done at the same site as the original biopsy when possible. If the first biopsy is non-diagnostic, a second pre-treatment biopsy should be performed if safe and acceptable to the patient and treating physician. The patient will not be eligible to enroll if no tumor tissue can be identified by the pathology team (touch prep evaluation will suffice). If the patient is enrolled and it is later determined that the first biopsy is not diagnostic or useful towards assessing the primary endpoint of increase in T cell infiltrate, then they will not be required to undergo the on-treatment biopsy. If the patient

is already on study treatment, they may continue on the study therapy even if no on-treatment biopsy is obtained.

The on-treatment biopsy should be done after at least 2 doses of radium-223 unless the second dose of radium-223 cannot be given due to safety/toxicity. If the latter, it should follow the protocol dictated time window (week 8 or within 3 weeks after week 8). If it cannot be performed for safety reasons or patient refusal (as long as patient initially agreed), it will not be considered a deviation.

Biopsies will be performed by an interventional radiologist under CT or PET-CT guidance. Blood samples will be drawn within 30 days of the biopsy to document an acceptable coagulation profile (INR < 1.5, PTT < 45, platelets >50,000). Aspirin and/or Plavix should be discontinued 5 days prior to the biopsy. On the day of the biopsy, a short physical exam will be performed by the radiologist including assessing the patient's general ASA score and airway rating. Informed written consent will be obtained following discussion of the risks (bleeding, infection, adjacent tissue injury, and pain) and benefits of the procedure. Biopsies will be performed using standard coaxial techniques under CT guidance without the administration of intravenous contrast. Several bone specimens (when possible, ideally at least 4-6 cores) will be obtained. Samples will be given to a dedicated technician present during the procedure for sample processing. Conscious sedation will be administered by a trained radiology nurse using small doses of Versed and fentanyl titrated to the patient's level of discomfort. Following the biopsy, patients will be observed for 2 hours following their last dose of conscious sedation for any complications (severe pain, hematoma, neurologic deficit) prior to departing with a chaperone.

See Laboratory Manual for further details.

9.1.2 Collection, Handling, and Shipping of Specimens

For the correlative studies, blood will be drawn and sent to the Fong Lab at UCSF at the designated timepoints, please refer to the Laboratory Manual for details.

9.2 Symptomatic Skeletal Events (SSEs)

SSEs will be defined as the use of external beam radiation to relieve bone pain, occurrence of new/symptomatic pathologic fracture, occurrence of spinal cord compression, or tumor-related orthopedic surgical intervention (secondary to a complication of a bone metastasis).

9.3 Quality of Life

Quality of life, pain and analgesic use will be measured via the FACT-P (Appendix B), Brief Pain Inventory (Short Form) (Appendix C), and analgesic diary (Appendix D) prior to treatment, at week 5, 9, and then every 6-8 weeks after week 9 (as schedules differ between the two arms), and at treatment progression/off study visit.

FACT-P: The FACT-P is a questionnaire with each item scored on a scale of 0-4 with higher scores indicating increased symptoms. Scoring instructions are detailed in Appendix B.

Brief Pain Inventory (Short Form): The Brief Pain Inventory (short form) is a 15 item questionnaire. The BPI allows patients to rate the severity of their pain and the degree to which their pain interferes with common dimensions of feeling and function. When scoring the Brief Pain Inventory, two scores are calculated: the pain severity and pain inference score. The pain severity score is calculated by adding the scores for questions 3, 4, 5 and 6 and then dividing by 4. This gives a severity score out of 10. The pain interference score is calculated by adding the scores for questions 9a, b, c, d, e, f and g and then dividing by 7. This gives an interference score out of 10. Changes in mean scores for the two domains will be analyzed over time.

Analgesic Use: Participants will record all narcotic analgesic medications taken for pain in an analgesic diary provided by the study team during the same assessment intervals in which pain scores will be measured. Prior to each interval, a member of the clinical research team will pre-populate the diary of each participant with the names and dosages of the narcotic medications prescribed, so that patients only have to indicate the number of doses taken by the end of each 24 hour period. For each interval, patients' mean narcotics use will be calculated by multiplying the daily dose unit by the number of units taken, averaged by the number of days with available data. Changes in narcotic use will be qualified as decreased (including discontinued), stable, or increased, based on the average daily narcotics use relative to the baseline interval.

Narcotics use will be considered stable if the average daily dose of a given narcotic was identical. Equianalgesia calculations will be required to quantify narcotic use if patients changed narcotic type or if dosages were changed in patients concomitantly receiving different narcotic types. In cases where equianalgesia calculations were required, narcotics use will be considered stable if the calculated equivalents were within 5% of the baseline dose.

9.4 Exploratory Bone Response Criteria

Quantitative Bone SPECT/CT
Substudy to be performed at DFCI only.

Bone scans will be routinely performed as part of the radiologic evaluations for measurement of tumor response. SPECT/CT will be obtained at the time of each bone scan required per protocol as part of standard of care for anatomic localization of lesions. Methods to quantitate bone tracer are not as established as the standardized uptake value (SUV) is for FDG-PET/CT, but new methods and software are now available to perform quantitation on SPECT/CT (Cachovan et al. EJNMMI; 3:45, 2013). Additional processing and quantitative analysis with newer software will be performed as part of research to explore if and how quantitative changes in bone tracer uptake on SPECT/CT correlate with markers of bone turnover, immune biomarkers, response on standard

imaging (CT or MRI).

If SPECT/CT is unable to be completed at screening or at on study timepoints for logistical reasons, it will not be considered a protocol violation.

9.5 Mutations in DNA Repair Genes in Prostate Cancer (to be funded by Bayer)

Prostate cancer represents a broad clinical and biological spectrum ranging from indolent tumors to aggressive disease resulting in significant morbidity and mortality. Uncovering differences in the genetic makeup of tumors may improve our understanding of its clinical heterogeneity. Recent studies have helped define the molecular taxonomy of prostate cancer using comprehensive genomic analyses. Robinson et al. demonstrated the presence of a potential driver mutation in nearly all metastatic CRPC tumor samples which included frequent alterations in the AR (71.3%), PI3K (49%) and DNA damage repair (DDR) pathways (22.7%).[25]

It is estimated that human cells encounter thousands of DNA damaging events on a daily basis.[26] These events can be mediated by endogenous (deamination and hydrolytic reactions, reactive oxygen species, DNA replication errors) and exogenous (ultraviolet light, ionizing radiation, alkylating agents and platinum compounds) sources.[27,28] Depending on the causative agent, the extent of DNA damage can range from base modifications and nucleotide mismatch to double stranded DNA breaks which is the most biologically hazardous form of DNA damage.[29] In order to maintain genomic stability and compensate for these damaging events, cells have evolved several repair mechanisms such as base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER) and double strand DNA repair pathways which includes homologous recombination (HR) and non-homologous end joining (NHEJ).[30] HR is the high fidelity mechanism preferentially used for repair of double strand DNA breaks and is mediated by proteins coded by genes such as BRCA-1, BRCA-2 and Rad52. The importance of these genes in maintaining genomic stability is highlighted by the high risk of familial breast and ovarian carcinoma and even some prostate cancers associated with germline BRCA-1 and BRCA-2 mutations (loss of function of these genes).

In addition to inherited germline mutations, these aberrations can be acquired through somatic changes. In advanced prostate cancer, germline and somatic mutations in DNA repair mechanisms have been reported in 11.8% and 22.7% of patients respectively.[25,31] In recent study by Annala et al, the presence of DDR mutations was associated with a shorter time to the emergence of castration resistance (11.8 months vs. 19.0 months; P=0.03) and an attenuated response to AR targeted therapy in castration resistant setting.[32] However, the presence of impaired DNA repair mechanisms also provides a therapeutic opportunity by targeting the inherent genomic instability of these tumor cells. Exploiting the biological principle of “synthetic lethality”, the use of PARP inhibitors has been associated with impressive responses in patients with advanced ovarian cancer with somatic or germline BRCA mutations.[33] Mateo et al recently demonstrated the presence of somatic or germline alterations in DDR alterations in 33% of patients with CRPC who were treated with the PARP inhibitor olaparib in a phase 2 study and that it highly

correlated with response.[34] Response rates were 33% in the overall study population compared to 88% in the cohort with DNA repair alterations, thus suggesting the potential role of these abnormalities as predictive biomarkers of response. The impact of these mutations on the efficacy of radium-223, which induces double stranded DNA breaks and tumor cell death through alpha particles, is unknown.

We hypothesize that tumor cells with germline or somatic mutations in DDR genes will be more susceptible to the DNA damaging effects of radium-223. Supporting this hypothesis, Steinberger et al recently reported a durable response with 2 courses of radium-223 in a patient with a frameshift mutation in BRCA-2.[35] Further, the presence of impaired DNA repair pathways in the tumor cells has been associated with higher tumor mutational load, neo-antigen expression and response to immune checkpoint blockade across several solid tumors.[36-38] Through this proposed correlative work, we aim to utilize whole exome sequencing (WES) to assess the impact of DNA repair and other genomic alterations on the clinical efficacy of radium-223 with or without the immune checkpoint inhibitor pembrolizumab.

Whole exome and transcriptome sequencing of tumor samples

Pre-treatment and on therapy biopsy specimens with matched germline samples will be used for whole exome and transcriptome sequencing in collaboration with the Van Allen Laboratory and the Center for Cancer Precision Medicine. Baseline alterations will be correlated with clinical outcomes. Induction of on-therapy changes will be characterized and correlated with efficacy. To be eligible for DNA isolation, tissue must contain at least 20% tumor nuclei. The DNA is then analyzed by massively parallel sequencing using a solution-phase Illumina hybrid capture kit and an Illumina HiSeq 2500 sequencer at the Broad Institute using established methodology. In parallel, capture based whole transcriptome sequencing, which can be performed from either frozen or formalin-fixed samples will be done on all tumor samples to determine gene expression quantification through orthogonal methods, as well as identify fusion products and alternative splicing events. Furthermore, RNA analysis through whole transcriptome sequencing will enable quantification of immune cell compartments that may be related to specific DNA repair mutations (e.g. mismatch repair) and may impact predictions of response, and this analysis can enable enrichment of likely pathogenic DNA repair mutations by distinguishing those that are present in DNA and also expressed in the RNA.

Circulating tumor derived cf-DNA in advanced prostate cancer

Genomic information derived from tissue based next generation sequencing is being increasingly employed in the clinic to guide treatment decisions. While it is an important step towards personalized medicine, there are limitations to tissue based sequencing assays. There can be significant genomic heterogeneity between the primary tumor and the metastatic sites.[39] As such, a biopsy of a single disease site might not be representative of the overall tumor clone. Further, genomic characteristics of tumors may change over the course of treatment with emergence of different resistant mechanisms.[40-42] Although biopsy of a metastatic site can provide genetic information at a given stage in

the disease course, it cannot provide insight into how subsequent therapies may induce clonal evolution or intrinsic dynamic changes in the biology of the tumor. While serial biopsies might accomplish this objective, they subject patients to repeated discomfort and increased risk of potential complications adding to the overall morbidity and anxiety of the disease.

Analyzing tumor derived cf-DNA in the blood can potentially circumvent the above challenges. cf-DNA is composed of fragments of coding and non-coding DNA which are released into the circulation during tumor cell apoptosis or necrosis.[43] Previous studies have shown that compared to normal controls, patients with prostate cancer have significantly higher levels of cf-DNA.[44] High pretreatment levels of tumor derived cf-DNA have been associated with poor response to taxane chemotherapy and worse clinical outcomes.[45] In addition to quantitative changes, cf-DNA has the potential to establish pretreatment mutational signatures that may predict resistance or sensitivity to established and investigational therapies. Supporting this concept, Salvi et al demonstrated that pretreatment copy number gain in AR and CYP17A1 genes detected in cf-DNA samples was predictive of poor overall survival and progression-free survival (PFS).[46] Further, by comparing the genomic alterations at baseline, on treatment, and at the time of disease progression, it may be possible to uncover novel mutations, copy number alterations and somatic variants which can be acquired as a result of clonal evolution and lead to treatment failure.[47,48] A major advantage of cf-DNA is that the sample is obtained by a simple blood draw and does not require a more invasive tumor biopsy.

Whole genome sequencing of circulating tumor derived cell free DNA

Blood samples will be obtained from patients enrolled in both arms prior to treatment initiation (week 1), on therapy at week 5 and week 13, and at the time of disease progression. Whole genome sequencing will be performed at 0.1x coverage, which we term ultra-low pass whole genome sequencing (ULPWGS). The sequencing information derived from ULPWGS will be used to estimate “tumor purity”, which is defined as percent of circulating DNA derived from tumor cells in the blood, and to identify copy number alterations in the tumor using an adaptation of a probabilistic model, called ichorCNA.[49] While other algorithms for assessing copy number changes from cf-DNA have been described,[50,51] ichorCNA has the advantage of accounting for mixtures of cell populations to assess for subclonal events and estimating tumor purity.

As this study was added during study enrollment, not all patients will have cfDNA analysis performed. Lack of cfDNA samples will not be considered a deviation.

The cfDNA samples will be analyzed by investigators at collaborating institutions, such as the Broad Institute, for patients enrolled on this trial. From these samples, the data will be submitted to the National Institutes of Health’s Database for Genotypes and Phenotypes (dbGaP) and other public databases. Samples and data will be de-identified.

Details regarding obtaining and processing cfDNA can be found in the Laboratory Manual.

10. STUDY CALENDAR

All baseline evaluations are to be conducted within 42 days prior to treatment initiation.

Assessments must be performed prior to administration of any study agent. On-study assessments should be administered within 72 hours of the protocol-specified date but prior to study drug administration. Imaging assessments may have a window of +/- 7 days but must remain on schedule even if doses are being held for toxicity or other reasons.

For participants on Arm A, if pembrolizumab is discontinued for toxicity, visits and assessments will then follow the schedule for Arm B. If radium-223 is discontinued, visits and assessments will be every 3 weeks at the time of the pembrolizumab dosing. The radium-223 visits on Arm A will be discontinued and not considered a protocol violation. The quality of life questionnaires and immune studies will be done on the pembrolizumab visit closest to their required visit if they had stayed on radium-223 (e.g., will be done on week 7 instead of week 8). Imaging assessments will remain the same on an every 12 week basis.

Table 15: Required Data Table

Study Calendar	Pre-Study[A]	Wk 1	Wk 4 (Arm A only)	Wk 5	Wk 7 (Arm A only)	Wk 8	Wk 9	Wk 10 (Arm A only)	Wk 13+ (R)	End of Treatment[S]
Informed consent	X									
Demographics	X									
Medical history	X									
Concurrent meds	X	X	X	X	X	X	X	X	X	
Physical exam[B]	X	X	X	X	X		X	X	X	X
Vital signs[C]	X	X	X	X	X		X	X	X	X
Height	X									
Weight	X	X	X	X	X		X	X	X	X
ECOG Performance status	X	X	X	X	X		X	X	X	X
CBC w/diff, plts[D]	X	X	X	X	X		X	X	X	X
Serum chemistry[E]	X	X	X	X	X		X	X	X	X
Testosterone	X									
PSA[F]	X	X[F]		X			X		X	X
TSH, Free T4[G]	X		X	X					X	
PT/INR, PTT[H]	X									
EKG	X									
Pembrolizumab[I] (Arm A only)		X	X		X			X	X	
Radium-223[J]		X		X			X		(X)[J]	
Adverse event evaluation[K]		X	X	X	X		X		X	X
Radiologic evaluation & Tumor measurements[L]	X								X[L]	
Correlative immune studies[M]		X		X			X		X*	
Bone biopsy[N]	X					X				
cfDNA[O]		X		X					X	
FACT-P Survey[P]		X		X			X		X[O]	X
Brief Pain Inventory[P]		X		X			X		X[O]	X
Analgesic Diary[P]		X		X			X		X[O]	X
Follow-up[Q]										X[Q]

A: All baseline evaluations are to be conducted within 42 days prior to treatment initiation. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. D1 labs and assessments do not need to be performed if have been done within 72hrs prior to study drug dosing. C1D1 labs do not need to re-meet eligibility criteria but do need to meet criteria as detailed in Section 5.2.1.

B: Physical examination should include general description of participant, head, eyes, and throat, chest, abdominal, extremities, neurologic, skin, and lymph node examination. Any other evaluation is up to the discretion of the practitioner. It will not be considered a violation if the exam is not described as outlined here. Patient will be seen in clinic the day of any study drug infusion (e.g., radium-223 and/or pembrolizumab)

C: Vital signs included blood pressure, heart rate, respiratory rate, body temperature

D: CBC with differential should be done before each dose of radium-223 and pembrolizumab.

E: Serum chemistry to include full CMP with sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, albumin. Liver function tests to include AST, ALT, total bilirubin and direct bilirubin, alkaline phosphatase.

F: PSAs should be done at baseline, week 1 day 1 (per treating investigator's discretion), every 4-6 weeks on therapy, and at study end.

G: TSH, Free T4 to be done a baseline, week 4 (week 5 for arm B), week 13, and then approximately every 12 weeks.

H: Coagulation factors to include PT and INR.

I: Pembrolizumab will be given by IV every 3 weeks (+/- 3 days) in Arm A only.

J: Radium-223 will be given by IV every 4 (+/- 7 days) weeks for a maximum of 6 doses.

Arm A: Radium-223 will be held after 3 doses. It may be restarted (to complete the final doses up to a maximum of 6 from study start) when there is progressive disease in bones as long as there is no new visceral disease.

Arm B: Radium-223 will be given as per standard of care monthly for 6 months in a row without breaks except for toxicity/intolerance as detailed in Section 6.2.

K: Adverse events will be monitored continuously

L: Diagnostic CT chest and CT or MRI of the abdomen and pelvis and bone scan including SPECT/CT should be obtained at baseline and every 12 weeks (+/- 7 days). (NOTE: SPECT/CT will be done only at DFCI.) Scans should remain on an every 12 week schedule (+/- 7 days) even if dose delays occur.

M*: Correlative immune studies: will be obtained and transported overnight at room temperature to the Fong Lab at UCSF. Samples for immune studies will be done at week 1 (pre-dose), week 5, week 9, week 13, week 21 and at the time of progression for those patients still on study.

N: Bone biopsy: archival tissue is permitted for baseline analysis. IR guided bone biopsy will be done at week 8 (+3 weeks) after the second dose of radium-223. If the second dose cannot be given for safety reasons, biopsy should still be performed within the window detailed here.

O: Blood samples for cell free DNA (cf-DNA) will be performed at treatment initiation (week 1, pre-dose), on treatment (week 5 and 13), and at time of progression.

P: Quality of life, pain and analgesic use will be measured via the FACT-P (Appendix B), Brief Pain Inventory (Short Form) (Appendix C), and analgesic diary (Appendix D) prior to treatment, at week 5, 9, then every 6-8 weeks after week 9 (as schedules differ between the two arms), and at treatment progression/off study visit.

Q: After progression/treatment discontinuation, follow the participant for survival, receipt of next line therapies, and post-treatment SSEs every 6 months until death or 2 years after treatment discontinuation.

R: Arm A study assessments will continue based on the same schedule as detailed for weeks 1-13 when pembrolizumab is given alone (e.g., visits, labs, assessments every 3 weeks only). If radium-223 is resumed upon progression, assessment schedule and radium-223 + pembrolizumab dosing will resume based on the schedule detailed for weeks 1-13. Arm B study assessments and radium-223 dosing will continue based on the same schedule detailed for weeks 1-13 up to 6 doses without breaks except for toxicity/intolerance (Section 6.2).
Note: For both arms: Immune studies will follow the schedule detailed in footnote M*

S: End of Treatment visit should occur within 30 days after last study treatment.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 12 weeks (+/- 7 days). In addition to a baseline scan, confirmatory scans should also be obtained 12 (not less than 6) weeks following initial documentation of progressive disease.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[52] and PCWG2[23].

11.1.1 Definitions

Eligibility requires lack of measurable disease by RECIST v1.1. As such, all baseline lesions would be considered non-target.

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease:

Note: Patients are not eligible if they have measurable disease for this study.

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area can still be considered measurable if they have grown since the last time of radiation on at least 2 subsequent scans.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Non-target lesions. All bone lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

Imaging studies will be reviewed by independent radiologists at the Tumor Imaging Metrics Core at DFCI. Ultimately, all scans will undergo central review at DFCI for final response analysis. However, for eligibility and ongoing response assessment while on study therapy, UCSF review will be per investigator assessment.

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and not more than 42 days before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases.

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.4 **Evaluation of Bone Lesions**

Existing bone metastases will be the only lesions in some patients. Because bone lesions are notoriously difficult to follow, **'progression' in non-target lesions is defined as the appearance of ≥2 new lesions, and, for the first reassessment only, a confirmatory scan performed 6 or more weeks later is required that shows a minimum of 2 or more additional new lesions (PCWG2 criteria).**

Complete Response (CR): Disappearance of all non-target lesions and normalization of PSA. All lymph nodes must be non-pathological in size (<10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

Definitions of Progression:

1. Bone Progression:
Appearance of 2 or more new lesions on bone scan, and, for the first reassessment only, a confirmatory scan performed 6 or more weeks later that shows a minimum of 2 or more additional new lesions. The date of progression is the date of the first scan that shows the change.

2. Soft Tissue Progression:
Soft tissue disease progression by modified RECIST v1.1 with additional requirement that progression at first assessment be confirmed by a second scan 6 or more weeks later. Note that for some treatments, a lesion may increase in size before it decreases.

3. Symptomatic Progression:
Symptomatic progression is defined as evidence of unequivocal symptomatic or clinical progression defined by at least 1 of the following:

- A marked escalation in cancer-related pain that is assessed by the Investigator to indicate the need for other systemic therapy or palliative radiotherapy. Ignore early changes (≤ 12 weeks) in pain or health-related quality of life in absence of compelling evidence of disease progression. Confirm progression of pain or health-related quality of life ≥ 3 weeks later
- An immediate need for initiation of new anticancer treatment or surgical intervention for complications due to tumor progression. Palliative radiation is allowed while on therapy to combat symptomatic/clinical progression and does not require that the patient discontinue study therapy unless treating investigator does not believe they are deriving any clinical benefit.
- A marked deterioration in ECOG performance status to Grade 3 or higher, or
- It is felt to be in the best interest of the patient to come off study due to clinical progression

11.1.5 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.6 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease): all patients in this study will have non-measurable bone only disease

Table 16: Evaluation of Overall Response

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.7 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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

11.1.8 Evaluation of Time to Event Endpoints

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to

any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

11.1.9 Methods for Evaluation of Changes in PSA

Biochemical Response:[53]

The laboratory will utilize a consistent method to check serum PSA at baseline, every other cycle while on treatment, and at study termination.

PSA decline ≥50% from baseline to 12 weeks of therapy and which has been confirmed with a second PSA at ≥ 3 weeks later.

Percent change in serum PSA:[23]

The percent change (rise or fall) from baseline at 12 weeks, and separately, the maximal change (rise or fall) at any time will be recorded using a waterfall plot.

PSA Progression:

PSA progression is defined as ≥25% increase and ≥2 ng/mL increase above the nadir or baseline value for patients who had a PSA decrease on treatment which is confirmed by a second value 3 or more weeks later.

For patients without a decrease on treatment, PSA progression is defined as ≥25% and a ≥2 ng/mL after 12 weeks.

PSA progression must be confirmed at the next study visit 4 weeks later.

PSA progression alone will not be considered diagnostic of disease progression, and will not be used as a reason to discontinue study treatment.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

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

12.1.2 Responsibility for Data Submission

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

12.2 Data Safety Monitoring

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

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is an open label phase 2 trial that randomizes 45 mCRPC patients (2:1 ratio) to radium-223 plus pembrolizumab combination therapy or to radium-223 monotherapy. The primary objective is to compare the increase in immune CD8+ and CD4+ T cell infiltration levels from baseline to 8 weeks on treatment between the two treatment arms.

Primary Endpoint:

Difference in tissue immune cell (e.g., CD4+/8+ T) infiltration in the bone metastasis biopsy specimens from baseline to 8 weeks on treatments in both the combination and monotherapy arms.

Secondary Endpoints:

- Safety and Tolerability: grade 3 or higher adverse events that are considered treatment related according to NCI CTCAE (version 4.0)

- Progression-free survival (PFS): is defined as the time from the first dose of study drug to the earlier of the first documentation of definitive disease progression [as

defined by Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) and PCWG2] or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation

- Overall survival as defined in 11.1.8

Exploratory Endpoints:

- Clinical-based endpoints

  - Changes in PSA

  - PSA response: defined as decline ≥50% from baseline to 12 weeks of therapy and which has been confirmed with a second PSA at ≥ 3 weeks later

  - Time to PSA progression as defined in 11.1.9

  - Time to normalization of alkaline phosphatase (if initially elevated)

  - Time to decrease in alkaline phosphatase (if initially elevated)

  - Symptomatic skeletal events (SSEs): Rate of SSEs and time to first SSE

  - Changes in osteoblastic activity on bone scan (assessed using the quantitative SPECT/CT)

  - Changes in quality of life, pain, and analgesic use, and time to introduction of analgesics

- Immune-based endpoints

  - Quantify the CD8, CD4, and FoxP3+ CD4 (Treg) cell infiltration in the bone biopsy specimens, and to subsequently quantify the CD8/Treg and CD4/Treg ratios

  - Change in circulating T cell clonality

  - Induction of IgG antibody response

  - Changes in PD-L1 expression and co-regulation of other immune checkpoints such as TIM-3, LAG-3, and PD-L2

  - Changes in immunosuppressive cell populations and cytokines (e.g., MDSCs, Tregs, TGF-β, and iNOS)

  - Change in serum chemokines: (e.g., CXCL9, CXCL10)

- Changes in soluble PD-L1 (Freeman Lab assay)

- Genomic-based endpoints

  - Correlation of DNA damage repair (DDR) mutations in baseline tumor samples and circulating cf-DNA with progression-free survival (PFS) on radium-223 ± pembrolizumab.

    - DDR encompasses genes such as BRCA1, BRCA2, FANCA, ATM, PALB2, HDAC2 and CK2

    - PFS will be defined as time from randomization to:

      - Appearance of 2 or more new bony lesions compared to baseline, confirmed on a repeat bone scan ≥6 weeks later

      - Appearance of new soft tissue lesions on CT/MRI or progression of existing ones per RECIST 1.1 criteria

      - Death from any cause

  - Prevalence of mutations in DDR genes in tissue or cf-DNA in patients with metastatic CRPC treated with Ra-223

  - Incidence of novel genomic alterations detected at the time of progression as mechanisms of treatment resistance

  - Impact of baseline cf-DNA tumor purity (defined as percentage of tumor derived circulating DNA; high: >7.5% vs. low: ≤ 7.5%) on subsequent PFS

  - Change in tumor purity from baseline to weeks 5 and 13

  - Impact of change in tumor purity at 13 weeks (increased/stable vs. decreased compared to baseline) on PFS

  - PSA response (defined as PSA decline ≥50% compared to baseline) and overall survival (OS: defined as time from randomization to death from any cause) in patients with and without DNA repair mutations on radium-223 ± pembrolizumab.

13.2 Accrual Rate and Study Duration

With two sites participating and an anticipated accrual rate of 4-5 patients per month, we expect to enroll 45 patients in approximately 1 to 1.5 years. With an additional 6 months of follow-up, the total study duration is expected to be 2 years. If more than 9 patients (6 on combination arm, 3 on radium arm) have inadequate bone biopsy specimens to assess the primary endpoint, patients will be replaced.

Table 17: Accrual Targets

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	0	+	8	=	8
Not Hispanic or Latino	0	+	37	=	37
Ethnic Category: Total of all subjects	0	+	45	=	45
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	0	+	8	=	8
Black or African American	0	+	3	=	3
Native Hawaiian or other Pacific Islander	0	+	1	=	1
White	0	+	33	=	33
Racial Category: Total of all subjects	0	+	45	=	45

13.3 Stratification Factors

Patients will be stratified based on:

- Baseline alkaline phosphatase <220 or ≥220
- Volume of bone metastases (high: ≥ 4 bone metastases including one beyond the axial skeleton vs. low: <4 bone metastases)
  -Based on CHAARTED (ECOG 3805) criteria[24]

13.4 Sample size justification

The study plans to enroll 45 patients to ensure 36 patients to include in the primary analysis after taking into consideration that 20% patients may be lost to follow-up or have inadequate serum and tissue specimens for analysis.

The sample size justification is based on comparisons between the treatment arms of the increase in CD4+ T or CD8+T cell infiltration levels measured in the bone biopsy specimen from baseline to 8 weeks. Our primary biological hypothesis is that patients receiving pembrolizumab plus radium-223 will have a higher increase in CD8+ and CD4+ T cell infiltration from baseline to 8 weeks on treatment than patients treated with radium-223 alone. While little is known of CD8+ T cell levels in metastatic prostate cancer specimens, a 50% to 80% difference between the treatment arms for the increase in CD4+ or CD8+ T cells would represent a biologically meaningful treatment effect. With a total of 36 patients with adequate tissue/specimens for analysis, the study would provide 24 patients treated with the combination and 12 patients treated with radium-223 monotherapy. There would be 91% statistical power to detect a difference of increase in CD4/8 cell infiltration levels

between the treatment arms that is 1.2 times the population standard deviation (i.e. an effect size of 1.2, defined as the difference as a fraction of population standard deviation) using Wilcoxon rank sum test with two-sided type I error α=0.05. This corresponds to a hypothesis test for a 70% difference between the two treatment arms of the increase in CD4/8 T cell infiltration levels, with a population standard deviation (SD) of 58%. The following table provides statistical power to be achieved from a few other scenarios with effect size assumptions.

	Table 18: Statistical power for Wilcoxon rank sum test				
	Effect size (Difference of the increase in CD4/8+ cell infiltration levels between arms/SD)				
Evaluable Sample sizes	0.8	0.9	1	1.1	1.2 (=.7/0.58)
100% enrolled N1=30, N2=15	69	79	86	92	96
80% enrolled N1=24, N2=12	**59**	**69**	**78**	**85**	**91**

13.5 Safety Monitoring Plan

Give there is no safety data for the combination therapy of pembrolizumab and radium-223, an early safety monitoring will be conducted to ensure that the combination therapy is well tolerated. A rate of targeted grade 3-5 AEs, particularly related to induction of immune phenomenon such as pneumonitis, colitis, and severe rash, in excess of 33% with combination therapy would be of concern. The safety assessments will be implemented after 15 (~50%) of the planned 30 subjects in the combination therapy arm have completed 12 weeks of combination therapy (including those who discontinued treatment because of toxicity), a focused safety review will be conducted. An estimated targeted AE rate with a one-sided binomial 90% CI will be constructed. If 5 or more of the first 15 subjects experience the targeted AEs (an observed 33% AEs rate, to have a upper bound of the CI <=53%), then the enrollment of combination therapy arm may be suspended pending the study team and DSMC review. The following table gives the operating characteristics of the early safety stopping; if the true unknown probability of grade ≥3 AEs rate is 40% or higher, the probability of observing 5 or more subjects with grade ≥3 AEs will be greater than 78.3% at the time of AE assessment.

Table 19: Operating characteristics of early safety monitoring

True/unknown G3+ AE rate (%)	Probability (%) of observing 5 or more ≥ grade 3 AEs in 15 subjects
20	16.4
30	48.5
40	78.3
50	94.1
60	99.1

The trial will be reviewed semi-annually by the DF/HCC Data and Safety Monitoring Board.

In addition to summarizing all AEs according to type and grade, all grade 4 (or higher) AEs will be listed for review.

13.6 Definition of Analysis Populations

Safety population: All patients who have received at least one dose of radium-223 or pembrolizumab will be included in safety assessment.

Primary analysis population: All patients who receive at least one dose of radium-223 and if in arm A, one dose of pembrolizumab and have bone biopsy assessment at baseline and 8 weeks will be included to assess primary, secondary, and exploratory endpoints.

13.7 Analysis of Primary Endpoints

The extent of immune cell infiltration (in the bone biopsy specimens): The comparison of an increase in CD4+ T or CD8+ T cell infiltration levels measured in the bone biopsy specimen from baseline and 8 weeks between the treatment arms will use Wilcoxon rank sum tests. The difference and the 95% confidence interval will also be provided.

13.8 Analysis of Secondary Endpoints

Safety and Tolerability: All adverse events recorded during the trial will be summarized for the safety population. The incidence of events that is new or worsening from the time of first dose of treatment will be summarized according to system organ class term, severity (based on CTCAE version 4.0 grade), type of adverse event, and relation to study treatment. For the combination therapy, special interests include toxicities related to induction of immune phenomenon such as pneumonitis, colitis, and severe rash. The frequency of AEs will be summarized separately according to treatment arms, with 90% exact binomial confidence intervals (CI). The proportion of patients in each arm with grade 4 or higher AEs will also be reported with an exact binomial 90% CI.

Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, and type of adverse event.

PFS and OS: The distributions of time to PSA progression, PFS and OS will each be summarized using the product-limit method of Kaplan-Meier according to treatment arms. Median times for each endpoint will be presented with two-sided 95% confidence intervals estimated using log(-log(survival)) methodology. Kaplan-Meier estimates of the endpoints at 6 or 12 months after treatment initiation may also be presented with two-sided 95% confidence intervals.

13.9 Analysis of Exploratory Clinical Endpoints

The endpoints will be summarized descriptively and graphically by treatment assignment. Tests for intra-patient changes in values while on combination treatment will use Wilcoxon signed-rank for continuous endpoints; categorical endpoints at two timepoints will be cross-tabulated and relevant proportions will be reported with confidence intervals. Changes in values will also be compared between treatment groups using the Wilcoxon rank sum tests for continuous endpoints. The Kaplan-Meier method will be employed to characterize the distributions of time to event endpoints.

Specifically,

Time to PSA progression: the distributions of time to PSA progression will be summarized using Kaplan-Meier according to treatment arms. Median times to PSA progression will be presented with two-sided 95% confidence intervals.

PSA response: the proportion of patients with a PSA response will be presented with a two-sided 95% confidence interval **(CI)** using exact binominal method according to the treatment arms. Change in PSA will also be summarized descriptively according to the treatment arms and the changes in PSA will also be compared between treatment groups using the Wilcoxon rank sum tests.

Symptomatic skeletal events (SSEs): the proportion of patients with SSEs will be presented with a two-sided 95% confidence interval (CI) using exact binominal method and the distributions of time to first SSEs will be summarized using Kaplan-Meier plots according to the treatment arms.

Quality of life: descriptive statistics at each time point and changes over time will be provided for quality of life, pain and analgesic use measures. Mixed models may be used to explore the impact of time on quality of life and pain adjusting for treatment arms and other relevant factors.

Time to introduction of analgesic use will be summarized using Kaplan-Meier plots according to the treatment arms.

13.10 Analysis of Exploratory Immune Endpoints

Will be done at the Fong Lab at UCSF and DFCI Center for Molecular Oncology Core.

We hypothesize that the number of CD8+ and CD4+ T cells will significantly increase following anti-PD-1 antibody treatment. IHC will be performed on bone metastasis biopsies obtained at baseline and on treatment to quantify tumor infiltration by CD8+ T cells, Tregs (FoxP3+ CD4+ cells), and total CD4 T cells (in both normal and tumor tissue).

We hypothesize that the treatment with Radium-223 will prime T cells and therefore lead to changes in the circulating T cell repertoire. We will assess this by performing TCR sequencing of circulating blood on baseline and on treatment blood samples. For each arm, the change in circulating TCR between pre-treatment and post- treatment will be assessed by calculating the number of unique clonotypes comprising the top 25th percentile of cumulative reads after sorting by clone abundance. Repertoire change between sequencing experiments will be measured using Morisita's distance.

To detect circulating antibodies in sera derived from the pretreatment and on therapy-timepoints, spotted antigen arrays will be used. After standard preprocessing of the protein array data, Cluster and Treeview software will be used for unsupervised clustering of the data with Pearson correlation and complete linkage. For each array, an antigen is identified as being detected if its value is above the median. To determine the number of up- and down modulated antibodies, the difference in log2 intensity values of pretreatment and post-treatment samples will be taken for each patient to identify antigens that are detected differentially due to treatment. Number of antibodies with at least 2- or 4-fold difference between pretreatment and post-treatment samples will be compared between the treatment arms by performing two-sided Wilcoxon rank sum test.

Tumor tissue from each patient's biopsy will be evaluated by IHC for PD-L1 levels prior to administration of the drug. Two independent pathologists who are blinded to the patients' clinical outcomes (e.g., response, survival) will score the percentage of tumor cells exhibiting cell-surface staining for PD-L1. PD-L1 positivity is defined per core by a 5% expression threshold.[54,55] Other levels of PD-L1 positivity will be explored. Patients with multiple cores will be considered PD-L1–positive if any specimen meets this criterion. Similarly, tumor will be stained for other immune checkpoints such as TIM-3, LAG-3, and PD-L2.

The bone metastasis biopsies will be evaluated to assess whether increased levels of MDSCs, TGF-β, iNOS, arginase, or Tregs which have been linked with T cell suppression[56] correlate with lower levels of effector T cell infiltrate post PD-1 blockade. If so, these may be surrogates for lack of efficacy with this strategy.

The local production of relevant cytokines and chemokines in serum and tumor tissue will also be examined using real-time PCR, western analysis, and commercially available RNase protection kits. The impact of the agent on the expression of potential immunosuppressive molecules such as indoleamine 2,3-dioxygenase, nitric oxide synthetase, and arginase in antigen presenting cells will also be evaluated. Post-treatment levels will also be characterized.

For continuous immunologic endpoints, the following table shows the statistical power to be achieved for the detectable effect sizes (difference as a fraction of population standard deviation) within group comparisons of values and assuming either 100% or 80% of patients have paired samples (two-sided α=0.05).

Table 20: Statistical Power assessments for comparisons between two timepoints within combination therapy (arm A)

	Statistical power (%) (bold)				
	Effect size within arms between two timepoints				
Evaluable Sample size	0.6	0.7	0.75	0.8	0.9
If 100% enrolled for analysis N=30	**61**	**75**	**80**	**85**	**92**
If 80% enrolled for analysis N=24	**52**	**65**	**71**	**76**	**85**

13.11 Analysis of Exploratory Genomic Endpoints

Mutations in DDR genes can be seen in around 30% of patients with metastatic CRPC.[34] We hypothesize that presence of these mutations would sensitize tumor cells to the DNA damaging effects of radium-223 and result in a longer PFS. The study is planned to enroll 45 patients who will receive radium-223. Assuming 10% will not have adequate tissue for sampling, we estimate that we will have adequate samples from at least 40 patients available for the correlative study. Using a log-rank test with a one-sided type I error of 0.15, there is 80% statistical power to detect an improvement in median PFS from 6 months (patients without DNA repair mutation) to 12 months (patients with a DNA repair pathway mutation) corresponding to hazard ratio of 0.50 (Table 21).

Table 21:

Assumptions:
- N=40 (evaluable samples)
- Median PFS in non-mutated DNA repair group = 6 months

DDR mutation prevalence	**Targeted median PFS (mos) in patients with patients with DDR mutation**	**HR**	**N (PFS event)**	**Power**
0.3	**12**	**0.5**	**36**	**0.8**
0.3	13	0.46	36	0.85
0.4	12	0.5	36	0.82
0.4	13	0.46	35	0.87

The distributions of PFS and OS will each be summarized using the product-limit method of Kaplan-Meier according to prevalence of baseline genomic alternations (e.g., with or without DNA repair mutation). Median times for each endpoint will be presented with two-

sided 95% CI estimated using log(-log(survival)) methodology. Log-rank test will be used to compare the median PFSs between the DDR mutation groups. Kaplan-Meier estimates of the endpoints at 6 or 12 months after treatment initiation may also be presented with two-sided 95% confidence intervals.

For the cfDNA analysis, patients will be dichotomized based on baseline tumor purity status into 2 groups: high (tumor purity >7.5%) vs. low (tumor purity ≤ 7.5%). The cutoff choice was based on the prior study that demonstrated 7.5% to be the lower limit of accurate detection for tumor derived cf-DNA.[57] Kaplan-Meier estimates will be used to assess the distribution of PFS according to tumor purity status. Medians of time to PFS will be shown with two-sided 95% CIs.

An exploratory landmark analysis will be performed and will include all patients who have not progressed (no PFS event) by 13 weeks and have available cf-DNA at baseline and week 13. Change in tumor purity from baseline to week 13 on therapy would be used as a binary variable (increase/stable tumor purity vs. decreased tumor purity) to assess its impact on subsequent PFS. The 13 week time point was chosen to allow for the first restaging scans (12 weeks) after initiation of therapy and allow for sufficient exposure to pembrolizumab considering prior studies in melanoma and NSCLC have demonstrated a median time to response of around 8-12 weeks.[58,59] Cox regression model will be employed to assess the association of PFS and change in tumor purity, with estimated hazard ratio (increase/stable tumor purity vs. decreased tumor purity) and corresponding 95% CI.

The proportion of patients with PSA response (≥ 50% decline from baseline) will be presented with a two-sided 95% CI using exact binominal method according to baseline genomic alternation (e.g., DDR mutations). Change in PSA will also be summarized descriptively according to the genomic alteration groups and the changes in PSA will also be compared between genomic alteration groups using the Wilcoxon rank sum tests

Other secondary objectives will be summarized descriptively.

13.12 Reporting and Exclusions

13.12.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment.

13.12.2 Evaluation of the Primary Efficacy Endpoint

There is no primary efficacy endpoint.

Analyses of secondary efficacy endpoints will be on an intent-to-treat basis. Specifically, all eligible participants included in the study must be assessed for response/outcome to therapy, even if there are major protocol therapy deviations.

Subanalyses may then be performed on the basis of a subset of participants,

excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding participants from the analysis should be clearly reported. If applicable to the endpoint, the 95% confidence intervals will be provided.

14. PUBLICATION PLAN

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

APPENDIX A: Performance Status Criteria

ECOG Performance Status Scale	Karnofsky Performance Scale

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

APPENDIX B: FACT-P (version 4)

Today's Date: _____ Participant Study ID: _____
Participant Name: _____ Cycle Number: _____

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some -what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some -what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my					

	illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box ☐ and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4

GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
C2	I am losing weight	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
P1	I have aches and pains that bother me	0	1	2	3	4
P2	I have certain parts of my body where I experience pain	0	1	2	3	4
P3	My pain keeps me from doing things I want to do	0	1	2	3	4
P4	I am satisfied with my present comfort level	0	1	2	3	4
P5	I am able to feel like a man	0	1	2	3	4

P6	I have trouble moving my bowels	0	1	2	3	4
P7	I have difficulty urinating	0	1	2	3	4
BL2	I urinate more frequently than usual	0	1	2	3	4
P8	My problems with urinating limit my activities	0	1	2	3	4
BL5	I am able to have and maintain an erection	0	1	2	3	4

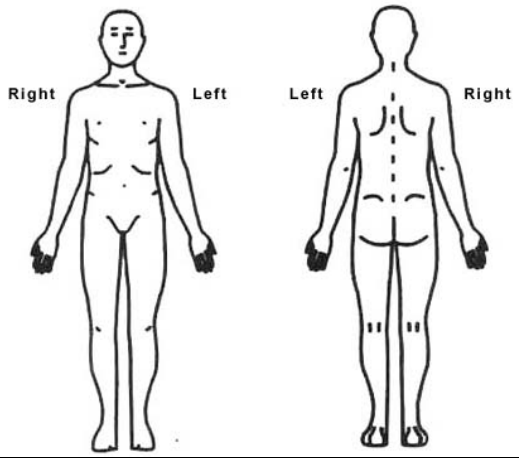
APPENDIX C: Brief Pain Inventory (Short Form)

Today's Date: _____Participant Study ID: _____
Participant Name: _____Cycle Number: _____

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

Yes No

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



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

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

4. Please rate your pain by circling the one number that best describes your pain at its LEAST in the past 24 hours.

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

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

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

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

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

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

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

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

Check box for N/A ☐

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

A. General activity:

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

B. Mood:

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

C. Walking ability:

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

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

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

E. Relations with other people:

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

F. Sleep:

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

G. Enjoyment of life:

0	1	2	3	4	5	6	7	8	9	10
Does not interfere										Completely interferes

APPENDIX D: Narcotic Analgesic Diary

Participant Name: _____ **Cycle Number:** _____

Study Site Staff: Write in pain medication names, routes, and strengths in the columns below. If the route is "other," specify the routine on the line after the "other" check box. List only one type and strength of medication on each row. Include all medications the participating is taking for pain or that may alleviate pain symptoms.

Study Participant: If on the day you complete this diary you start a new medication for pain or change the strength of the pills, write the new information on a new row. Do not change the information about the medication you have already taken. If the drug is given in some other way than orally or via patch, check "other" and specify how the drug is given on the line next to the "other" check box.

Pain Medication Use

Study Participant: Please complete this diary for the 24 hour period before your study clinic visit.

Record the number of units you have taken of each medication over the prior 24 hours.

DATE: _____ / _____ / _____
(mm/dd/yy)

Medication Name	Route (check one box)	Strength per Unit (example 10 mg per tablet)	Number of Units Taken
	□ Oral □ Patch □ Other		
	□ Oral □ Patch □ Other		
	□ Oral □ Patch □ Other		
	□ Oral □ Patch □ Other		
	□ Oral □ Patch □ Other		
	□ Oral □ Patch □ Other		

References:

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**(1): 9-29.
2. Parker C, Nilsson S, Heinrich D, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. *The New England journal of medicine* 2013; **369**(3): 213-23.
3. Pharmaceuticals BH. Xofigo Prescribing Information. 2013.
4. Corp MSD. Keytruda Prescribing Information. 2014.
5. Corp MSD. MK-3475 Investigators Brochure Edition 7. 2014.
6. Thompson RH, Dong H, Lohse CM, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2007; **13**(6): 1757-61.
7. May KF, Jr., Gulley JL, Drake CG, Dranoff G, Kantoff PW. Prostate cancer immunotherapy. *Clin Cancer Res* 2011; **17**(16): 5233-8.
8. Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol* 2014; **32**(10): 1020-30.
9. Thompson RH, Gillett MD, Cheville JC, et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. *Proc Natl Acad Sci U S A* 2004; **101**(49): 17174-9.
10. Latchman YE, Liang SC, Wu Y, et al. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. *Proc Natl Acad Sci U S A* 2004; **101**(29): 10691-6.
11. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and Activity of Anti-PD-L1 Antibody in Patients with Advanced Cancer. *The New England journal of medicine* 2012.
12. Barsoum IB, Smallwood CA, Siemens DR, Graham CH. A mechanism of hypoxia-mediated escape from adaptive immunity in cancer cells. *Cancer research* 2014; **74**(3): 665-74.
13. Frederick DT, Piris A, Cogdill AP, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* 2013; **19**(5): 1225-31.
14. Han SJ, Ahn BJ, Waldron JS, et al. Gamma interferon-mediated superinduction of B7-H1 in PTEN-deficient glioblastoma: a paradoxical mechanism of immune evasion. *Neuroreport* 2009; **20**(18): 1597-602.
15. Jiang X, Zhou J, Giobbie-Hurder A, Wargo J, Hodi FS. The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. *Clin Cancer Res* 2013; **19**(3): 598-609.
16. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med* 2007; **13**(1): 84-8.
17. Spranger S, Spaapen RM, Zha Y, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. *Sci Transl Med* 2013; **5**(200): 200ra116.
18. Wongkajornsilp A, Wamanuttajinda V, Kasetsinsombat K, et al. Sunitinib indirectly enhanced anti-tumor cytotoxicity of cytokine-induced killer cells and CD3(+)CD56(+) subset through the co-culturing dendritic cells. *PLoS One* 2013; **8**(11):

e78980.
19. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 2013; **369**(2): 134-44.
20. Patnaik A KS, Tolcher AW, Rasco DW, Papadopoulos KP, Beeram M, Drengler R, Chen C, Smith L, Perez C, Gergich K, Lehnert M Phase I study of MK-3475 (anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *J Clin Oncol 30, 2012 (suppl; abstr 2512)*

.
21. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment with Response to Anti-PD-1 Therapy. *Clin Cancer Res* 2014; **20**(19): 5064-74.
22. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015; **520**(7547): 373-7.
23. Scher HI, Halabi S, Tannock I, 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. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2008; **26**(7): 1148-59.
24. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. *The New England journal of medicine* 2015; **373**(8): 737-46.
25. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* 2015; **161**(5): 1215-28.
26. Lindahl T, Barnes DE. Repair of endogenous DNA damage. *Cold Spring Harb Symp Quant Biol* 2000; **65**: 127-33.
27. De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis* 2004; **19**(3): 169-85.
28. Wogan GN, Hecht SS, Felton JS, Conney AH, Loeb LA. Environmental and chemical carcinogenesis. *Semin Cancer Biol* 2004; **14**(6): 473-86.
29. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* 2001; **27**(3): 247-54.
30. Altieri F, Grillo C, Maceroni M, Chichiarelli S. DNA damage and repair: from molecular mechanisms to health implications. *Antioxid Redox Signal* 2008; **10**(5): 891-937.
31. Pritchard CC, Mateo J, Walsh MF, et al. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. *N Engl J Med* 2016; **375**(5): 443-53.
32. Annala M, Struss WJ, Warner EW, et al. Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer. *Eur Urol* 2017.
33. Matulonis UA, Penson RT, Domchek SM, et al. Olaparib monotherapy in patients with advanced relapsed ovarian cancer and a germline BRCA1/2 mutation: a multistudy analysis of response rates and safety. *Ann Oncol* 2016; **27**(6): 1013-9.
34. Mateo J, Carreira S, Sandhu S, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *N Engl J Med* 2015; **373**(18): 1697-708.
35. Steinberger AE, Cotogno P, Ledet EM, Lewis B, Sartor O. Exceptional Duration

of Radium-223 in Prostate Cancer With a BRCA2 Mutation. *Clin Genitourin Cancer* 2017; **15**(1): e69-e71.
36. Lee V, Murphy A, Le DT, Diaz LA, Jr. Mismatch Repair Deficiency and Response to Immune Checkpoint Blockade. *Oncologist* 2016; **21**(10): 1200-11.
37. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; **348**(6230): 124-8.
38. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 2015; **350**(6257): 207-11.
39. Carreira S, Romanel A, Goodall J, et al. Tumor clone dynamics in lethal prostate cancer. *Sci Transl Med* 2014; **6**(254): 254ra125.
40. Burrell RA, Swanton C. Tumour heterogeneity and the evolution of polyclonal drug resistance. *Mol Oncol* 2014; **8**(6): 1095-111.
41. Iwasa Y, Michor F. Evolutionary dynamics of intratumor heterogeneity. *PLoS One* 2011; **6**(3): e17866.
42. McGranahan N, Swanton C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell* 2015; **27**(1): 15-26.
43. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer* 2011; **11**(6): 426-37.
44. Boddy JL, Gal S, Malone PR, Harris AL, Wainscoat JS. Prospective study of quantitation of plasma DNA levels in the diagnosis of malignant versus benign prostate disease. *Clin Cancer Res* 2005; **11**(4): 1394-9.
45. Kienel A, Porres D, Heidenreich A, Pfister D. cfDNA as a Prognostic Marker of Response to Taxane Based Chemotherapy in Patients with Prostate Cancer. *J Urol* 2015; **194**(4): 966-71.
46. Salvi S, Casadio V, Conteduca V, et al. Circulating cell-free AR and CYP17A1 copy number variations may associate with outcome of metastatic castration-resistant prostate cancer patients treated with abiraterone. *Br J Cancer* 2015; **112**(10): 1717-24.
47. Frenel JS, Carreira S, Goodall J, et al. Serial Next-Generation Sequencing of Circulating Cell-Free DNA Evaluating Tumor Clone Response To Molecularly Targeted Drug Administration. *Clin Cancer Res* 2015; **21**(20): 4586-96.
48. Wyatt AW, Azad AA, Volik SV, et al. Genomic Alterations in Cell-Free DNA and Enzalutamide Resistance in Castration-Resistant Prostate Cancer. *JAMA Oncol* 2016; **2**(12): 1598-606.
49. Ha G, Roth A, Khattra J, et al. TITAN: inference of copy number architectures in clonal cell populations from tumor whole-genome sequence data. *Genome research* 2014; **24**(11): 1881-93.
50. Heitzer E, Ulz P, Geigl JB, Speicher MR. Non-invasive detection of genome-wide somatic copy number alterations by liquid biopsies. *Mol Oncol* 2016; **10**(3): 494-502.
51. Ulz P, Belic J, Graf R, et al. Whole-genome plasma sequencing reveals focal amplifications as a driving force in metastatic prostate cancer. *Nature communications* 2016; **7**: 12008.
52. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer* 2009; **45**(2): 228-47.
53. Bubley GJ, Carducci M, Dahut W, et al. Eligibility and response guidelines for

phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1999; **17**(11): 3461-7.
54. Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Science translational medicine* 2012; **4**(127): 127ra37.
55. Thompson RH, Kuntz SM, Leibovich BC, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. *Cancer research* 2006; **66**(7): 3381-5.
56. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive Strategies that are Mediated by Tumor Cells. *Annu Rev Immunol* 2006.
57. Romanel A, Gasi Tandefelt D, Conteduca V, et al. Plasma AR and abiraterone-resistant prostate cancer. *Sci Transl Med* 2015; **7**(312): 312re10.
58. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* 2015; **372**(26): 2521-32.
59. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387**(10027): 1540-50.