Original Study

Immune Analysis of Radium-223 in Patients With Metastatic Prostate Cancer

Joseph W. Kim,[1,2] Min Sun Shin,[1] Youna Kang,[1] Insoo Kang,[1] Daniel P. Petrylak[1,2]

Abstract

Radium-223 delivers high-energy radiation to osseous metastases. Its effect on the immune system is known. We observed a decrease in the mean frequency of programmed cell death protein 1-expressing cytotoxic T cells after 1 treatment of radium-223 in patients with prostate cancer. Further investigation is warranted to define the clinical significance of this finding and to elucidate the immunologic aspect of how radium-223 mediates its anti-tumor activity.

Background: Radium223 (Ra223) delivers high-energy radiation to osteoblastic metastasis of prostate cancer, resulting in irreparable double-stranded DNA damage. The effects of Ra223 on CD8+ T cell subsets in patients with prostate cancer is unknown. **Patients and Methods:** Fifteen men with metastatic prostate cancer with clinical indication for Ra223 without any autoimmune or immune deficiency conditions were enrolled. Patients received a course of Ra223 50 kBq/kg. Concurrent use of prednisone $\leq$ 10 mg a day was allowed. Peripheral blood samples were collected before and 3 to 4 weeks after the first dose of Ra223 50 kBq/kg. Peripheral blood mononuclear cells were purified and analyzed for the phenotypic and functional characteristics of $CD8^+$ T cells using flow cytometry. **Results:** One Ra223 treatment did not result in significant change in the overall frequencies of $CD8^+$ T cells and their subsets including naive, central memory, and effect memory cells. However, the mean frequency of programmed cell death protein 1-expressing EM $CD8^+$ T cells decreased after 1 Ra223 treatment from 20.6% to 14.6% ($P = .020$), whereas no significant change was observed in the frequencies of CD27-, CD28-, or CTLA4-expressing T cells. One Ra223 treatment was not associated with any significant change in the frequencies of $CD8^+$ T cells producing IFN-γ, TNF-α, and IL-13. **Conclusion:** One Ra223 treatment is associated with a decreased mean frequency of programmed cell death protein 1-expressing effect memory $CD8^+$ T cell without affecting other immune checkpoint molecules or cytokine production. Further investigations are warranted to elucidate the immunologic and clinical significance of our observations and its long-term effects after multiple treatments.

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Introduction

Radium-223 (Ra223) is an alpha-radiation emitting radioisotope approved in treatment of metastatic castration resistant prostate cancer (mCRPC). A phase III randomized, placebo-controlled study demonstrated that Ra223 improves survival in men with mCRPC by 3.6 months (median, 14.9 months vs. 11.3 months, in Ra223 treated group vs. placebo group, respectively) with a hazard ratio of death, 0.70 (95% confidence interval, 0.58-0.83; $P < .001$), and delays time to first skeletal event by 5.8 months (hazard ratio, 0.66; 95% confidence interval, 0.52-0.83; $P = .00037$) with a favorable safety profile.[1,2] Ra223, as a calcium mimetic, localizes to osteoblastic metastases of prostate cancer, and emits alpha particles, leaving irreparable double-stranded DNA damage and eventual cell death.

In addition to its conventional cytotoxic effect via damaging DNA, radiation induces immunogenic cell death by affecting various compartments of the immune system.[3,4] Preclinical studies have shown that radiation upregulates the expression of antigen-presenting MHC molecules and affects cytokine production and various subpopulations of immune cells as well as stromal cells.[5-8] A

I.K. and D.P.P. have contributed equally to this article as senior authors.

[1]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT
[2]Prostate and Urological Cancers Program, Yale Comprehensive Cancer Center, New Haven, CT

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Address for correspondence: Joseph W. Kim, MD, 333 Cedar St, New Haven, CT 06520
E-mail contact: joseph.w.kim@yale.edu

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beta particle-emitting radioisotope, Samarium-153-EDTMP (Sm-153), has been shown to induce immunogenic modulation by altering the phenotype of tumor cells such that they are more susceptible to T-cell mediated killing.[7] A randomized phase II study of Sm-153 in combination with a prostate-specific antigen-targeted vaccine, versus Sm-153 alone, showed a trend toward improvement in the 4-month progression-free rate (23.8% vs. 11.1%; $P = .27$), and an improvement in median progression-free survival of 3.7 months versus 1.7 months ($P = .046$).[9] In addition, several other clinical trials incorporated external beam radiation therapy, in combination with an immune-based therapy to capitalize on its immune modulating effects.[10-13]

The effect of Ra223 on human immune systems is unknown. We hypothesized that Ra223 would affect the CD8+ T cell and its subsets in patients with metastatic prostate cancer. We investigated the immunologic effect of Ra223 in patients with mCRPC by examining the phenotypic and functional characteristics of $CD8^+$ T cells that include the expression of co-stimulatory, co-inhibitory molecules, and cytokines.

Patients and Methods

Patient Population

A total of 15 men with castration-resistant prostate cancer with osseous metastases with clinical indication for Ra223 were enrolled. Patients with active autoimmune disease requiring therapy, or with known immunodeficiency syndrome were excluded. Patients who had been on low-dose corticosteroids (≤ 10 mg prednisone daily) were allowed. Patients received a course of Ra223 50 kBq/kg intravenously every 4 weeks as a standard practice. Table 1 shows clinical characteristics of the study subjects. Research blood was collected at 2 time points: (1) before and (2) 3 to 4 weeks after the first dose of Ra223 50 kBq/kg.

Purification and Stimulation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMCs were isolated from heparinized peripheral venous blood using Ficoll-Hypaque gradient (GE Healthcare, Piscataway, NJ). PBMCs were washed with phosphate buffered saline (PBS) and resuspended in RPMI 1640 media supplemented with 10% fetal calf serum and 1% glutamine/penicillin/streptomycin. Cells were stimulated for 4 hours with PBS (control) or PMA (50 ng/ml; Sigma, St. Louis, MO) and ionomycin (1 μg/ml; Sigma) in the presence of Golgiplug (BD Biosciences, San Jose, CA) in a tissue culture incubator at 37°C as previously done.[14-16]

Table 1 Baseline Patient Characteristics

Patient Characteristics	Median (Range) N = 12
Age, y	72 (55-85)
PSA at entry, ng/mL	78 (10.6-808)
Number of prior therapies for mCRPC	3 (1-4)
Prior chemotherapy	
Yes	8
No	4
Concurrent use of prednisone	
Yes	4
No	8

Abbreviations: mCRPC = metastatic castration-resistant prostate cancer; PSA = prostate-specific antigen.

Flow Cytometry

Fresh PBMCs were stained with anti-CD3-APC-Cy7, CD8-APC, CD45RA-PE-Cy5, CCR7-PE-Cy7, PD-1-Pacific Blue, IL-7Rα-FITC, CD27-PE, CD28-PE, CTLA4-PE, CX3CR1-PE antibodies, or isotype control (all from BioLegend, San Diego, CA). PBMCs that had been stimulated with PMA/ionomycin were stained with anti-CD3-APC-Cy7, CD8-APC, CD45RA-PE-Cy5, and CCR7-PE-Cy7 antibodies followed by fixation, permeabilization (Cytofix/Cytoperm Kit, BD Biosciences), and staining with anti-IFNγ-PE, TNF-α-FITC, or IL-13-PE antibodies (BioLegend).[14] Stained cells were analyzed on an LSRII flow cytometer (BD Biosciences). Collected data were analyzed using FlowJo software (Tree Star, Ashland, OR).

Statistical Analysis

Statistical analysis was done using the GraphPad Prism 6 (GraphPad Software, La Jolla, CA). *P* values less than .05 were considered statistically significant.

Results

The Frequency of PD-1-Expressing Effector Memory (EM) $CD8^+$ T Cells Decreased in Patients With mCRPC After a Treatment of Ra223

To determine the possible effect of Ra223 on the expression of co-stimulatory and -inhibitory molecules by $CD8^+$ T cells in patients with mCRPC, we analyzed the expression CD27, CD28, PD-1, and CTLA-4 on different subsets of $CD8^+$ T cells in these patients before and after Ra223 administration. Based on the expression of the T cell receptor co-receptor CD45RA and lymphoid tissue homing chemokine receptor CCR7, we identified naive ($CD45RA^+CCR7^+$), central memory (CM) ($CD45RA^-CCR7^+$), and EM ($CD45RA^{+/-}CCR7^-$) $CD8^+$ T cells in peripheral blood (Figure 1A). The overall frequency of lymphocytes, total $CD8^+$ T cells, naive, CM, and EM $CD8^+$ T cells did not change after Ra223 treatment (Table 2). Most naive and CM $CD8^+$ T cells expressed CD27 and CD28 but not PD-1 (data not shown). In EM $CD8^+$ T cells, some cells expressed CD27, CD28, and/or PD-1 (Figure 1B). The frequency of PD-1^+ EM $CD8^+$ T cells decreased after Ra223 treatment (mean frequency [%] ± standard error of the mean [SEM], 20.6 ± 2.13 vs. 14.6 ± 2.37; $P = .020$), whereas the frequency of $CD27^+$ and $CD28^+$ EM $CD8^+$ T cells remained unchanged (Figure 1C). The individual data showing the changes of PD-1-expressing CD8+ T cells are shown in Supplemental Figure 1 in the online version. CTLA-4-expressing naive, CM, and EM $CD8^+$ T cells were hardly detected in most samples (data not shown). In naive and CM $CD8^+$ T cells, most cells continued to express CD27 and CD28 at similar levels after Ra223 (mean frequency [%] ± SEM, naive CD27, 96 ± 1.53 vs. 94.8 ± 1.76; naive CD28, 95.0 ± 1.74 vs. 93.0 ± 2.11; CM CD27, 89.0 ± 1.64 vs. 86.3 ± 2.51; CM CD28, 98.0 ± 0.94 vs. 95.1 ± 1.95; $P > .05$ for all).

A Decrease in the Frequency of PD-1 Expression Was Also Observed Among IL-7Rα^{high} EM $CD8^+$ T Cells

The cytokine IL-7 and its receptor IL-7 receptor alpha (IL-7Rα) play a major role in the generation and maintenance of memory T