Immune-Checkpoint Blockade Enhances ^{225}Ac-PSMA617 Efficacy in a Mouse Model of Prostate Cancer

Johannes Czernin, Kyle Current, Christine E. Mona, Lea Nyiranshuti, Firas Hikmat, Caius G. Radu, and Katharina Lückerath

Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, UCLA, Los Angeles, California

Prostate-specific membrane antigen (PSMA)–targeted radionuclide therapy (RNT) may increase tumor immunogenicity. We aimed at exploiting this effect by combining RNT with immunotherapy in a mouse model of prostate cancer (PC). **Methods:** C57BL/6-mice bearing syngeneic RM1-PGLS tumors were treated with ^{225}Ac-PSMA617, an anti-PD-1 antibody, or both. Therapeutic efficacy was assessed by tumor volume measurements (CT), time to progression (TTP), and survival. **Results:** PSMA RNT or anti-PD-1 alone tended to prolong TTP (isotype control, 25 d; anti-PD-1, 33.5 d [$P = 0.0153$]; RNT, 30 d [$P = 0.1038$]) and survival (control, 28 d; anti-PD-1, 37 d [$P = 0.0098$]; RNT, 32 d [$P = 0.1018$]). Combining PSMA RNT and anti-PD-1 significantly improved disease control compared with either monotherapy. TTP was extended to 47.5 d ($P \leq 0.0199$ vs. monotherapies), and survival to 51.5 d ($P \leq 0.0251$ vs. monotherapies). **Conclusion:** PSMA RNT and PD-1 blockade synergistically improve therapeutic outcomes in our PC model, supporting the evaluation of RNT and immunotherapy combinations for PC patients.

Key Words: ^{225}Ac-PSMA; immunotherapy; prostate cancer; PD-1; syngeneic mouse model

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Prostate-specific membrane antigen (PSMA)–targeted radionuclide therapy (RNT) is effective but not curative in approximately 50% of patients with metastatic castration-resistant prostate cancer (mCRPC). However, PSMA RNT may increase PC immunogenicity; this might be exploited by combining RNT with immunotherapy to improve both RNT and immunotherapy outcomes.

Immunotherapies have been used successfully against several cancer types (*1,2*). Two immunotherapies were approved by the Food and Drug Administration for PC. The cancer vaccine sipuleucel-T (Provenge; Dendreon Pharmaceuticals) improves overall survival by 4 mo in patients with nonsymptomatic or minimally symptomatic mCRPC but neither lowers prostate-specific antigen levels nor improves radiographic or progression-free survival (*3*). PD-1 immune-checkpoint blockade (pembrolizumab) was approved for the approximately 5% of mCRPC patients presenting with mismatch-repair gene defects or microsatellite instability (*4*). In the Keynote-199 trial (NCT02787005), modest objective response rates to pembrolizumab (3%–5%) were observed in genetically unselected PC patients (*5*). Overall, the efficacy of immunotherapies in PC, especially when applied as monotherapy, has been limited. This lack of efficacy has been attributed to the low immunogenicity of PC that might result from a low mutational burden, impaired T cell activation, and an immunosuppressive tumor microenvironment that limits cytotoxic but increases regulatory T cell infiltration and activation (*6–10*).

The cytotoxicity of ionizing radiation may enhance tumor immunogenicity by inducing immunogenic cell death; this, in turn, can lead to the release of tumor-associated antigens and to an inflammatory phenotype (*1,11–13*). Indeed, several clinical trials explore the combination of radiotherapy with immunotherapy in PC (*11*). However, little is known about the immunogenicity induced by RNT.

Here, we demonstrate synergy between PSMA RNT and PD-1 blockade in a syngeneic PC mouse model.

MATERIALS AND METHODS

Cell Culture

RM1-PGLS cells were a gift from Michel Sadelain (Memorial Sloan Kettering Cancer Center). Parental RM1 cells were derived by transduction of mouse prostate cells with *ras* and *myc* oncogenes (*14*); transduction of parental RM1 cells with human PSMA and SFG-Egfp/Luc yielded RM1-PGLS (*15*). This cell line represents CRPC. Cells were maintained in Rosewell Park Memorial Institute-1640 with 5% fetal bovine serum (Omega Scientific) at 37°C, 5% CO_2. *Mycoplasma* contamination was excluded using the Venor GeM *Mycoplasma* detection kit (Sigma-Aldrich).

Mice

Animal studies were approved by the UCLA Animal Research Committee (approval 2005-090). Male 6- to 8-wk-old C57BL/6 mice (Department of Radiation Oncology, UCLA) were housed under pathogen-free conditions (12-h/12-h light/dark cycle; food and water ad libitum). Animal welfare was ensured daily by vivarium staff and investigators.

^{225}Ac-PSMA617

^{225}Ac was supplied by the U.S. Department of Energy Isotope Program, Office of Science for Nuclear Physics. ^{225}Ac-PSMA617 was synthesized as previously described (*16*) with more than 92% purity by radio thin-layer chromatography at 130 MBq/μmol (UCLA Biomedical Cyclotron Facility).

CT

Tumor volumes were monitored by CT. Scans were analyzed using OsiriX (version 10.0.2; Pixmeo SARL) (*16*).

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For correspondence or reprints contact: Katharina Lückerath, UCLA, 650 Charles E. Young Dr. S., Los Angeles, CA 90095.
E-mail: klueckerath@mednet.ucla.edu
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Flow Cytometry

PSMA expression was verified using an anti–hPSMA-APC antibody (5 μL/test, REA408; Miltenyi) (Fig. 1A) (*16*). Interferon-γ–induced (10 ng/mL) or radiation-induced (x-ray, 10 Gy) PD-L1 expression was determined 24 h after treatment using an anti–mPD-L1-PE antibody (1:20, clone 10F.9G2; Biolegend). Samples were measured on an LSRII flow cytometer (BD) and analyzed using FlowJo (Three Star).

Therapy Study

RM1-PGLS cells (0.1×10^6 cells in 50 μL of phosphate-buffered saline plus 50 μL of Matrigel) were subcutaneously inoculated into the shoulder region of mice. When tumors reached 97 ± 34 mm^3 (day 11), the mice were randomized on the basis of tumor volumes into 4 groups (8–9 mice per group). Group 1 comprised the rat-IgG2a isotype control (clone 2A3, BE0089; bxcell); group 2, anti-PD-1 (clone RMP1-14, BE0146; bxcell); group 3, ^{225}Ac-PSMA617; and group 4, ^{225}Ac-PSMA617 plus anti-PD-1. The mice were treated with 30 kBq of ^{225}Ac-PSMA617 (intravenously) on day 12 and with anti-PD-1 or isotype control (10 mg/kg in phosphate-buffered saline, intraperitoneally) on days 13, 16, 20, and 23. Tumor volumes and body weights were measured twice weekly until the tumors reached ≥ 3 cm^3 or the mice reached a humane endpoint.

Statistics

Data were analyzed by investigators who were masked to the interventions. Data are expressed as mean ± SD. Statistical significance was determined using 1-way ANOVA with Tukey correction and set to a P value of less than 0.05. Time to progression (TTP) to 1.5 cm^3 tumor volume and survival were analyzed using the log-rank test. Prism (version 8; GraphPad Software) was used for all statistical calculations.

RESULTS

Induction of PD-L1 Expression on RM1-PGLS Cells by IFN-γ

As a prerequisite for investigating the efficacy of anti-PD-1 in vivo, we tested the responsiveness of RM1-PGLS cells to IFN-γ. IFN-γ is released by activated T cells and can induce a reciprocal upregulation of PD-L1 expression on tumors cells to facilitate immune evasion (*17*). Treatment of RM1-PGLS with IFN-γ resulted in a 5.4 ± 2.3-fold increase in PD-L1 expression ($P = 0.0056$; Fig. 1B). This finding indicates that tumor control in vivo might be enhanced by PD-1 blockade.

Similarly, radiation-induced PD-L1 expression may contribute to immune suppression and radioresistance (*18*). In our model, PD-L1 expression did not significantly increase in response to irradiation (1.6 ± 0.02-fold; $P = 0.8010$) (Fig. 1C).

Efficacy of ^{225}Ac-PSMA617 and PD-1 Blockade

We investigated whether PD-1 blockade is effective in our model and whether combining ^{225}Ac-PSMA617 and PD-1 blockade improves therapy responses. Both anti-PD-1 and RNT monotherapy tended to enhance tumor control. Median TTP increased from 25 d (control) to 33.5 d (anti-PD-1; $P = 0.0153$) and 30 d (RNT; $P = 0.1038$). Survival tended to improve from 28 d (control) to 37 d (anti-PD-1; $P = 0.0098$) and 32 d (RNT; $P = 0.1018$) (Figs. 2A, 2B, 2D, and 2E).

Combining RNT and anti-PD-1 synergistically improved therapeutic efficacy. Median TTP and survival were 47.5 d ($P \leq 0.0199$ vs. monotherapies) and 51.5 d ($P \leq 0.0251$ vs. monotherapies), respectively, in the combination therapy group (Figs. 2A, 2B, 2F, and 2G). In addition, 2 of 8 mice in the RNT and anti-PD-1 group had stable disease and remained alive at the end of the observation period; all mice in the monotherapy groups, and 6 of 8 mice in the RNT and anti-PD-1 group, exhibited tumor growth delay. None of the mice exhibited signs of toxicity (e.g., weight loss) or had to be euthanized prematurely (Fig. 2C).

DISCUSSION

To our knowledge, this is the first report demonstrating synergistic antitumor efficacy between PSMA-targeted RNT and PD-1 blockade in a syngeneic PC mouse model.

The immunologic consequences of RNT have not been well documented. In NCI-H727 neuroendocrine tumors in NMRI mice (deficient in T and mature B cells), increased numbers of tumor-infiltrating antigen-presenting and natural killer cells after ^{177}Lu-DOTATATE were observed (*19*). Treatment of a syngeneic lymphoma mouse model with ^{90}Y-NM600 (targeting alkylphosphocholine) increased CD8-positive T cell infiltration and ratios of CD8-positive T cells to regulatory T cells and generated tumor-specific immunologic memory (*20*). Two studies demonstrated improved tumor control with RNT and immune-checkpoint blockade in MC38 colorectal cancer (integrin $\alpha_v\beta_3$–targeted ^{177}Lu-EB-RGD plus anti-PD-L1) (*21*) and B16F10 melanoma (very late antigen 4–targeted ^{177}Lu-LLP2A plus anti-CTLA-4 plus anti-PD-1 or anti-PD-L1) (*22*). The present study is in line with these findings and supports the exploration of RNT and immunotherapy combinations for the treatment of advanced PC.

The anti-PC synergy between PSMA RNT and PD-1 blockade might be explained by mechanisms similar to those observed when external-beam radiotherapy was combined with PD-1 blockade. On the basis of these observations, RNT might cause immunogenic cell death leading to the release of tumor-associated antigens and the enhancement of T cell diversity, priming, and activation (*23,24*). Because RNT delivers radiation to all PSMA-positive metastases, it would rely less than radiotherapy on abscopal effects for launching successful

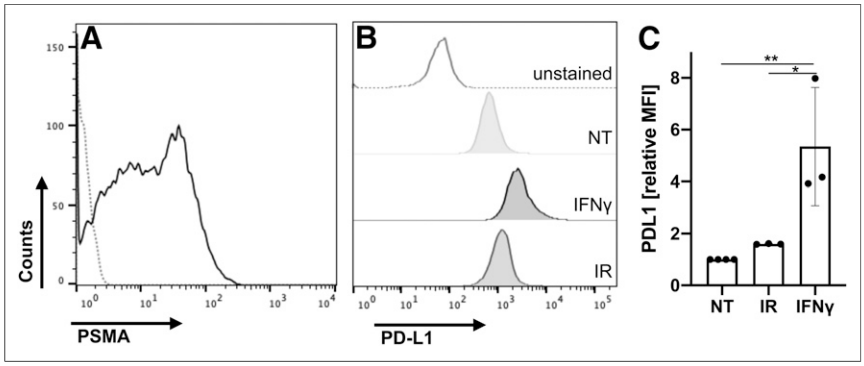

FIGURE 1. IFN-γ induces PD-L1 expression on RM1-PGLS cells. (A) PSMA expression. (B) Basal (NT), IFN-γ–, and radiation (IR)–induced PD-L1 expression. One of 3 representative experiments is shown. (C) Quantification of PD-L1 expression shown in B ($n = 3$). Columns represent mean values, bars represent SD, and individual values are shown as dots. Relative MFI = mean fluorescent intensity normalized to NT.

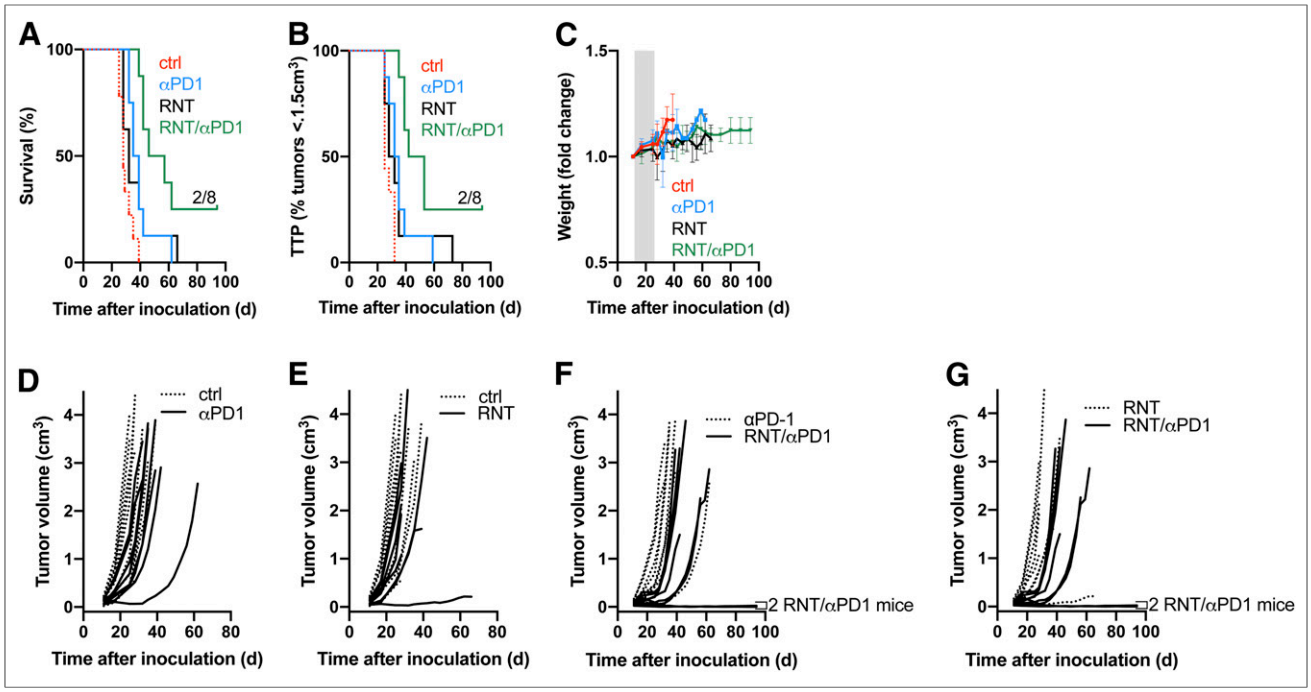

FIGURE 2. Synergy between ^{225}Ac-PSMA617 and anti-PD-1. (A) Survival: control, 28 d; anti-PD1, 37 d (P = 0.0098 vs. control); RNT, 32 d (P = 0.1018 vs. control); RNT and anti-PD-1, 51.5 d ($P \leq 0.0251$ vs. monotherapies). (B) TTP to half-maximal tumor volume: control, 25 d; anti-PD1, 33.5 d (P = 0.0153 vs. control); RNT, 30 d (P = 0.1038 vs. control); RNT and anti-PD-1, 47.5 d ($P \leq 0.0199$ vs. monotherapies). (C) Body weights (mean ± SD). Shaded area indicates treatment duration. (D–G) Tumor volumes for individual mice (8–9 mice per group). Two mice in RNT and anti-PD-1 group had stable disease and remained alive at end of observation period.

systemic antitumor immune responses (*23*). Blockade of PD-1 and PD-L1 signaling may prevent exhaustion of tumor-infiltrating cytotoxic T cells and their conversion to immunosuppressive regulatory T cells. In addition, PD-1 blockade might counteract the upregulation of PD-L1 expression that has been observed after radiation-induced activation of ATR, a key effector kinase in the DNA damage and replication stress response (*18*). In future studies, we will investigate the exact mechanisms underlying PSMA RNT-induced immunogenicity and the synergy between RNT and PD-1 blockade in PC.

The RNT and immunotherapy regimen was effective but not curative in the current PC mouse model. One explanation might be activation of immunosuppressive mechanisms beyond the reactive PD-L1 upregulation on PC tumors exploited in the current study. These include mechanisms mediated by the release of intracellular adenosine triphosphate from dying cells (*25*). Another reason for the noncurative outcome might be the pretreatment tumor volume (97 ± 34 mm^3), which might negatively correlate with immune cell infiltrates (*26*). Clinically, the intra- and interpatient spatial and temporal heterogeneity of PC might prove to be an additional challenge for generating curative outcomes after RNT and immunotherapy.

We studied the efficacy of immune-checkpoint blockade combined with RNT in a single PC mouse model that is based on murine hPSMA-overexpressing PC cells with basal and IFN-γ–induced PD-L1 expression. A large fraction of PC is immune-desert or immune-excluded, 2 phenotypes associated with resistance to PD-1 and PD-L1 blockade and reduced IFN-γ signaling (*1*). However, the presence of an IFN-γ–responsive gene signature has recently been reported in 9 mCRPC patients responding to ipilimumab but not in 10 nonresponders (*27*). In line with this study, the Keynote-199 trial concluded that anti-PD1 therapy is effective in some mCRPC patients, while highlighting the need to identify predictive biomarkers (*5*). Therefore, RM1-PGLS cells present a relevant subset of patients with mCRPC. Moreover, RNT might change the immune phenotype of PC to an inflammatory one in which immune-checkpoint blockade is likely effective. The recent registration of 2 clinical trials (NCT03658447 and NCT03805594) testing ^{177}Lu-PSMA617 plus pembrolizumab in genetically unselected patients with mCRPC underscores the high interest in combining RNT with immunotherapy. Therefore, and despite investigating only 1 mouse model, the current study is timely and strongly supports exploration of RNT and immunotherapy combinations for PC patients.

CONCLUSION

Combining PSMA RNT and PD-1 blockade synergistically reduces tumor burden and improves TTP and survival. Although the mechanistic explanation for this synergy has to be elucidated in future studies, RNT might support the conversion of PC from an immunologic cold tumor to a hot tumor. Thus, RNT and immunotherapy combinations represent promising therapeutic options even for those PC patients without microsatellite instability or mismatch-repair deficiencies and with heterogeneous PSMA expression (*16*).

DISCLOSURE

This study was partially funded by the Prostate Cancer Foundation (19CHAL09). Johannes Czernin and Caius Radu are cofounders and hold equity in Sofie Biosciences, Trethera Therapeutics. Intellectual property has been patented by UCLA and licensed to Sofie Biosciences, Trethera Therapeutics, but was not used in the current

study. No other potential conflict of interest relevant to this article was reported.

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KEY POINTS

QUESTION: Does ^{225}Ac-PSMA617 RNT synergize with PD-1 blockade in a mouse model of PC?

PERTINENT FINDINGS: In this therapeutic efficacy study, PSMA RNT or anti-PD-1 alone tended to prolong TTP and survival. Combining PSMA RNT and anti-PD-1 synergistically improved disease control and survival compared with either monotherapy.

IMPLICATIONS FOR PATIENT CARE: Our data suggest that RNT can promote PC immunogenicity and strongly support exploration of RNT and immunotherapy combinations for PC patients.

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