

DIFFUSING ALPHA-EMITTERS RADIATION THERAPY PROMOTES A PRO-IMMUNOGENIC TUMOR MICROENVIRONMENT AND SYNERGIZES WITH PD-1 BLOCKADE

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Background Diffusing alpha emitters radiation therapy (DaRT) utilizes Ra-224 loaded seeds that continuously release alpha-emitting atoms inside the tumor.¹ The treatment effectively ablates many types of human and mice xenografts² and shows 100% response rates in patients with recurrent and locally advanced squamous cell carcinoma (SCC) of the skin and head and neck.³ DaRT was previously shown to trigger specific- and systemic- antitumor immune responses in mice, that synergize with immunomodulation and immune stimulation.⁴⁻⁶ Nevertheless, the gene expression profile induced by DaRT treatment and its local and systemic immune response in combination with immune checkpoint inhibition by programmed cell death protein 1 (PD-1) blockade were not yet investigated.

Methods Balb/c mice bearing intracutaneous SCC murine tumors (SQ2 cells) were treated with an inert seed, a Ra-224 loaded DaRT seed, aPD-1or DaRT+aPD-1. Anti-PD-1or an isotype control was administered intraperitoneally at the dose of 10 mg/kg. Flow Cytometry Analysis (FACS) of dendritic cells, myeloid-derived suppressor cells (MDSCs) and lymphocytes was performed in tumors and spleens 16 days after DaRT/ inert seed insertion. Furthermore, tumors were subjected to IHC analysis for the detection of tumor infiltrating lymphocytes (TILs). In addition, similar FACS analysis and transcriptional profiling of immune-related target genes in tumor tissues were characterized using the Nanostring technology seven days post-DaRT seed insertion. Combination of DaRT with aPD-1 was also tested for retardation of tumor growth.

Results DaRT in combination with aPD-1 delayed tumor development, induced T lymphocytes infiltration and granzyme B secretion, and reduced systemic MDSCs more efficiently than each of the monotherapies. Gene expression and gene set enrichment analysis of mRNA levels 7 days after DaRT insertion indicated that DaRT upregulated apoptosis, p53 signalling, interferon signalling and myeloid related transcription, while downregulating DNA repair, cell proliferation and notch related transcription. Immunophenotyping analysis at this time-point showed that DaRT induced dendritic cell activation and affected the distribution of myeloid-derived suppressor cells populations.

Conclusions DaRT exerts a synergistic effect with aPD-1 in inhibiting tumor growth in a murine model of SCC and promotes a pro-immunogenic state both locally and systemically. DaRT potentiates not only a higher T-effector cells infiltration but also their cytotoxic potential under checkpoint molecule blockade. Therefore, the current study provides a rationale for a potential combination treatment in SCC patients.

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