1118 THE IODINATED FLUORESCEIN DERIVATIVE PV-10 ENHANCES THE ANTIVIRAL ACTIVITY OF CD8+ T-CELLS BY INDUCING STING DIMERIZATION: IMPLICATIONS FOR ENHANCED VACCINE APPLICATIONS

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Background Rose Bengal sodium (PV-10) has recently garnered attention for its anti-tumor cytotoxicity, safety profile, and efficacy in several cancer types. In a murine model of melanoma, PV-10 administration led to regression of untreated lesions and distant lung metastases. In the melanoma model, PV-10 treatment significantly enhanced CD8+ T-cell response, increased TNF-α production by CD8+ T cells, and improved overall survival. While these results demonstrate the potential of PV-10 as a vaccine adjuvant, significant knowledge gaps exist regarding the underlying mechanism of action behind this phenomenon. In this study, we investigate the involvement of the stimulator of interferon (IFN) genes (STING), a central innate immunity pathway, in PV-10-mediated immune activation.

Methods PV-10 (10% solution of Rose Bengal sodium in 0.9% saline) was provided by Provectus Biopharmaceuticals, Inc. and stored at room temperature in light-proof containers. PV-10-mediated STING induction and dimerization were assessed using THP-1 human leukemia monocytic cells. CD8+ T-cells and dendritic cells (DCs) were cultured in the presence of cytokines, as described previously. Cytotoxicity analyses were performed by Alamar Blue assays. STING dimerization was determined by western blot and mass spectroscopy analysis. CD8+ T-cells were stimulated with synthetic peptides representing hepatitis B surface antigen (HBsAg) and T-cell response in primed CD8+ T-cell and HBsAg-expressing PLC/PRF/5 hepatoma cell co-culture was analyzed by ELISpot assay.

Results THP-1 cells treated with 100 mM PV-10 showed STING dimerization with a 70 kDa band, in addition to the normal 35 kDa monomeric band. Additionally, pro-inflammatory cytokines and chemokines were significantly upregulated with longer PV-10 incubation times compared to untreated controls, including MCP-1, IP-10, VEGF-a, IL-18, IL-6, IL-8, and GM-CSF. A measurable increase in IFN-γ was also observed, altogether indicating that PV-10 treatment induces STING activation. Importantly, PV-10 treatment also significantly increased the number of IFN-γ ELISpots produced by HBsAg-primed CD8+ T-cells when exposed to the antigen-presenting PLC/PRF/5 cells.

Conclusions This report demonstrates, for the first time, the ability of PV-10 to function as an effective agent to enhance targeted immune therapeutics, including antiviral vaccines. Our studies show its unique modulation of the STING pathway as a potential mechanism for this activity. We discuss, in detail, the experimental findings and the development of PV-10 for future clinical studies.

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Ethics Approval All study specimens were collected following informed consent and approval by the Health Research Ethics Board of Alberta (HREBA) (Ethics ID: HREBA.CC-16–0286).

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