PH 894: AN INTASYL SELF-DELIVERING SIRNA TARGETING BRD4 HAS DUAL FUNCTIONS TO SENSITIZE TUMOR CELL KILLING AND ACTIVATE CD8 T CELLS

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Background Bromodomain containing proteins such as BRD4 play critical roles during cancer development and progression. BRD4 regulates oncogenes such as MYC and contributes to escape from immunosurveillance by decreasing tumor cell immunogenicity. Therefore, BRD4 is an attractive target for cancer therapy. Current clinical studies have focused on small molecule inhibitors of BRD4, however, these are not selective for BRD4 but inhibit other BRD proteins and are associated with toxicity and development of resistance. PH-894 is a self-delivering RNAi compound that specifically silences the BRD4 gene. We previously demonstrated potent antitumor activity of PH-894 in syngeneic mouse tumor models. Here we use B16ova melanoma cells which express ovalbumin peptide (OVA) and OT-1 T cells which recognize OVA to further explore the antitumor mechanism of PH-894.

Methods OT-1 T cells were stimulated with irradiated EG7 cells, followed by treatment with either PH-894 or a chemically matched non-targeting RNAi control (NTC). Seventy-two hours post-treatment, the levels of secreted IFN-γ were assayed by ELISA and BRD4 protein expression determined by flow cytometry. B16ova tumor cells were pre-treated with IFN-γ for 18 hours, followed by treatment with PH-894 or NTC for 3 days, and BRD4 protein expression assessed. To investigate whether PH-894 pretreatment could sensitize the killing of B16ova cells by OT-1 T cells, B16-OVA cells treated with IFN-γ were pre-treated with PH-894 or NTC-647 for 3 days. The treated B16ova cells were cocultured with anti-CD3-activated OT-1 CD8+ T cells for 20 hours. OT-1 T cells were removed and B16ova cell viability was measured using CellTiter-Glo. Specific killing activity was calculated by normalizing luminescence in wells with cocultured B16ova/OT-1 T cells to those with B16ova cells only.

Results The expression of BRD4 was downregulated by PH-894 in both IFN-γ treated B16ova tumor cells and EG7 activated OT-1 T cells. Treatment with PH-894 resulted in a significant increase of IFN-γ production by the OT-1 cells. PH-894 pretreatment of the B16ova tumor cells significantly increased cell killing activity of the tumor cells by activated OT-1 T cells (90.8% killing activity with PH-894-treated B16ova cells compared to 68% in untreated cells).

Conclusions We demonstrate that PH-894 potently activates CD8+ T cells, and PH-894 pretreatment of tumor cells can sensitize tumor cell killing by CD8+ T cells. These data provide additional mechanistic insight and support further development and clinical investigation of PH-894 as a new class of antitumor therapeutic.