PH-894, an INTASYL self-delivering siRNA targeting BRD4 has dual functions to sensitize tumor cell killing and activate CD8+ T cells

Abstract: 1402

Shenghua Zhou, Benjamin Cuiffo, Melissa Maxwell, Dingxue Yan, Brianna Rivest, Andrew Boone, James Cardia and Simon P. Fricker

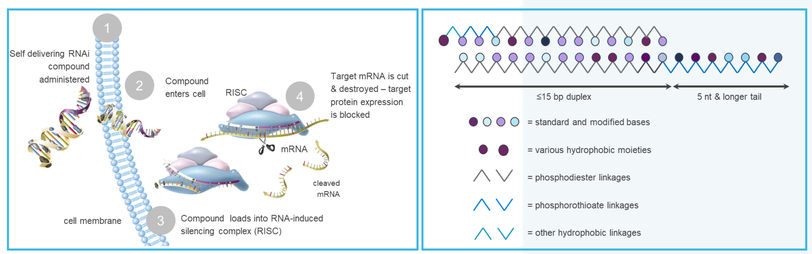
SITC 2022 NOVEMBER 8-12 BOSTON MASSACHUSETTS

Abstract

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[1,2]. 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.

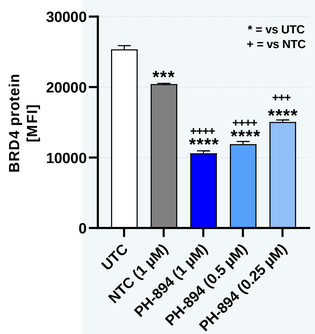
We demonstrate that PH-894 potently activates CD8+ T cells, and PH-894 pretreatment of tumor cells can sensitize tumor cell killing by CD8+ T cells. These data provide additional mechanistic insight and support further development and clinical investigation of PH-894 as a new class of antitumor therapeutic.

INTASYL™ mechanism of silencing and structure



PH-894 downregulates expression of BRD4 protein in B16-OVA cells

Figure 1. B16-OVA cells were treated with PH-894 or a non-targeting RNAi control (NTC) for 72h. The expression of BRD4 was determined using anti-BRD4 antibody (intracellular staining) and flow cytometric analysis. UTC: untreated control. Results are shown as mean fluorescence intensity (MFI). Means ± SEM (n = 2) are shown. Means were compared to UTC (*) and NTC (+) by one way ANOVA and Tukey's test. **** or ++++ p<0.0001.



PH-894 silences the expression of BRD4 in OT-1 T cells

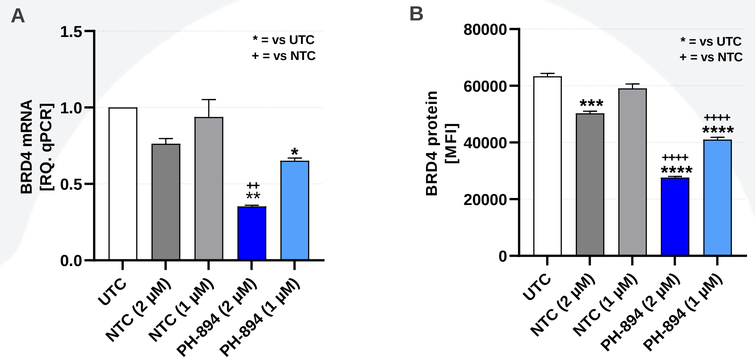


Figure 2. To mimic the tumor microenvironment where T cells are chronically interacting with tumor cells, OT-1 T cells were repeatedly stimulated with irradiated E.G7-OVA lymphoma cells, followed by treating with PH-894 or a non-targeting RNAi control (NTC) for 3 days. The expression level of BRD4 mRNA was determined by RT-qPCR (A) and the level of BRD4 protein was determined by intracellular staining with anti-BRD4 antibody and flow cytometry analysis (B). Means ± SEM (n = 2) are shown. Means were compared to UTC (*) and NTC (+) by one way ANOVA and Tukey's test. ** or ++ p<0.002; * or + p< 0.01; **** or ++++ p<0.0001.

Silencing of BRD4 increases IFN-γ production in OT-1 T cells

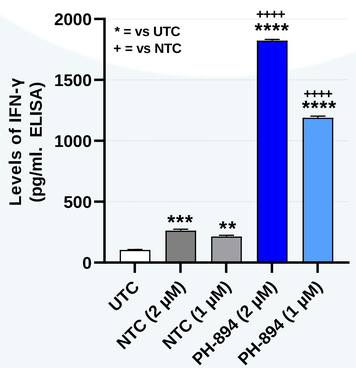
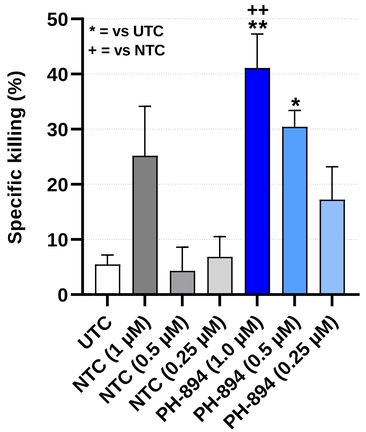


Figure 3. OT-1 T cells were repeatedly stimulated with irradiated E.G7-OVA cells, followed by treating with PH-894 or a non-targeting RNAi control (NTC) for 3 days. The level of secreted IFN-γ in cell culture supernatants was measured using ELISA. Means ± SEM (n = 2) are shown. Representative results of more than 3 experiments. Means were compared to UTC and NTC by one way ANOVA and Tukey's test. **** or ++++ p<0.0001, ** p<0.0006 ** p<0.005.

Silencing of BRD4 sensitizes B16-OVA cell killing to OT-1 T cells

Figure 4. B16-OVA cells were treated with PH-894 or a non-targeting RNAi control (NTC) with indicated concentrations or left untreated (UTC) for 72h. B16-OVA cells were then cocultured with OT-1 T cells (activated with plate-bound anti-CD3/anti-CD28 for 3 days) at effector-to-target ratio of 0.2:1. The survival of B16-OVA cells was measured using CellTiter-glo. The B16-OVA cell killing rate was calculated by normalizing the luminescence to those in UTC wells without OT-1 T cells. The spontaneous killing rate caused by compound treatment itself (without OT-1 T cells) was <10%. Means ± SEM (n = 3) are shown. Means were compared to UTC (*) and NTC (+) by one way ANOVA and Tukey's test. ** or ++ p<0.005; *p< 0.05.



Summary and Conclusions

- **PH-894 significantly silenced the expression of BRD4 in both B16-OVA tumor cells and OT-1 CD8+ T cells.**
- **Silencing of BRD4 expression in OT-1 CD8+ T cells chronically exposed to E.G7-OVA-tumor cells induced over 10-fold production of IFN-γ.**
- **PH-894 pre-treatment of B16-OVA cells sensitized B16-OVA tumor cell killing to OT-1 T cells.**
- **These studies provide new mechanistic insights into the dual mechanism of antitumor activity of PH-894**
  - **Targeting tumor cells to make them more prone to be killed by T cells**
  - **Targeting T cells to better recognize and kill tumor cells**
- **These data further support the clinical development for PH-894 either by local immunotherapy or T cell adoptive cell transfer.**

References

1. Cuiffo et al. AACR 2022.
2. Maxwell et al. AACR-NCI-EORTC 2021.