Combinatorial antitumor effects of CD3-based trispecific T cell activating constructs (TriTACs) and checkpoint inhibitors in preclinical models

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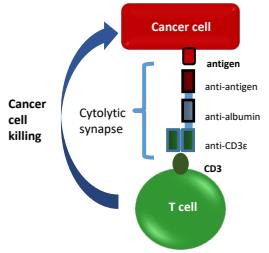
INTRODUCTION

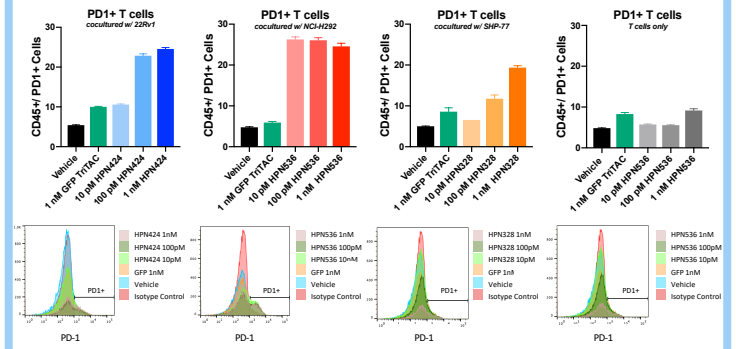
- T cell-engagers function by forming an immunological synapse between cancer target cells and T cells, leading to T cell directed tumor killing
- Blinatumomab (Blincyto®) has demonstrated clinical activity in homological malignancies and is the first T-cell engager approved by the FDA
- Harpoon has developed a proprietary half-life extended T cell engager format (TriTAC™)



- TriTAC molecules are currently being investigated in multiple phase 1/2 clinical trials in solid and liquid tumors, including HPN424 targeting PSMA in prostate cancer (NCT03577028), HPN536 targeting MSLN in multiple malignancies (NCT03872206), HPN217 targeting BCMA in multiple myeloma (NCT04184050) and HPN328 targeting DLL3 in small cell lung cancer (SCLC) (NCT04471727)
- Upregulation of co-stimulatory and/or inhibitory receptors during activation can potentially affect the cytolytic functions of TriTAC-activated T cells
- PD1/PDL1 blockade may enhance the potency of TriTAC-mediated tumor cell killing

TriTAC Molecules Induce PD-1 Expression on T Cells

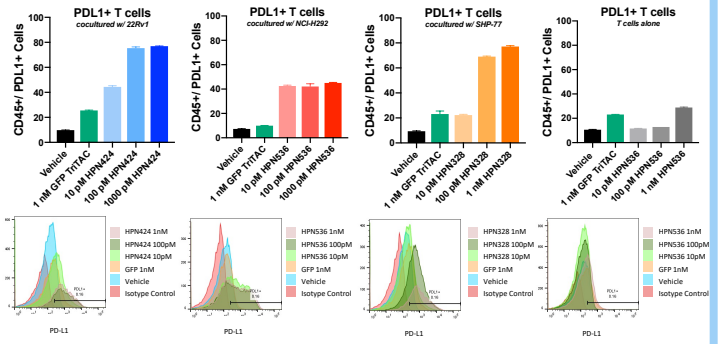
PD-1 expressed on T cells can potentially interact with PD-L1 expressing cells within the tumor microenvironment leading to inhibition of cytolytic T cell activity. We therefore assessed the PD-1 expression on T cells following TriTAC treatment in the presence of target expressing tumor cell lines.



Increased PD-1 on T cells following TriTAC treatment.
T cells were co-cultured with cancer cells at a ratio of 10:1 and treated with TriTAC (10pm, 100pm, 1nM), GFP targeting TriTAC or vehicle for 48 hrs. Expression of PD-1 and PD-L1 was then measured by FACS analysis.

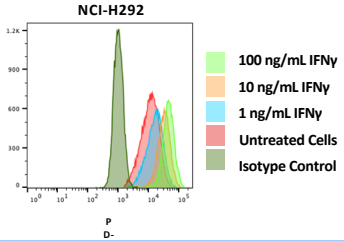
TriTAC Molecules Induce PD-L1 Expression on T cells

TriTAC treated T cells may also upregulate PD-L1 in response to their activation. PD-L1 expressed on T cells can potentially interact with PD-1 on other T cells within the tumor microenvironment leading to inhibition. We therefore assessed the PD-L1 expression on T cells following TriTAC treatment.



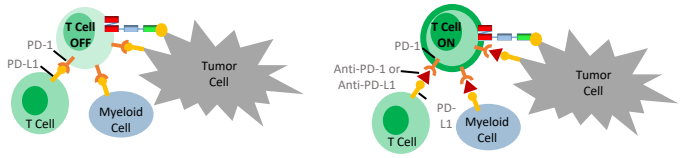
Increased PD-L1 on T cells following TriTAC treatment
T cells were co-cultured with cancer cells at a ratio of 10:1 and treated with TriTAC (10pm, 100pm, 1nM), GFP targeting TriTAC or vehicle for 48 hrs. Expression of PD-L1 was then measured by FACS analysis.

PD-L1 Expression on Tumor Cells



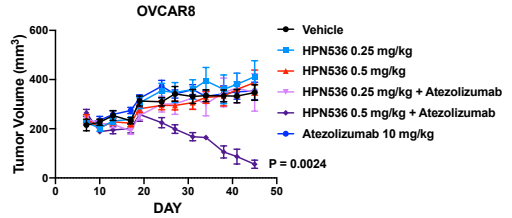
NCI-H292 lung and OVCAR-8 ovarian (data not shown) cancer cells express basal levels of PD-L1 and expression is further increased following IFNγ treatment.
IFNγ is a cytokine released upon T cell activation and can upregulate PD-L1 expression. NCI-H292 lung cancer cells were treated with 1, 10, or 100 ng/mL IFNγ for 48 hrs. Expression of PD-L1 was then measured by FACS analysis.
In concordance with other studies, expression of PD-L1 on tumor cells may down-modulate the cytolytic activity of TriTAC-activated T cells.

PD-1/PD-L1 Blockade May Enhance TriTAC-Mediated T Cell Activation & Tumor Cell Killing

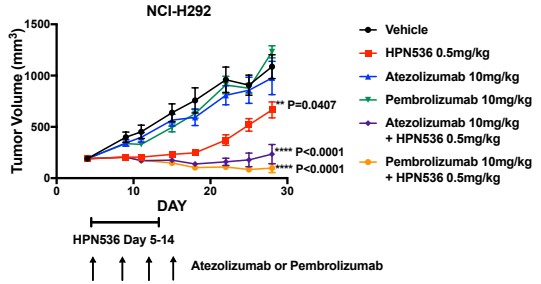


Engagement of PD-1 on T cells with PD-L1 overexpressed on tumor cells, myeloid cells or other activated T cells within the tumor microenvironment may lead to suppression of cytolytic T cell function. T cell activation by TriTACs may further increase PD-1/PD-L1 expression and signaling in the tumor microenvironment. Blockade of PD-1/PD-L1 may enhance the cytolytic functions of TriTAC-activated T cells.

Enhanced Antitumor Activity of TriTAC and PD-1/PD-L1 Blockage in Xenograft Models



Antitumor Activity of HPN536 combined with PDL1 blockade therapy in the OVCAR8 ovarian cancer model.
NSG mice were subcutaneously implanted with a mixture of OVCAR8 and T cells at a ratio of 2:1 (10 x 10^6 OVCAR8 : 5 x 10^6 T cells). Mice were randomized on day 6 when tumors reached ~220 mm^3. Treatment was initiated the following day by i.p. injection (HPN536 q.d. x 10 doses on days 7-16, then 5 days/ week for the duration of the study, atezolizumab biw) n=8.



Antitumor Activity of HPN536 combined with PD1/PDL1 blockade therapy in the NCI-H292 lung cancer model.
NSG mice were subcutaneously implanted with a mixture of NCI-H292 and T cells at a ratio of 1:1 of 5 x 10^6 cells each. Mice were randomized on day 4 when tumors reached 180 mm^3. Treatment was initiated the following day by i.p. injection (HPN536 q.d. x 10 doses on days 5-14, atezolizumab and pembrolizumab biw x 4 doses on days 5,9,12,16) n=10.

SUMMARY

- PD-1 can be readily detected on T cells subsequent to the engagement of the TCR by the TriTAC molecules HPN424, HPN536, and HPN328 in the presence of tumor cells expressing the target antigens PSMA, MSLN, and DLL3, respectively
- TriTAC molecules upregulated PD-L1 on T cells in a dose-dependent manner
- The combination of HPN536 with PD-L1 inhibitor led to more potent antitumor activity in the MSLN-expressing OVCAR8 ovarian cancer model
- MSLN-expressing NCI-H292 lung cancer model that co-expresses constitutive, high levels of PD-L1, both anti-PD1 and anti-PDL1 antibodies significantly enhanced the antitumor effects of the MSLN-targeting TriTAC HPN536 *in vivo*
- Together these results demonstrate the potential utility of PD1/PDL1 blockade to enhance the potency of TriTAC-mediated tumor cell killing, supporting further investigation of these combinatorial approaches in patients.

Abstract 1573 AACR Annual Meeting 2021