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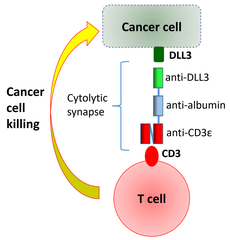
Anti-tumor Activity of HPN328, a DLL3-targeting, Trispecific, Half-life Extended T Cell Engager, is Enhanced by Combining with an Anti-PD-L1 Antibody in an Immunocompetent Mouse Model

Laura Valenzuela, Chi-Heng Wu, Wade Aaron, Mabel Bush, S. Jack Lin, Sony Sotelo Rocha, Subramanian Thothathri, Holger Wesche, Mary Ellen Molloy, Banmeet S. Anand

Harpoon Therapeutics, South San Francisco, CA

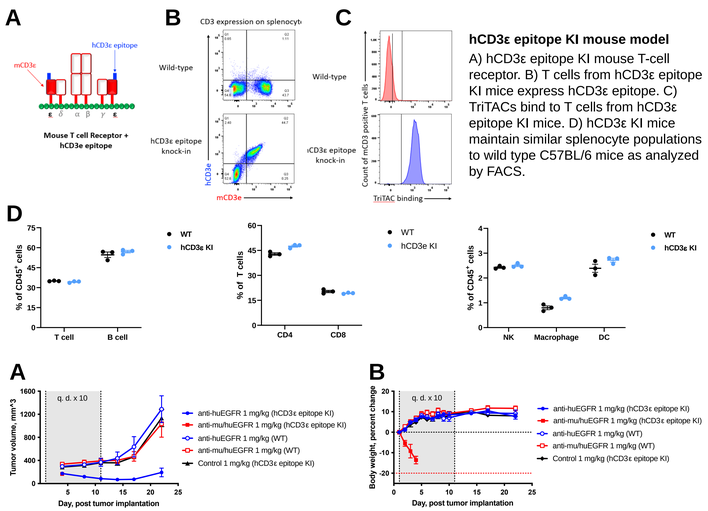
INTRODUCTION

- T cell engagers form immunological synapses between cancer target cells and T cells, leading to tumor killing
- Harpoon has developed a proprietary half-life extended T cell engager format (TriTAC™)
- HPN328 is a Delta-like ligand 3 (DLL3)-targeting TriTAC currently being evaluated in a phase 1/2 clinical trial for patients with advanced cancers associated with DLL3 expression, including small cell lung cancer (SCLC) and other neuroendocrine malignancies (NCT04471727)



- Upregulation of co-stimulatory and/or inhibitory receptors during activation have effects on the cytolytic function of TriTAC-activated T cells
- PD1/PD-L1 blockade may enhance TriTAC-mediated tumor cell killing
- Harpoon developed a humanized CD3ε (hCD3ε) immunocompetent mouse model which has the epitope of human CD3ε recognized by TriTACs, knocked-in (KI) to the mouse CD3ε gene

In Vivo and In Vitro Proof of Concept of hCD3ε Epitope KI Mouse Model

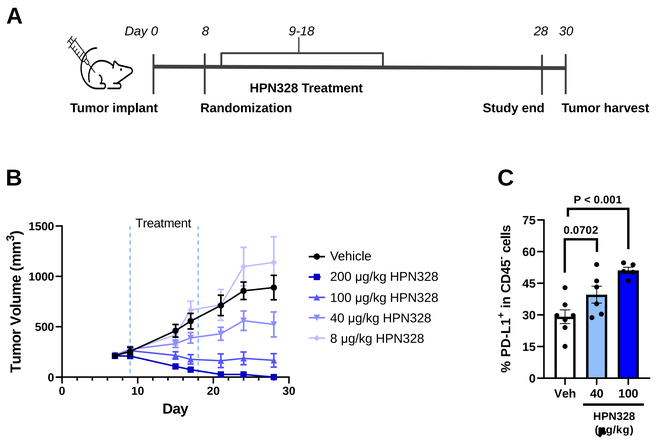


hCD3ε epitope KI mouse model

A) hCD3ε epitope KI mouse T-cell receptor. B) T cells from hCD3ε epitope KI mice express hCD3ε epitope. C) TriTACs bind to T cells from hCD3ε epitope KI mice. D) hCD3ε KI mice maintain similar splenocyte populations to wild type C57BL/6 mice as analyzed by FACS.

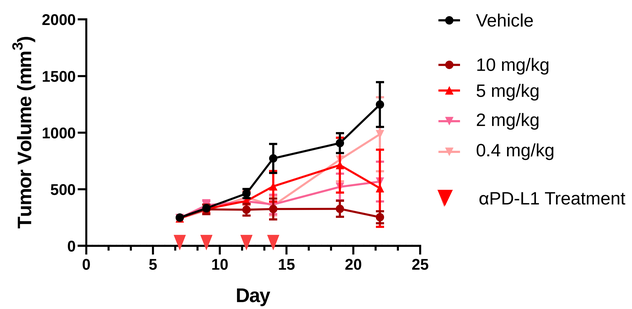
TriTACs engage and activate endogenous T cells from hCD3ε epitope KI mice in vivo, leading to anti-tumor activity and on-target toxicity to mouse EGFR expressing normal tissues. In vivo anti-tumor activity and toxicity was assessed using a hCD3ε epitope syngeneic mouse model. Tumor cells, 2e6 MC38-huEGFR, were implanted subcutaneously into the right flank of mice. The following day anti-EGFR TriTACs were dosed intraperitoneally q.d.x10. A) Anti-tumor activity of anti-huEGFR TriTAC. B) Body weight loss and toxicity (clinical observations, e.g., piloerection, hunched posture) induced by anti-mu/huEGFR TriTAC (hu: human; mu: mouse; WT: wild-type).

Anti-tumor Activity of HPN328 in the hCD3ε KI Mouse Model



HPN328 inhibited the growth of tumors in a dose-dependent manner but the treatment increased the expression of PD-L1 on tumor cells. A, B) hCD3ε KI mice (n=9) were implanted s.c. with 2 x10^6 MC38-huDLL3 tumor cells and randomized when tumors reached ~280 mm^3. Treatment began on day 9 (HPN328 q.d. x 10, i.p.). Study terminated on day 28. C) Tumors were removed 2 days after study termination and the expression of PD-L1 was analyzed by flow cytometry.

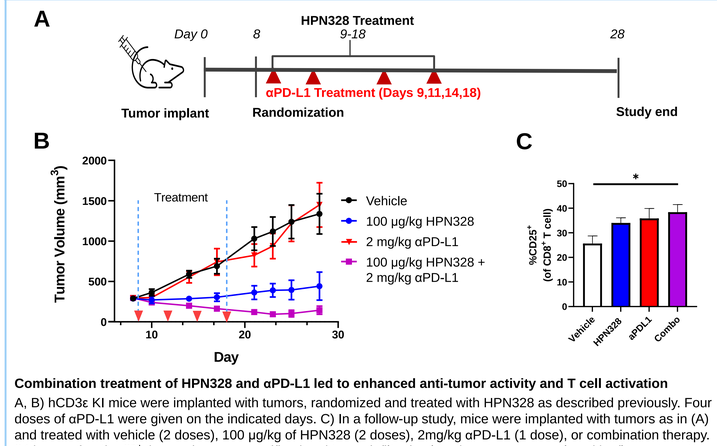
Anti-tumor Activity of αPD-L1 Antibody in the hCD3ε KI Mouse Model



Anti-PD-L1 mAb inhibited tumor growth in a dose-dependent manner

hCD3ε KI Mice (n=8) were implanted s.c. with 2 x10^6 MC38-huDLL3 tumor cells and randomized when tumors reached ~280 mm^3 on day 7. Treatment with anti-PD-L1 began on the same day via i.p. injection.

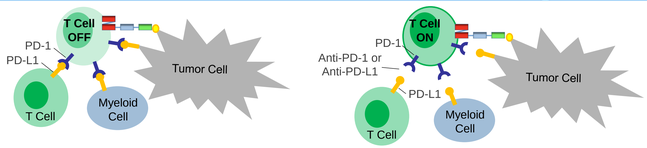
PD-L1 Blockade Enhanced the Anti-tumor Activity of HPN328 and T cell Activation



Combination treatment of HPN328 and αPD-L1 led to enhanced anti-tumor activity and T cell activation

A, B) hCD3ε KI mice were implanted with tumors, randomized and treated with HPN328 as described previously. Four doses of αPD-L1 were given on the indicated days. C) In a follow-up study, mice were implanted with tumors as in (A) and treated with vehicle (2 doses), 100 µg/kg of HPN328 (2 doses), 2mg/kg αPD-L1 (1 dose), or combination therapy. 24 hours after the 2nd dose, mice were sacrificed and tumor infiltrating lymphocytes were analyzed by flow cytometry.

PD1/PD-L1 Blockade Enhances TriTAC-mediated T Cell Activation & Tumor Cell Killing



Engagement of PD-1 on T cells with PD-L1 on tumors, myeloid cells and other activated T cells within the tumor microenvironment leads to suppression of T cell functions. Upregulation of these molecules are expected in response to treatment with T cell engagers. A combination therapy consists of PD-1/PD-L1 blockade and TriTAC is an effective way to counter these inhibitory mechanisms and enhance the cytolytic functions of TriTAC-activated T cells.

SUMMARY

- We generated hCD3ε KI mice with a comparable immune cell repertoire as wild type animals. This model not only uses endogenous mouse T cells as effector cells but also enables us to study the immunomodulating activities of TriTAC molecules.
- We demonstrated a dose dependent anti-tumor activity of HPN328 in hCD3ε KI mice.
- PD-L1 expression on MC38-hDLL3 tumor cells was upregulated after HPN328 treatment.
- Treatment with an αPD-L1 in combination with a sub-therapeutic dose of HPN328 led to enhanced anti-tumor activities and T cell activation compared to either treatment alone.
- These results support further investigation of this combination approach in patients. A clinical study of HPN328 in combination with atezolizumab is being planned.

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