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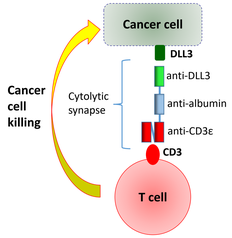
Long-term Anti-tumor Immunity Induced by HPN328, a DLL3-targeting, Trispecific, Half-life Extended T Cell Engager, in a Preclinical Immunocompetent Mouse Model

Mary Ellen Molloy, Laura Valenzuela, Chi-Heng Wu, Sony Sotelo Rocha, S. Jack Lin, Holger Wesche, Banmeet S. Anand

Harpoon Therapeutics, South San Francisco, CA

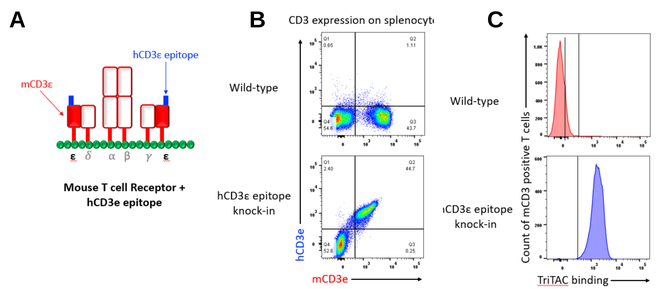
INTRODUCTION

- 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)

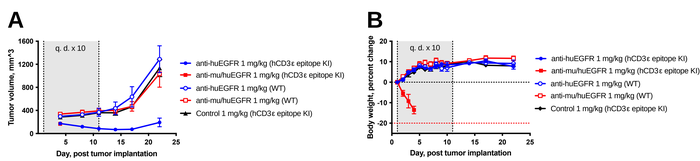


- Harpoon developed a humanized CD3ε (hCD3ε) immunocompetent mouse model which has the epitope of human CD3ε, as recognized by TriTACs, knocked-in (KI) to the mouse CD3ε gene
- We used the hCD3ε KI mouse model to test the ability of HPN328 to induce epitope spreading and to study long-term anti-tumor immunity

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

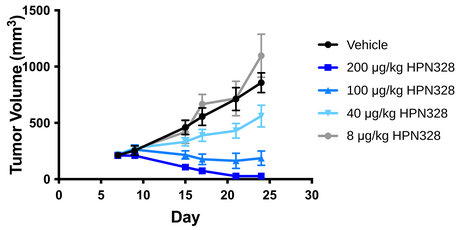


hCD3ε epitope KI mouse model A) hCD3ε epitope KI mouse T-cell receptor. B) T cells from hCD3ε epitope KI mice express hCD3ε epitope. C) TriTACs bind to T cells from hCD3ε epitope KI mice.

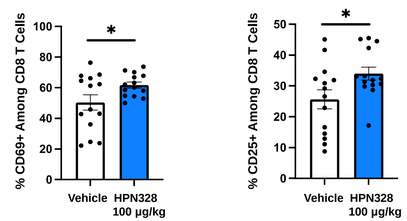


TriTACs engage and activate endogenous T cells from hCD3ε epitope KI mice in vivo, leading to anti-tumor activity and on-target toxicity to mouse EGFR expressing normal tissues. In vivo anti-tumor activity and toxicity was assessed using a hCD3ε epitope syngeneic mouse model. Tumor cells, 2e6 MC38-huEGFR, 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 Immunocompetent hCD3ε KI Mouse Model



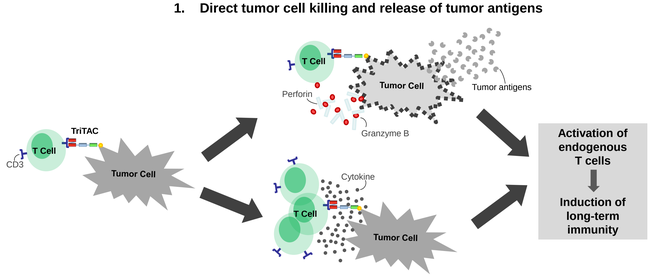
Dose-dependent anti-tumor activity of HPN328 in hCD3ε KI mice implanted with MC38-hDLL3 tumors. Mice (n=9) were randomized on day 8 when tumors reached ~280 mm³ and began treatment on day 9 (HPN328 q.d. x 10).



HPN328 increased the number of activated tumor-infiltrating CD8+ T cells. Tumors were harvested 2 days after dosing and the percentages of CD69 or CD25 positive CD8+ T cells were determined by flow cytometry.

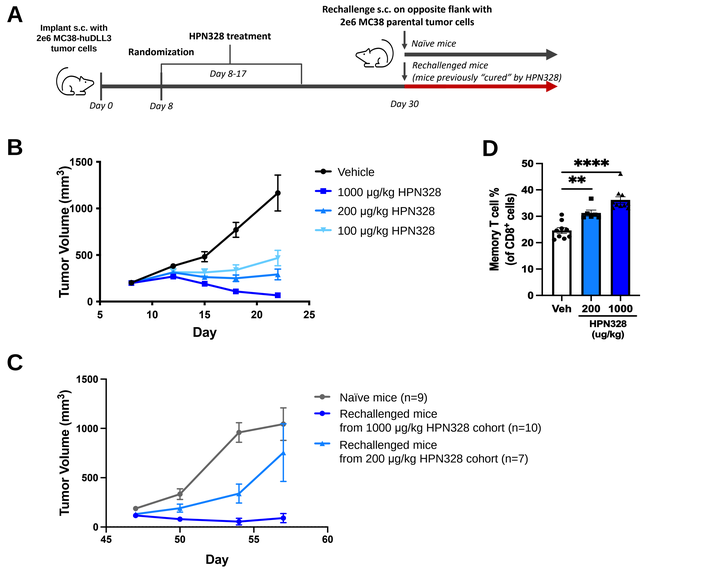
Potential Mechanism of TriTAC-induced Epitope Spreading and Long-term Anti-tumor Immunity

1. **Direct tumor cell killing and release of tumor antigens**



2. **T cell proliferation and inflammatory cytokine production**

HPN328 Treatment Induced Epitope Spreading and Long-Term Anti-tumor Immunity



HPN328 induced epitope spreading and long-term anti-tumor immunity. A) Study design. B) Dose-dependent anti-tumor activity of HPN328 in hCD3ε KI mice (5 per group) treated with the indicated doses daily for 10 days. C) Tumor-free mice from the study in B were rechallenged with parental MC38 cells that lack human DLL3 and were shown to be protected from the tumors. D) Increase in memory CD8 T cells in the spleens of these rechallenged mice. On study day 60, splenocytes from mice from the study in C were analyzed by flow cytometry.

SUMMARY

- We generated the hCD3ε KI mouse model that enables us to study the immune modulating activities of TriTACs
- We demonstrated a dose-dependent anti-tumor activity of HPN328 in mice implanted with MC38-hDLL3 tumors
- Mice treated with HPN328 had increased number of activated tumor-infiltrating CD8 T cells and memory CD8+ T cells in the spleens
- HPN328 induced epitope spreading and prolonged anti-tumor immunity, these are novel in vivo mechanism of action not previously reported
- Long-term anti-tumor immunity induced by HPN328 can potentially lead to more durable clinical responses in patients

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