The first-in-class PD1-IL18 conjugate BPT567 induces potent anti-tumor immunity by preferentially activating PD1⁺IL18R⁺ intratumoral effector T cells in *cis*

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BRIGHT PEAK THERAPEUTICS

ABSTRACT

Antibody-cytokine conjugates leverage orthogonal mechanisms of action (MoA) in one molecule to induce potent antitumor immune responses. PD-1-targeting conjugates are of particular interest since they preferentially target antigen-experienced PD-1⁺ CD8⁺ T cells enriched in the tumor microenvironment (TME) while simultaneously blocking the PD-(L)1 pathway and inducing potent cytokine receptor stimulation in the same CD8⁺ T cell in *cis* (*cis*-signaling). Interleukin 18 (IL-18) is a proinflammatory cytokine able to integrate both innate and adaptive immunity resulting in profound anti-tumor immune responses mediated by T effector and NK cells. BPT567 has been generated via chemical conjugation of the anti-PD-1 Ab Lipustobart™ to an enhanced human IL-18 variant of increased potency and reduced sensitivity to IL-18 binding protein (IL-18BP). BPT567 is designed to specifically target and activate a subset of tumor-infiltrating PD-1⁺IL18R⁺ CD8⁺ T effector cells recently described to exhibit superior cytotoxic and proliferative activity (Codarri Deak et al., Nature 2022).

Due to its ability to signal in *cis*, BPT567 shows enhanced potency as well as increased resistance to IL-18BP when analyzing IFNγ release in vitro in PD-1⁺ cells. Subsequent PD-1 receptor occupancy analyses showed that it is sufficient for BPT567 to engage a low number of PD-1 receptors to induce maximum IFNγ release in NK92 cells expressing human PD-1. Strikingly, despite sharing overlapping epitopes in PD-1, even a 200-fold excess of pembrolizumab has no impact on the *in vitro* potency of BPT567. *In vivo*, BPT567 exhibits strong anti-tumor efficacy at significantly lower IL-18 doses compared to the combination of an untargeted antibody-IL-18 conjugate and an anti-PD1 Ab further corroborating the importance of *cis*-signaling as a unique MoA of this PD1-IL18 conjugate. Detailed analyses of tumor-infiltrating immune leukocytes (TIL) revealed that BPT567 triggers a preferential expansion of CD8⁺ T effector memory cells within the TME. In line with this finding, activity in the MC38 tumor model is dependent on the presence of CD8⁺ T cells while depletion of other immune cell subsets (e.g. NK cells, CD4⁺ T cells, macrophages) had no impact on efficacy. Although not required for anti-tumor efficacy, CD4⁺ T cells are essential for proficient formation of an immunological memory.

In an effort to confirm findings generated in murine models in relevant human ex vivo systems, we analyzed the release of cytokines and chemokines in dissociated cells isolated from primary human tumor explants. Also in this human model, BPT567 is able to induce significantly stronger IFNγ release compared to either single agents or the combination of untargeted IL-18 and an anti-PD1 Ab, thus confirming the unique MoA of the PD1-IL18 conjugate triggering superior TIL activation in *cis*.

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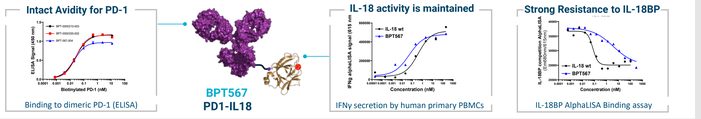
Section 4

AACR American Association for Cancer Research

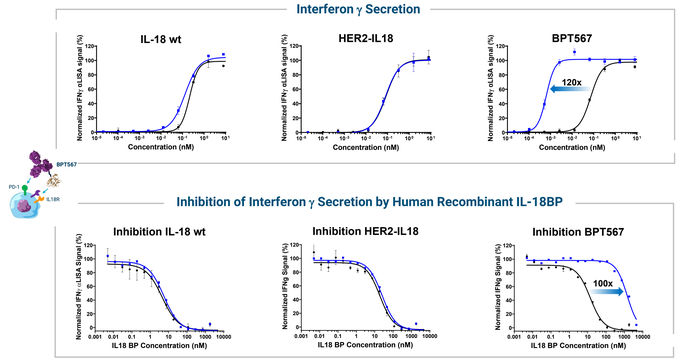
IMMUNOCONJUGATE PROPERTIES

Antibody and IL-18 Payload Properties are Fully Preserved in BPT567



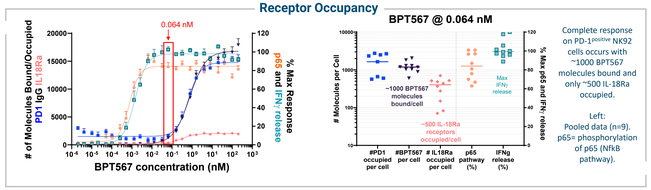
cis-SIGNALING

Cis-Signaling Results in Enhanced Potency and Increased IL-18BP Resistance of BPT567 in PD-1^{high} Cells



Top row: IFNγ secretion by either PD-1^{negative} or PD-1^{positive} NK92 cells stimulated for 16 h with indicated molecules.
Bottom row: Inhibition of IFNγ secretion by PD-1^{negative} or PD-1^{positive} NK92 cells in presence of 1nM of the respective compound and indicated concentrations of recombinant human IL-18BP.

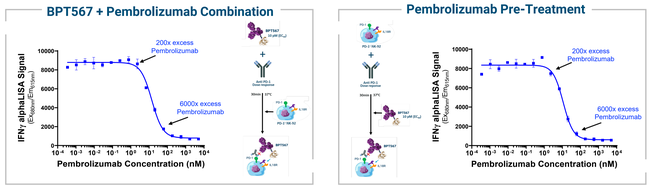
Full IL-18R Activation Requires Minimal Receptor Occupancy



Complete response on PD-1^{positive} NK92 cells occurs with ~1000 BPT567 molecules bound and only ~500 IL-18Ra occupied.

Left: Pooled data (n=9). p65= phosphorylation of p65 (NfkB pathway).

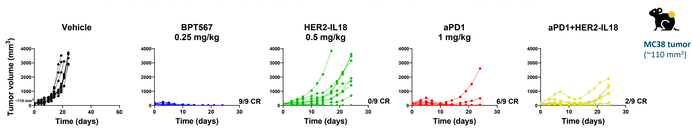
BPT567 is Fully Active in the Presence of a Large Excess of PD-1 Ab



Inhibition of *cis*-signaling (10 pM BPT567) by Pembrolizumab on PD-1^{positive} NK92 cells

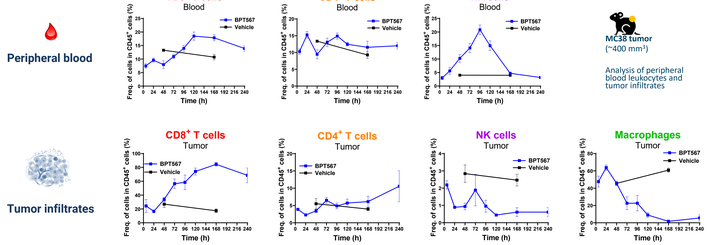
IN VIVO MODE OF ACTION

Efficacy of BPT567 is Superior to Anti-PD-1, Non-Targeted HER2-IL18, and to the Combination of Anti-PD-1 + HER2-IL18



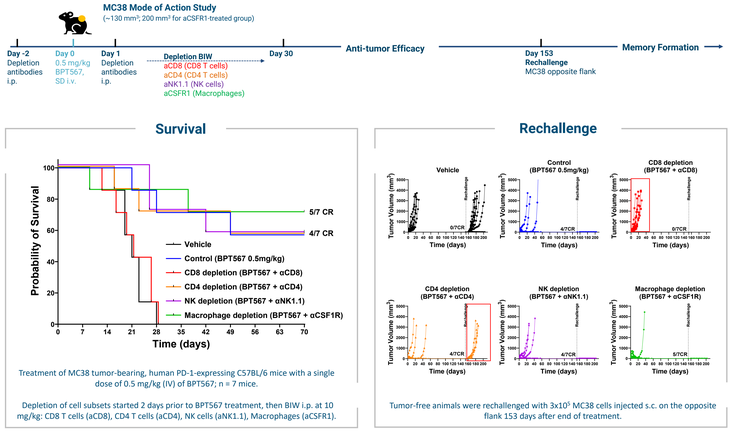
MC38 tumor-bearing transgenic huPD-1 C57BL/6 mice treated with two doses of the indicated molecules (QWx2, IV). Tumor volume over time. Each line represents an individual animal.

BPT567 Preferentially Expands PD1⁺IL-18Rα⁺ CD8⁺ T Cells in the TME



Treatment of MC38 tumor-bearing, human PD-1-expressing C57BL/6 mice with a single dose of 0.25 mg/kg (IV) of BPT567; n = 3 mice. Shown is frequency within CD45⁺ cells isolated from peripheral blood or from tumors harvested on indicated timepoints after treatment.

CD8⁺ T Cells Mediate the Anti-Tumor Efficacy of BPT567 While CD4⁺ T Cells Are Required for the Induction of Immunological Memory



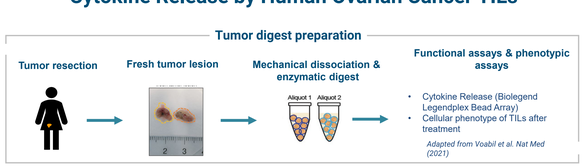
Treatment of MC38 tumor-bearing, human PD-1-expressing C57BL/6 mice with a single dose of 0.5 mg/kg (IV) of BPT567; n = 7 mice.

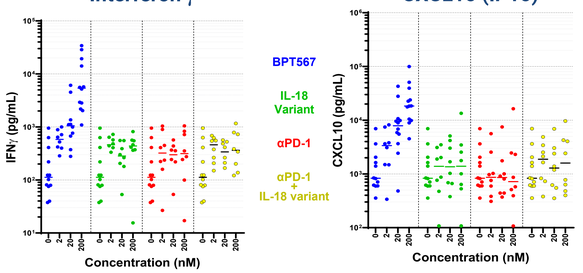
Depletion of cell subsets started 2 days prior to BPT567 treatment, then BIW i.p. at 10 mg/kg: CD8 T cells (aCD8), CD4 T cells (aCD4), NK cells (aNK1.1), Macrophages (aCSFR1).

Tumor-free animals were rechallenged with 3x10^5 MC38 cells injected s.c. on the opposite flank 153 days after end of treatment.

EX VIVO ACTIVITY IN HUMAN TILs

BPT567-Mediated *Cis*-Signaling is Required for Induction of Cytokine Release by Human Ovarian Cancer TILs





Ovarian Cancer Omentum metastasis digests were incubated for 48h with indicated doses of BPT567 (aPD1-IL18), a-PD1 mAb, rhIL-18 variant or the combination of the rhIL-18 variant + a-PD1 mAb. Cytokine levels were assessed from supernatants using a cytokine bead array kit (Legendplex). Each dot represents one donor (n=11).

CONCLUSIONS

- BPT567 is generated using Lipustobart™, a clinical stage anti-PD-1 Ab, and Bright Peak's IL-18BP-resistant enhanced IL-18 variant. Following conjugation, BPT567 retains activity of the engineered IL-18 payload as well as full PD-1 affinity and functional PD-1/PD-L1 blockade.
- *In vitro*, BPT567 shows enhanced potency and increased IL-18BP resistance in PD-1^{high} cells due to simultaneous binding to IL18R and PD-1 on the same cell (*cis*-signaling).
- BPT567 triggers maximal IFNγ release with as few as ~1000 molecules bound.
- Consequently, competition with BPT567 requires a large excess of anti-PD1 mAb such as Pembrolizumab.
- BPT567 is well tolerated and exhibits remarkable single agent anti-tumor efficacy that is superior to that of single agent anti-PD-1 Ab or non-targeted IL-18 IC as well as to the combination of both single agents.
- *In vivo*, BPT567 induces a massive rearrangement of tumor immune infiltrates, characterized by a strong depletion of macrophages and a striking expansion of effector memory CD8 T cells, including IL18Ra⁺PD-1⁺ T cells.
- BPT567 efficacy in the MC38 model requires the presence of CD8 T cells, while CD4 T cells are essential for generation of a long-lasting immunological memory.
- The dual mode of action of BPT567 is essential for potent release of pro-inflammatory cytokines in human tumor digests.