Using Site-Specific Chemical Conjugation to Generate Superior Half-Life Extended or PD1-Targeted Formats of a Potent IL-18 Variant Resistant to IL-18 Binding Protein

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Poster #1080
9) Immune-Stimulants and Immune Modulators.
b. Cytokines

BRIGHT PEAK THERAPEUTICS

INTRODUCTION

- **Interleukin-18 (IL-18)** is a pro-inflammatory cytokine able to trigger both innate as well as adaptive immune responses and is a potent amplifier of IFNγ, making it an attractive candidate for cancer immunotherapy.
- However, **IL-18 binding protein (IL-18BP)**, a secreted antagonist that binds IL-18 with high affinity, is induced by IFNγ in a natural negative feedback loop that neutralizes IL-18 activity.
- Hence, we set forth to generate a **potent IL-18Rα-enhanced, IL-18BP-resistant IL-18 variant ($IL18_{res}$)** that has a further ability to be conjugated for use as a "**payload**" in optimized therapeutic formats.
- We then aimed to exploit our **unique chemical conjugation platform** to develop two innovative IL-18-based therapeutics using this payload:
  - **BPT543 - a half-life extended** PEGylatedL-18_{res}.
  - **BPT567 - a first-in-class, multi-functional immunocytokine (IC)** to target PD-1⁺ antigen-experienced T cells and simultaneously deliver combined PD-1 blockade and potent IL-18 agonism (*cis*-signaling) in one molecule.

CONCLUSIONS

- Bright Peak has generated an optimized, conjugatable human IL-18 variant payload ($IL18_{res}$) with enhanced potency and strongly reduced sensitivity to IL-18BP.
- Half-life extended **BPT543** resulting from conjugation of $IL18_{res}$ to a 30kD PEG, exhibits significantly improved PK properties and triggers potent, durable IFN*γ* release in mice when administered IV.
- **BPT543** demonstrates antitumor efficacy as a single agent and potent synergy in combination with PD-1 blockade while being well tolerated in mice.
- **BPT567** generated by site-specific conjugation to the Fc domain of LZM009, an anti-PD-1 Ab in late-stage clinical development, retains activity of the enhanced $IL18_{res}$ payload and full PD-1 affinity and functional PD-1/PD-L1 blockade.
- *In vitro*, **BPT567** shows enhanced potency in PD-1⁺ cells, presumably due to binding IL18R and PD-1 on the same cell (*cis*-signaling).
- **BPT567** exhibits striking single agent antitumor efficacy, with induction of complete responses in ~90% of mice, while being well tolerated.

① ENGINEERED VARIANT $IL18_{res}$

$IL18_{res}$ Exhibits Strongly Reduced Binding to IL-18BP Compared to wtIL-18

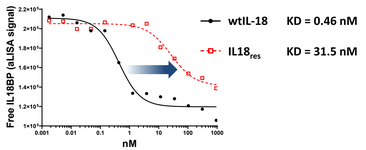
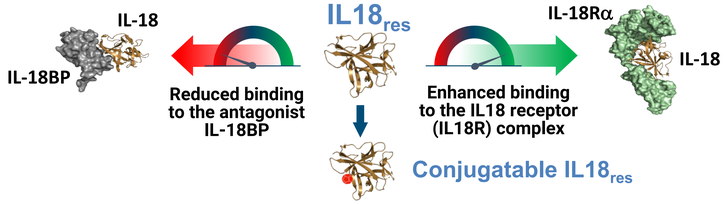


Figure 1. Human IL-18BP alphaLISA binding competition assay

$IL18_{res}$ is a Rationally Designed, Enhanced and Conjugatable IL-18 Payload



$IL18_{res}$ Payload is Significantly More Potent than wtIL-18

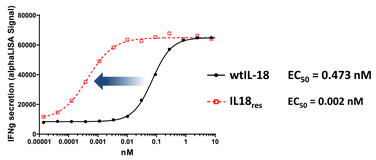


Figure 2. IFNγ secretion by NK92 cells following 16h treatment

② HALF-LIFE EXTENDED PEG-$IL18_{res}$ (BPT543)

PEGylation Has No Impact on IL-18BP Resistance of $IL18_{res}$

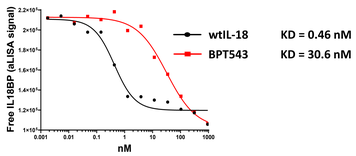
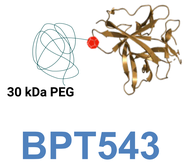


Figure 3. Human IL-18BP alphaLISA binding competition assay



BPT543 is a More Potent IFNγ Inducer Compared to wtIL-18

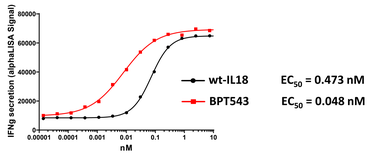


Figure 4. IFNγ secretion by NK92 cells following 16h treatment

PEGylation of $IL18_{res}$ Results in Superior PK Properties and Stronger and More Durable PD Effects

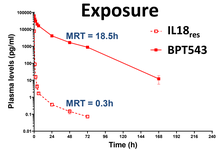


Figure 5. Plasma exposure of BPT543 and $IL18_{res}$ in C57BL/6 mice (single dose of 3mg/kg, i.v., average ± SEM of 3 mice). MRT, mean residence time

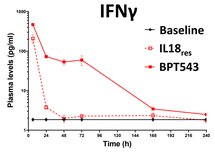


Figure 6. IFNγ plasma levels in C57BL/6 mice following treatment with $IL18_{res}$ or BPT543 (single dose of 3mg/kg i.v., average ± SEM of 3 mice; baseline: n=6 vehicle-treated mice)

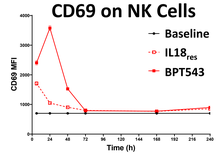


Figure 7. Upregulation of CD69 activation marker on NK cells in blood of C57BL/6 mice following treatment with $IL18_{res}$ or BPT543 (single dose of 3mg/kg i.v., average ± SEM of 3 mice; baseline: n=6 vehicle-treated mice)

BPT543 is Efficacious *In Vivo* and Has Potent Synergy with PD-1 Blockade

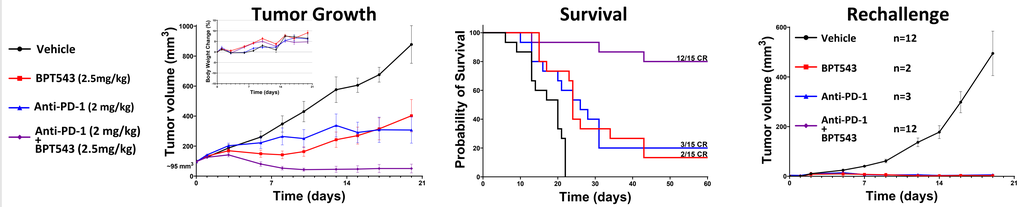


Figure 8. MC38 tumor-bearing C57BL/6 mice were treated with BPT543 (QWx2, 2.5 mg/kg i.v.), anti-murine PD-1 antibody RPM1-14 (BIWx6, 2 mg/kg i.p.), or with a combination of RPM1-14 at 2 mg/kg and BPT543 at 2.5mg/kg (average ± SEM of 9 mice per group).

③ PD1-$IL18_{res}$ IMMUNOCYTOKINE (BPT567)

Antibody Conjugation Further Improves IL-18BP Resistance of $IL18_{res}$

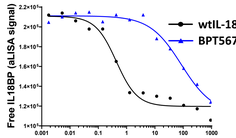
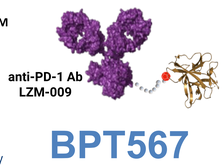


Figure 9. Human IL-18BP alphaLISA binding competition assay



Activity of $IL18_{res}$ is Fully Preserved Following Antibody Conjugation

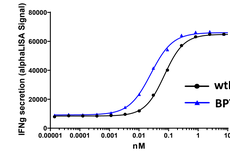


Figure 10. IFNγ secretion by NK92 cells following 16h treatment

PD-1-Dependent *Cis*-Signaling Results in Enhanced IL-18 Signaling by BPT567

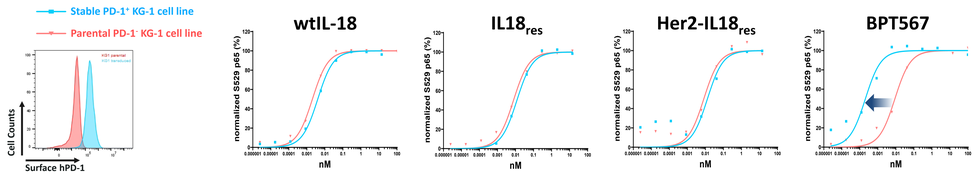


Figure 11. NFκB pathway activation (p65-S529 phosphorylation) in parental PD-1⁻ KG-1 cells and KG-1 cells transduced to overexpress human PD-1 following treatment with indicated molecules for 30 min at 37°C.

BPT567 Exhibits Striking *In Vivo* Efficacy That is Far Superior to PD-1 Blockade

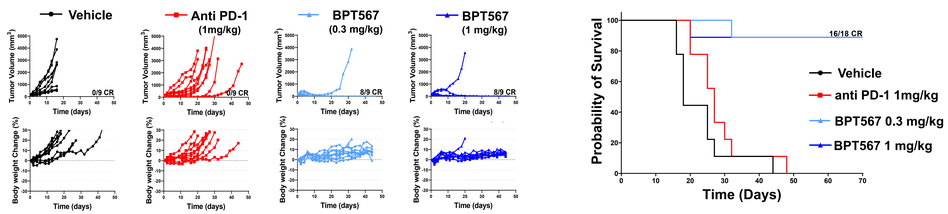


Figure 12. MC38 tumor-bearing huPD1 KO/KI C57BL/6 mice were treated with BPT567 IC (QWx2, i.v.) or anti-human PD-1 Ab LZM009 (QWx2, i.p.) at the indicated dose levels (average ± SEM of 9 mice per group).