Identification of BPT323 - An immunocytokine for treatment of autoimmune diseases combining orthogonal modes of action of TNFα blockade and selective T_{reg} expansion

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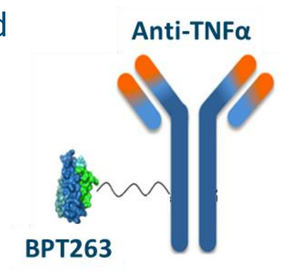
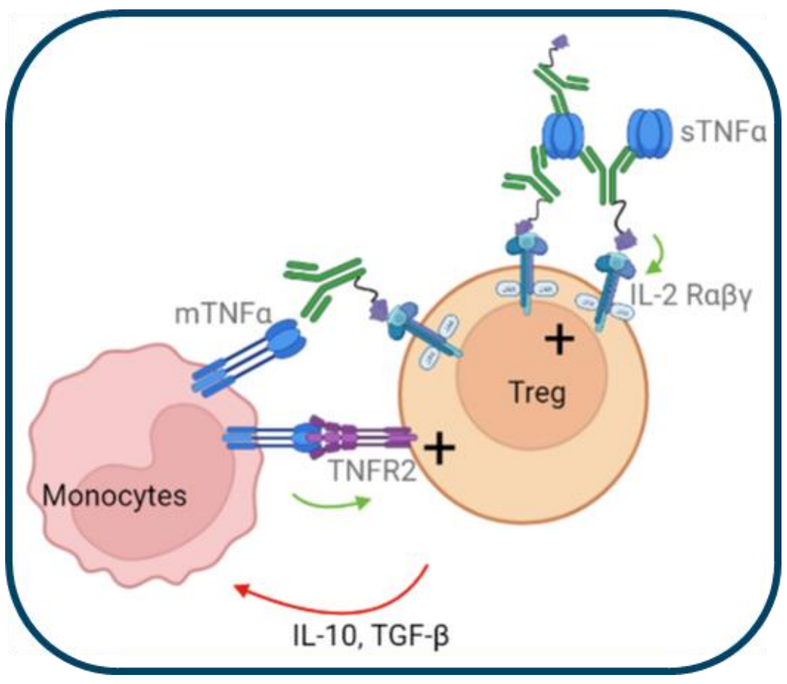
ABSTRACT

Immunocytokines (IC) provide the advantage of combining two orthogonal, i.e. non-overlapping modes of action of a cytokine and a monoclonal antibody whilst extending the plasma half-life of the conjugated cytokine. Using a proprietary chemical conjugation technology, we have site-specifically conjugated a single IL-2 variant that selectively activates and expands regulatory T cells (T_{regs}) to the Fc region of the well-characterized anti-TNFα antibody adalimumab to generate the TNFα-IL2 IC BPT323. TNFα binding and functional blockade were unaffected by IL-2 conjugation and Fc effector functions were preserved. Similarly, chemical conjugation to the antibody had only minimal impact on potency and selectivity of the IL-2 payload. *Ex vivo*, BPT-323 induced a strong activation and proliferation of Tregs which was further potentiated in the presence of soluble TNFα. This ligand-mediated increase in potency is most likely due to the formation of immunocomplexes with the TNFα trimer leading to increased avidity. This may provide a targeting approach in vivo, whereby the potency of the IC is selectively increased in inflamed tissues where the expression of TNFα is elevated. In mice, an Anti-TNFα-IL2 IC had a PK profile which resulted in a robust and durable expansion of T_{regs}. BPT323 treatment practically abolished paw inflammation in a keyhole limpet hemocyanin-induced delayed type hypersensitivity mouse model, whereas the parental antibody had no effect. In a human TNFα-driven mouse arthritis model, BPT323 suppressed disease on par with the parental antibody, thereby confirming that both MoAs of the IC were fully functional *in vivo*. Overall, these results highlight the capability of Bright Peak's cytokine engineering platform to generate potent, multi-modal IC therapeutics to potentially synergize complementary mechanisms of action and target enhanced cytokines to specific cells or tissues.

RATIONALE

BPT263: Synthetic IL-2 engineered to lack binding to IL2Rβ and with enhanced affinity to IL2Rα for T_{reg} selectivity
BPT323: BPT263 site-specifically conjugated to Fc region of anti-TNFα mAb adalimumab





- **Anti-TNFα-IL2 IC Combines Complimentary MoAs:**
  - Neutralization of pro-inflammatory TNFα
  - T_{reg}-mediated resolution of inflammation
- **Potential for synergistic efficacy:**
  - Clustering of IC by trimeric soluble and membrane TNFα (mTNFα) potentiates IL-2 payload signaling on T_{regs}
  - Stabilization of expanded T_{reg} phenotype by dampening proinflammatory environment
  - Physical bridging of T_{regs} and pro-inflammatory monocytes:
    - Activation of T_{regs} via TNFR2 by mTNFα on monocytes
  - Exposure of pro-inflammatory monocytes to T_{reg}-derived IL-10 & TGFβ may drive conversion to anti-inflammatory phenotype

IL-2 FUNCTION

BPT323 Selectively Activates T_{regs} with no Activity on $CD8^+$ T cells

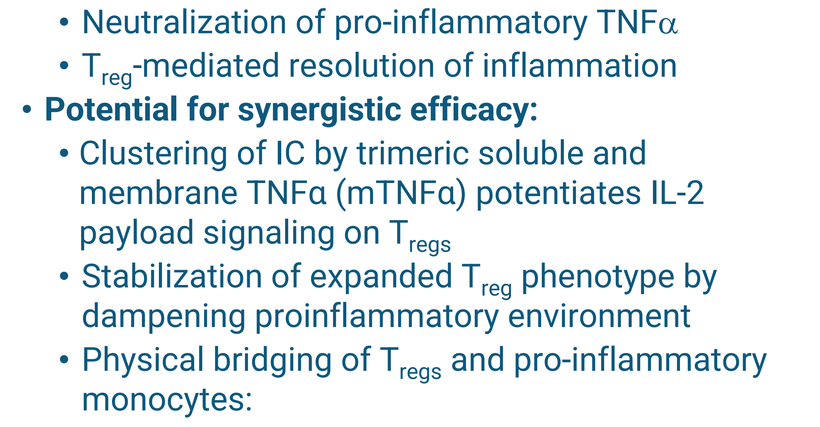


Figure 1. STAT5 phosphorylation in primary human T_{regs} and $CD8^+$ T cells after stimulation of pan T cells with BPT263 and BPT323

ANTI-TNFα FUNCTION

BPT323 Binds and Inhibits TNFα on Par with Adalimumab

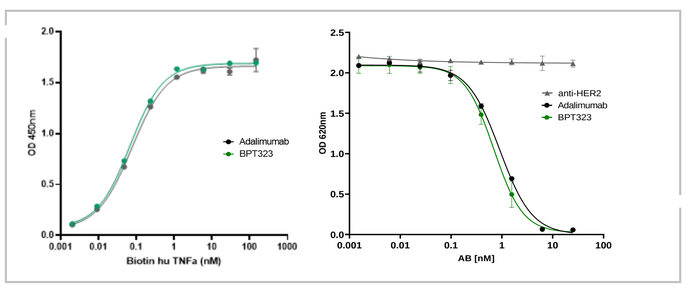


Figure 2. Binding of biotinylated TNFα to immobilized adalimumab or BPT323 (left panel). Inhibition of TNFα-induced reporter activity in HEK-Blue TNFα reporter cells by adalimumab, BPT323 or anti-HER2 as a negative control (right panel)

Fc FUNCTION

BPT323 Binds FcγRI and FcRn on Par with Adalimumab

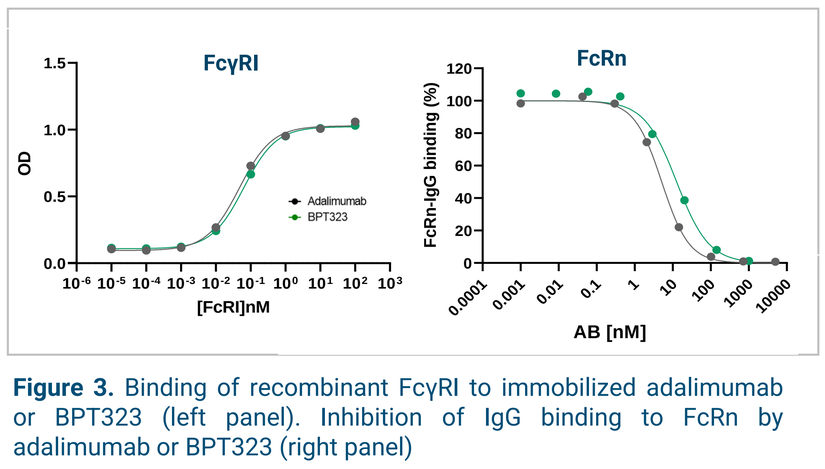


Figure 3. Binding of recombinant FcγRI to immobilized adalimumab or BPT323 (left panel). Inhibition of IgG binding to FcRn by adalimumab or BPT323 (right panel)

SYNERGY

BPT323 Enhances its IL-2 Potency in Presence of Soluble TNFα

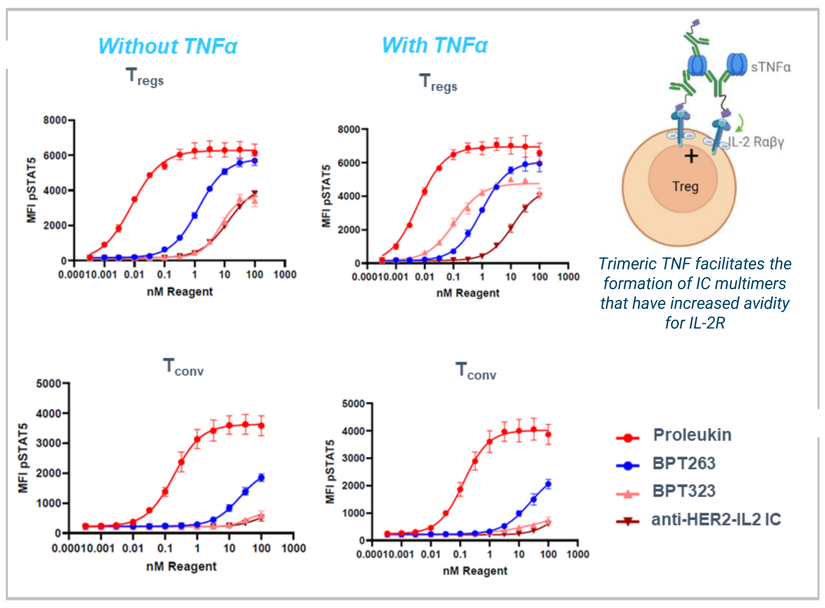


Figure 4. STAT5 activation in the indicated T-cell subsets induced by Proleukin (wild-type IL-2), BPT263, BPT323 or HER2-IL2 IC (non-TNFα binding IC control) with or without recombinant TNFα pre-incubation. Data is reported as mean ± SD (n=2)

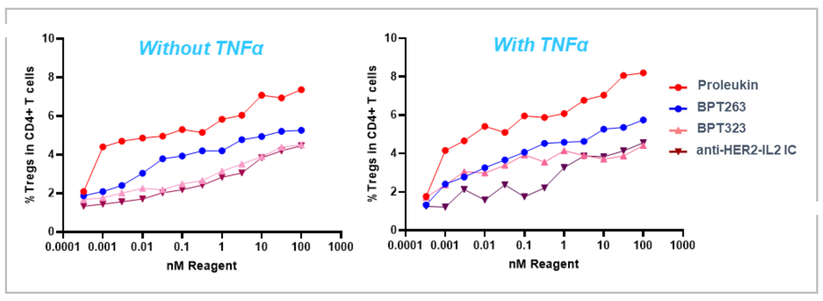
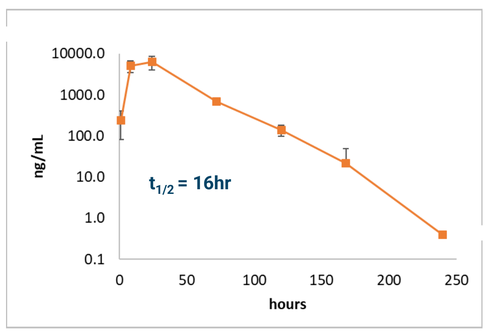


Figure 5. Proportion of T_{regs} in the $CD4^+$ T cell population after a 5 day ex vivo incubation of pan-T cells with Proleukin, BPT263, BPT323 or HER2-IL2 IC in the absence (left panel) or presence (right panel) of a TNFα pre-incubation

- **TNFα-mediated increase of potency may facilitate augmented activity in inflamed tissues where TNFα is produced**

MOUSE PK/PD

An Anti-TNFα-IL2 IC has a Suitable PK Profile that Results in a Robust and Selective Expansion of T_{regs}



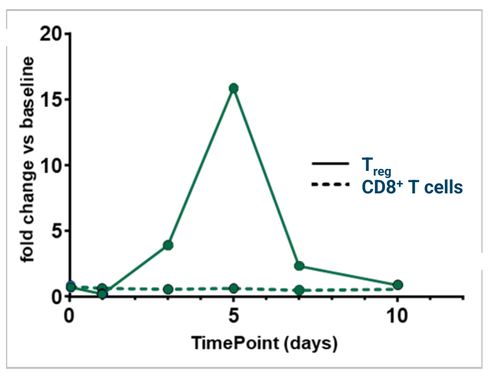


Figure 6. Human TNFα transgenic mice received a single sub-cutaneous (s.c.) injection of a TNFα-IL2 IC at 1 mg/kg. Plasma exposure (upper panel) and fold change in absolute counts relative to baseline (non-treated) of T_{regs} or $CD8^+$ T cells (lower panel)

IL-2 *IN VIVO* EFFICACY

BPT323, but not Adalimumab, Significantly Suppresses Keyhole Limpet Hemocyanin (KLH)-Induced Delayed Type Hypersensitivity

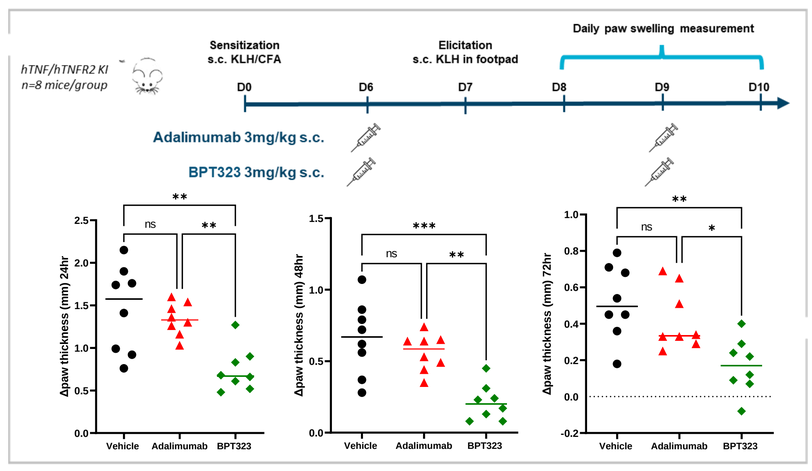


Figure 6. As adalimumab is not rodent cross-reactive, human TNFα knock-in mice were used. Paw thickness difference between the left paw challenged with KLH compared to baseline (pre-treatment) was measured at 24, 48 and 72hrs post-challenge. ns: non-significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, one way ANOVA with multiple comparison with the Tukey test. Data is reported as a scatter plot with mean

ANTI-TNFα *IN VIVO* EFFICACY

BPT323 Shows Equivalent Efficacy to Adalimumab in a Human TNFα-Driven Arthritis Model

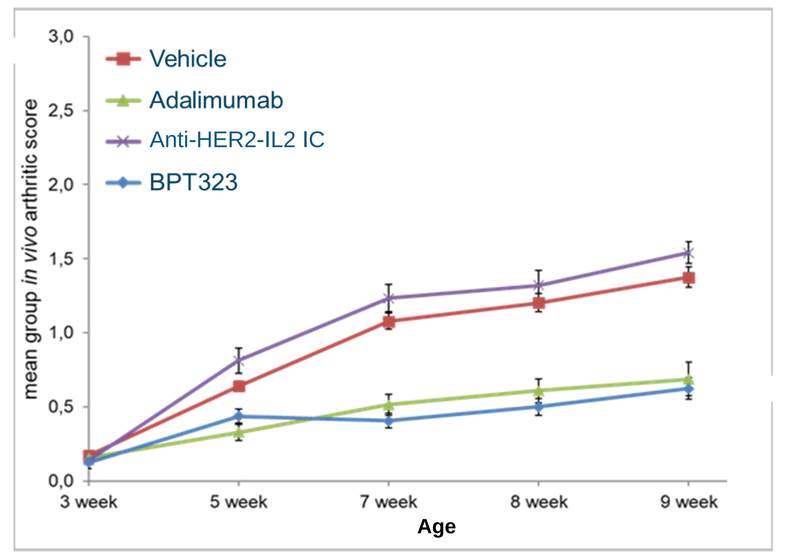


Figure 7. Transgenic mice overexpressing human TNFα that spontaneously develop arthritis were treated at the onset of disease with vehicle, adalimumab, BPT323 or anti-HER2-IL2 IC (non-TNFα binding IC control) at 1 mg/kg Q3D s.c. until week 7 and the arthritic score over the course of the study reported as mean ± SEM (n=8)

CONCLUSIONS

- Conjugation of a T_{reg}-selective IL-2 variant to the Fc region of the anti-TNFα mAb adalimumab generated an IC that fully retained the functions of the parent molecules *in vitro* and *in vivo*
- This combination demonstrated a novel synergy in that T_{reg} activation and expansion was augmented by engagement of the IC with TNFα, most likely via trimeric TNFα driving the formation of IC multimers that have increased avidity for the IL-2 receptor
- This TNFα-mediated increase in potency would be expected to facilitate targeted activity of the IC in inflamed tissues where TNFα is produced at high levels.

ABOUT BRIGHT PEAK

Bright Peak is a privately held biotechnology company based in Basel, Switzerland and San Diego, CA. We are rapidly advancing a robust portfolio of next-generation, multi-functional, cytokine-based immunotherapeutics for the treatment of patients with cancer and autoimmune disease. We accomplish this by leveraging our world-class protein-engineering capabilities, and our unique cell-free technology-platform to chemically synthesize and conjugate novel protein therapeutics that reflect state-of-the-art insights into cytokine and T-cell checkpoint biology. Our pipeline stretches from discovery to IND-enabling, and encompasses enhanced cytokines, antibody-cytokine conjugates and other novel formats. Bright Peak is funded by a syndicate of leading healthcare investors