Identification of BPT264 - a potent alpha-enhanced/beta-dead and half-life-extended IL-2 T_{reg} enhancer for treatment of autoimmune diseases

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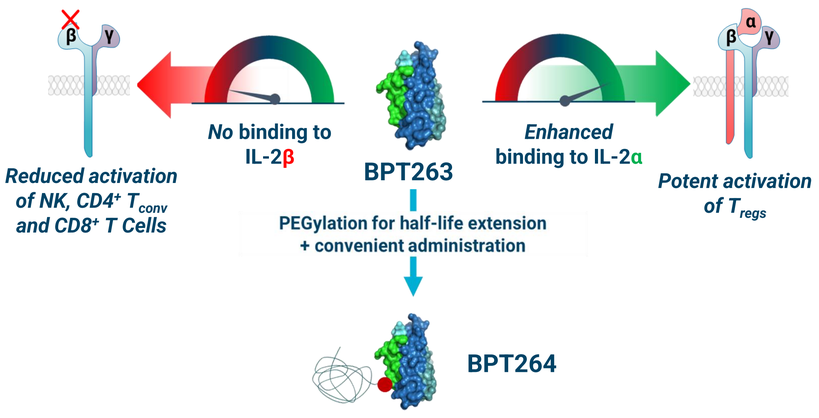
ABSTRACT

Low-dose IL-2 therapy has shown promising preliminary clinical efficacy in a wide range of autoimmune diseases by preferentially expanding regulatory T cells (T_{reg}) due to their expression of the high affinity IL-2 receptor, IL-Rαβγ, whilst avoiding the reciprocal expansion of CD4⁺ T_{conv}, CD8⁺ T cells and NK cells which express the intermediate affinity IL-2 receptor, IL-Rβγ. However, due to the low dose used and the short half-life of IL-2, the level and duration of T_{reg} expansion is sub-optimal. To this end, we have rationally designed BPT264, a fully synthetic, site-specific 30kDa PEGylated IL-2 variant which has been engineered to completely ablate binding to IL-2Rβ whilst uniquely augmenting binding to IL-2Rα. This translates *ex vivo* into a highly selective activation of T_{regs} with almost no activation of CD4⁺ T_{conv}, CD8⁺ T cells or NK cells. Correspondingly, a single dose of BPT264 in mice induced a robust 34-fold expansion of T_{regs} with only minor effects on the numbers of CD4⁺ T_{conv}, CD8⁺ T cells, NK cells and eosinophils. BPT264 also exhibited an improved PK profile over wildtype IL-2 which will facilitate a convenient dosing schedule in patients. In terms of efficacy, BPT264 treatment almost abolished antigen-driven and T cell-mediated ear inflammation in a keyhole limpet hemocyanin-induced delayed type hypersensitivity mouse model. In non-human primates, a single dose of BPT264 led to an unprecedented 57-fold expansion of T_{regs} to a level where they constituted 66% of the CD4⁺ T cell population. BPT264 therefore represents a half-life extended, β-dead, α-enhanced IL-2 variant with a best-in-class profile compared to other T_{reg}-selective IL-2 variants currently in development. IND-enabling studies have been initiated and a first in human trial is planned to start early 2024.

RATIONAL DESIGN

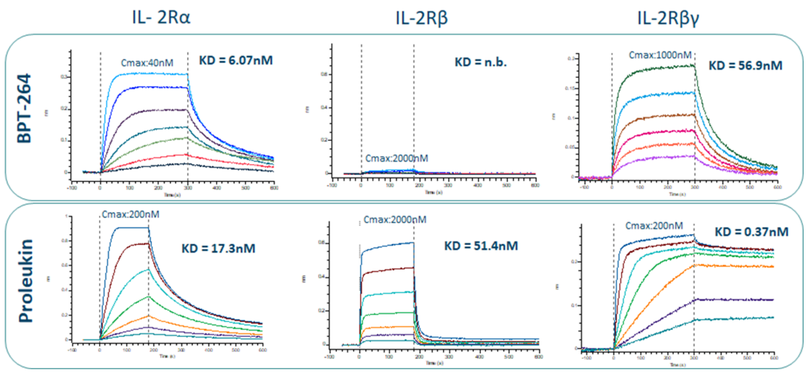
BPT263: Base synthetic cytokine engineered to lack binding to IL2Rβ and with enhanced affinity to IL2Rα

BPT264: BPT263 site-specifically conjugated to 30 kDa PEG to improve PK properties



IN VITRO PROFILE

BPT264 Shows Uniquely Augmented Binding to IL-2Rα, no β Binding and Weak Binding to βγ Compared to Wild-Type IL-2 (Proleukin)



	Affinity (nM)		
	α	β	βγ
BPT264	6.07	n.b.	56.9
Proleukin	17.3	51.4	0.37

Figure 1. Binding affinities of BPT264 and Proleukin to IL-2 receptor subunits α, β and βγ heterodimer determined by Biolayer Interferometry analysis (n.b.- no binding)

BPT264 Selectively Activates T_{regs} with Minimal Activity on CD8⁺ T cells, CD4⁺ T_{conv} and NK cells in Human, Cyno Monkey and Mouse

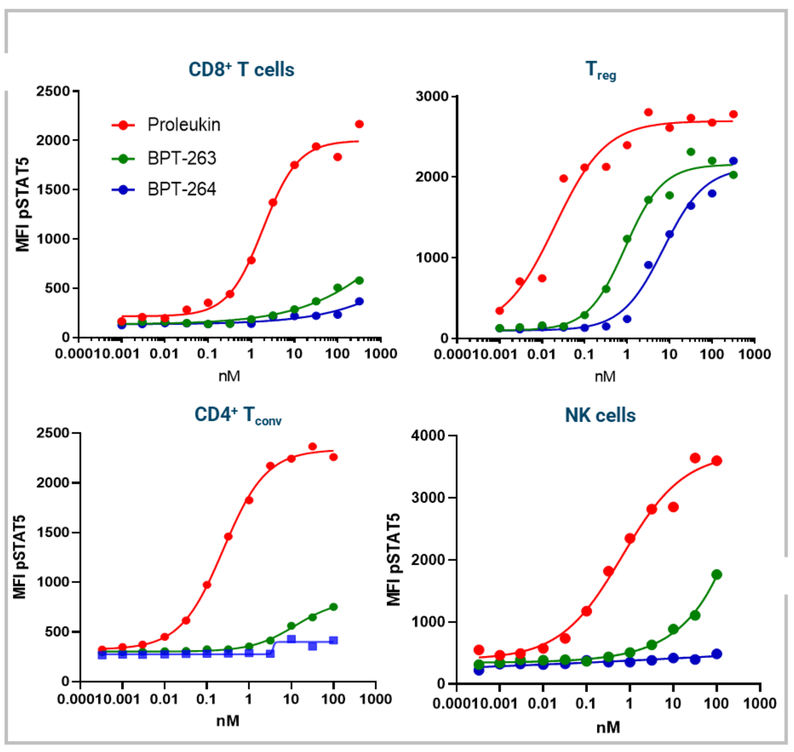


Figure 2. STAT5 phosphorylation in primary human CD8⁺ T cells, T_{regs}, CD4⁺ T_{conv} and NK cells after stimulation with Proleukin, BPT263 and BPT264

Species	BPT264 EC_{50} (nM)		
	Treg	CD4⁺ T_{conv}	CD8⁺ T cell
Human	15.8	>10000	>10000
Cyno	9.17	>10000	>10000
Mouse	45.45	>10000	>10000

Table 1. EC_{50} for STAT5 phosphorylation in T_{regs}, CD4⁺ T_{conv} and CD8⁺ T cells from human, cynomolgus monkey and mouse

MOUSE PK/PD

BPT264 has an Extended PK Profile in Mouse

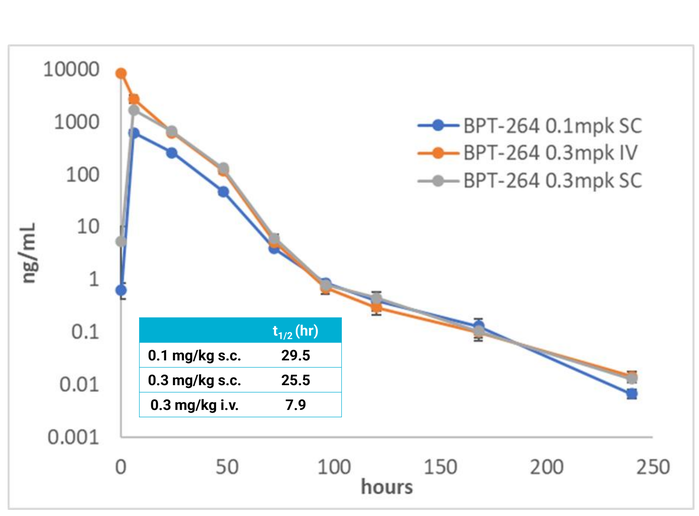
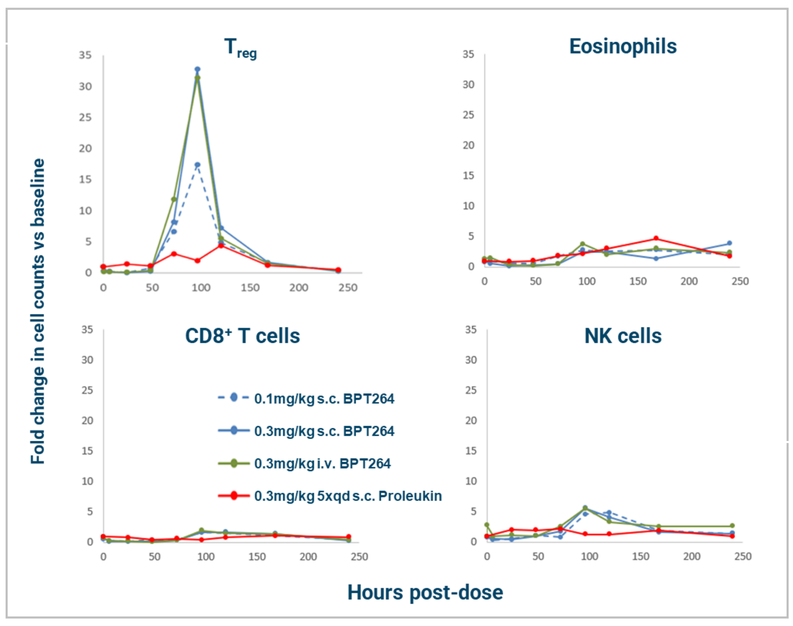


Figure 2. Plasma exposure after single sub-cutaneous (s.c.) or intravenous (i.v.) injections of BPT264 at 0.1 or 0.3 mg/kg in C57BL/6 mice

BPT264 Selectively Expands T_{regs} >30x with Minimal Effect on CD8⁺ T cells, NK cells and Eosinophils



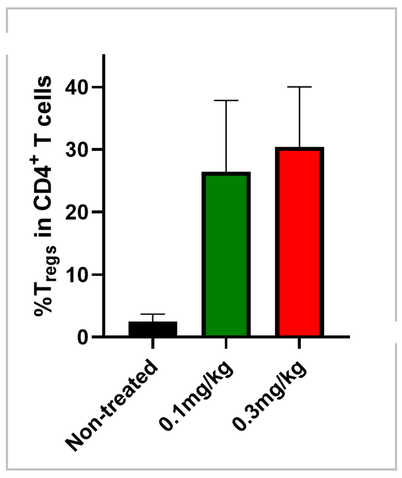


Figure 3. Upper panel: Fold change in absolute counts relative to baseline (non-treated) of T_{regs}, eosinophils, CD8⁺ T cells and NK cells in mice treated as described in Fig. 2. Lower panel: Maximum proportion of T_{regs} in the CD4⁺ T cell population. Data is reported as mean ± SD

EFFICACY

BPT264 Demonstrates Strong and Long-Lasting Efficacy in a Keyhole Limpet Hemocyanin (KLH)-Induced Delayed Type Hypersensitivity Model

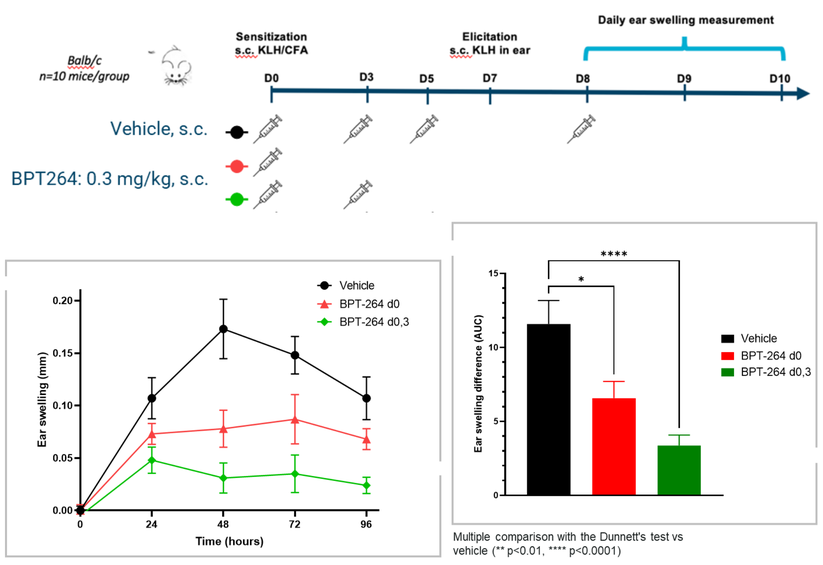
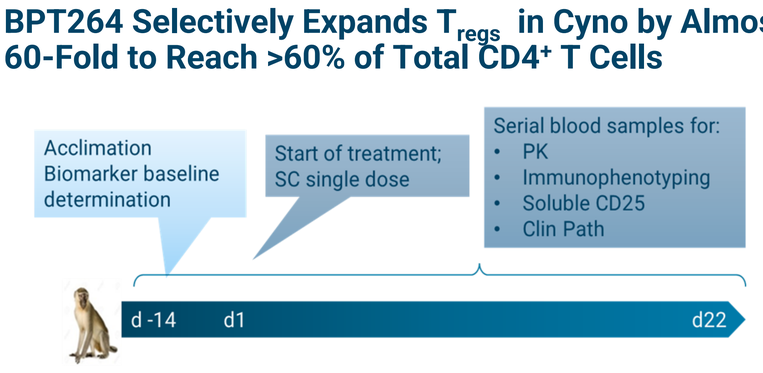


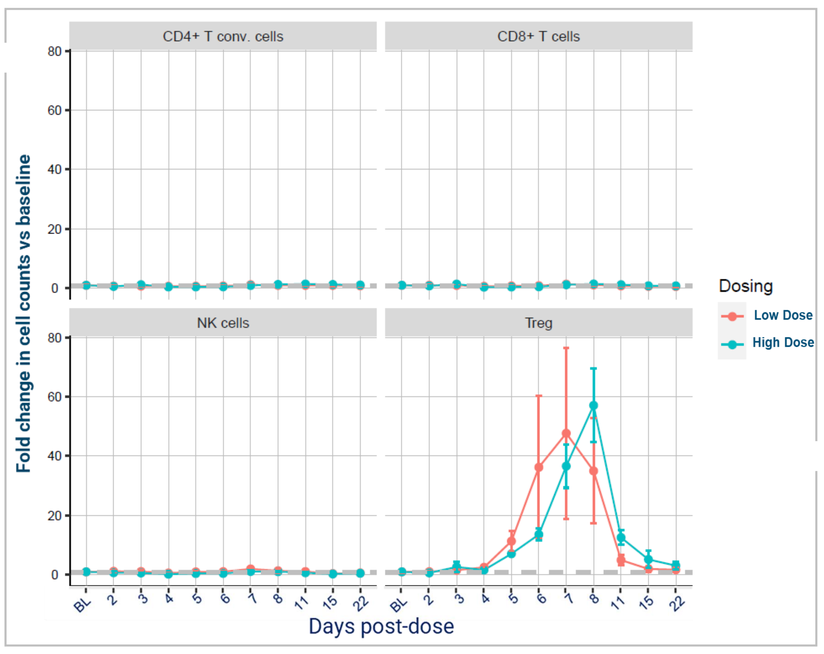
Figure 4. Ear thickness difference between the right ear (challenged with KLH) and the contralateral ear (injected with saline) reported in mm over time (left panel) or as area under the curve (AUC, right panel). Data is reported as mean ± SEM

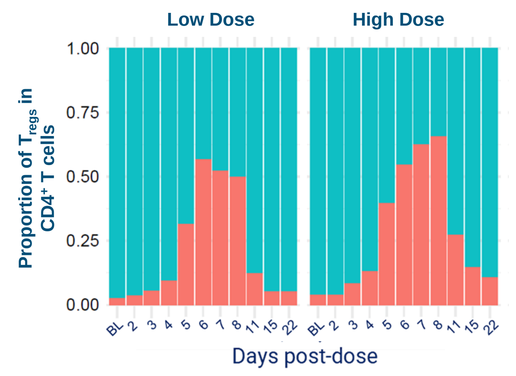
CYNO IMMUNO PD

BPT264 Selectively Expands T_{regs} in Cyno by Almost 60-Fold to Reach >60% of Total CD4⁺ T Cells



- **BPT264 was well tolerated at all doses with no effects on clinical signs or blood biochemistry**





	Max %T_{reg} in CD4
Low dose	57
High dose	66

Figure 5. BPT264 was administered to cynomolgus monkeys with a single s.c. injection. Cell expansion is shown as fold change in absolute counts relative to baseline (pre-treatment) of CD4⁺ T_{conv}, CD8⁺ T cells, NK cells and T_{regs} (upper graph) or as the proportion of T_{regs} in the total CD4⁺ T cell population (red bars, lower graph). Data is reported as mean ± SEM. Table: Maximum percentage of T_{regs} in the CD4⁺ T cell population.

CONCLUSIONS

- BPT264 is a uniquely α-enhanced/β-dead half-life extended IL-2 generated using our novel chemical protein synthesis technology with best-in-class properties
- In vitro, BPT264 highly selectively activates T_{regs} from human, cynomolgus monkey and mouse, with almost no activation of CD4⁺ T_{conv}, CD8⁺ T cells or NK cells
- In mouse, BPT264 selectively expands T_{regs} >30x and strongly suppresses antigen-driven inflammation
- In cynomolgus monkey, BPT264 is well tolerated and selectively expands T_{regs} nearly 60-fold (constituting 66% of the CD4⁺ T cell population) with no effect on CD8⁺ T cells, NK cells and CD4⁺ T_{conv}
- Based on these best-in-class properties, IND-enabling studies have been initiated.

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 immunotherapies 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.