BPT-143: A fully synthetic alpha-dead IL-2 with a best-in-class preclinical pharmacodynamic and efficacy profile supporting first-in-human clinical development

Jean-Philippe Carralot, Rubén Alvarez Sanchez, Matilde Arévalo Ruiz, Magali Muller, Vijaya Pattabiraman, and Bertolt Kreft
Bright Peak Therapeutics, Basel. Email: science@brightpeaktx.com

BRIGHT PEAK THERAPEUTICS

ABSTRACT

High-dose recombinant IL-2 (aldesleukin) is approved for treatment of advanced melanoma and renal cell carcinoma; however, major limitations restrict its therapeutic use. Wild-type IL-2 acts via binding to the medium-affinity IL-2 receptor βγ (IL2Rβγ) expressed in CD8+ T effector cells and NK cells. At the same time, its efficacy is dampened due to strong activation of regulatory T cells (Treg) expressing the high-affinity IL-2 receptor αβγ (IL2Rαβγ). Furthermore, binding to CD25/IL2Rα on endothelial cells and type 2 innate lymphoid cells is thought to be involved in the induction of severe toxicity including vascular leak syndrome (VLS). In addition, aldesleukin exhibits a very short half-life that, combined with its safety risks, requires a burdensome inpatient treatment schedule.

We set out to rationally design a variant of human IL-2 that addresses and overcomes the major limitations of aldesleukin. Using our chemical protein synthesis technology, we introduced select modified amino acids including site-specific chemical conjugation handles to optimize the properties of IL-2 for cancer therapy while maintaining high homology to the wild-type IL-2 sequence. Bright Peak's enhanced cytokine shows increased binding to CD122/IL2Rβ and does not interact with CD25/IL2Rα to improve safety and prevent the preferential activation of Tregs compared to CD8+ T effector cells. Site-specific conjugation to a 30 kDa PEG for half-life extension resulted in the generation of BPT-143, which is equipotent to aldesleukin in activating CD8+ T cells *in vitro*. In mice, BPT-143 induces a strong expansion of CD8+ T cells with only transient and minor effects on Tregs *in vivo* and exhibits improved PK properties allowing for a convenient dosing schedule.

In the syngeneic CT26 tumor model, BPT-143 showed strong anti-tumor efficacy as a single agent as well as enhanced efficacy in combination with an anti-PD-1 antibody. BPT-143 induced a high rate of complete responses and, upon tumor re-challenge, all cured animals rejected CT26 tumor cells indicating the development of immunologic memory. In multiple-dose PK/PD studies in non-human primates (NHP), BPT-143 was well tolerated and induced robust and repeated expansion of CD8+ T cells, CD4+ conventional T cells and NK cells while showing only negligible effects on Tregs and eosinophils. Both *in vivo* efficacy studies in murine tumor models as well as PD effects observed in NHP indicate that BPT-143 has a best-in-class profile among "not-alpha" IL-2 compounds currently in development. BPT-143 is currently in IND-enabling studies.

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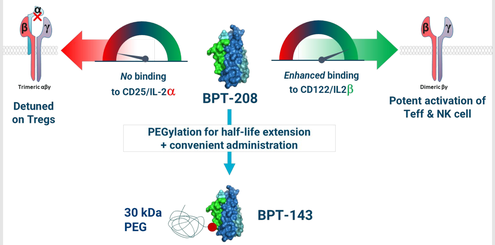
TWO-STEP RATIONALLY DESIGNED IL-2

BPT-208: Base cytokine engineered not to bind to CD25/IL2Rα and with enhanced affinity to CD122/IL2Rβ
BPT-143: BPT-208 site-specifically conjugated to 30 kDa PEG to improve PK properties



IN VITRO PROFILE

BPT-143 is CD25/IL-2Rα-dead and binding to CD122/IL2Rβ is comparable to aldesleukin

K_D (nM)	Aldesleukin	BPT-208	BPT-143
IL-2Rα (CD25)	15.35	No binding*	No binding*
IL-2Rβ (CD122)	709	133	942

Table 1. Binding affinities to IL-2 receptors α and β determined by Surface Plasmon Resonance (*K_D> 2500μM).

BPT-143 shows reduced potency towards Tregs and equipotency to aldesleukin with CD8+ Teff

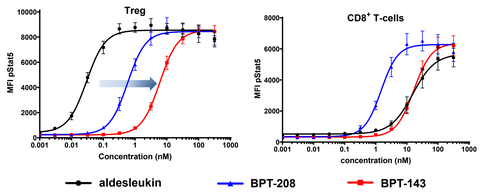


Figure 1. STAT5 phosphorylation in primary human CD8+ Teff and Tregs after stimulation with aldesleukin, BPT-208 and BPT-143

MOUSE PK/PD

BPT-143 has improved PK properties in mice

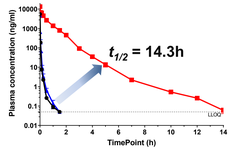


Figure 2. Plasma exposure after single equimolar intravenous (i.v.) injections of aldesleukin, BPT-208, and BPT-143 at 0.8 mg/kg *(average ± SEM of three mice)*

BPT-143 has negligible effects on Tregs while triggering strong & durable CD8+ Teff expansion

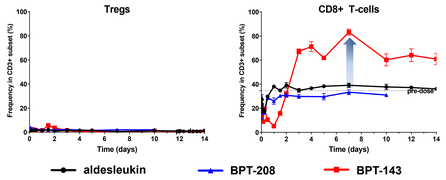


Figure 3. Frequency of CD4+ Treg and CD8+ T-cells within CD3+ lymphocytes after single equimolar intravenous injections of aldesleukin, BPT-208, and BPT-143 at 0.8 mg/kg *(average ± SEM of three mice)*

TUMOR GROWTH INHIBITION

BPT-143 exhibits single agent *in vivo* efficacy

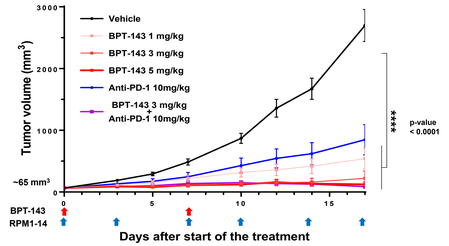


Figure 4. CT26 tumor-bearing BALB/c mice (n=9) were treated with BPT-143 (injected i.v. Q1Wx2 at 1, 3, 5 mg/kg), anti-murine PD1 antibody RPM1-14 (injected intraperitoneally (i.p.) at 10 mg/kg BiWx6), or with a combination of RPM1-14 and BPT-143 at 3mg/kg. *(average ± SEM of nine mice per group)*

SURVIVAL

BPT-143 induces complete responses in mono-therapy and shows enhanced *in vivo* efficacy in combination with anti-PD-1

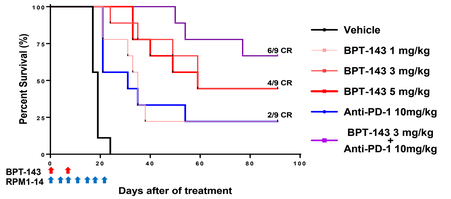
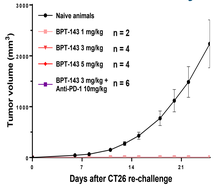


Figure 5. Survival plot of CT26 tumor-bearing BALB/c mice corresponding to TGI data shown in Fig. 4

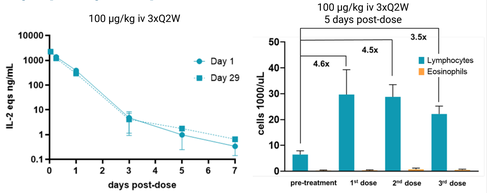
BPT-143 promotes durable anti-tumor immunity

Figure 5b: On day 90, tumor-free mice were re-challenged with CT26 Tumor cells, and all BPT-143-treated mice rejected tumors while (0/16) of naïve mice grew tumors (9/10).



NHP PK/PD

BPT-143 exhibits consistent PK and sustained lymphocyte expansion in NHP



BPT-143 was administered to cynomolgus monkeys as i.v. infusion in single and repeated dose (Q2W) settings.

Figure 6a: PK was dose proportional and remained sustained across all three dosing cycles. All animals tested negative for anti-BPT-143 antibody formation.

Figure 6b: Treatment resulted in pronounced and sustained lymphocyte expansion with no or minimal effect on eosinophils. BPT-143 was well tolerated with no signs of vascular leakage syndrome (VLS) or hypotension.

NHP IMMUNOPD

BPT-143 induces strong T and NK cell expansion

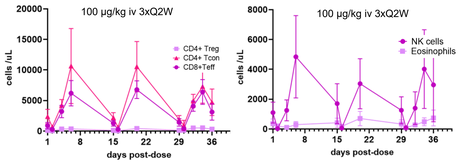


Figure 7a: Flow cytometry analysis showing activation of the lymphoid compartment resulting in strong expansion of CD8+ T effector, CD4+ T conventional and NK cells.

Figure 7b: CD8+ memory cells were the most expanded subsets showing maximal increases of about 15-30x for CD8+ TEM and TCM, respectively (data not shown).

CONCLUSIONS

- BPT-143 is an α-dead, βγ-preserved IL-2 generated using our novel chemical protein synthesis technology.
- BPT-143 exhibits robust single agent activity and induces complete tumor regression *in vivo*.
- In NHP, BPT-143 induces pronounced and sustained expansion of CD8+ Teff, NK, and CD4+ Tconv cells with no or only minimal effects on Treg and eosinophils.
- BPT-143 is well tolerated, and no major clinical findings or signs of VLS have been observed.
- GMP production was completed producing sufficient drug product to support a Phase 1 clinical study.

REFERENCES

1. Creating next-generation biologics using a novel chemistry platform technology. **AACR 2022. Abstract #2138**
2. Cis-activation of PD-1+ effector T cells with dual-targeting immunocytokines generated using a novel chemical conjugation platform. **AACR 2022. Abstract #589**

ABOUT BRIGHT PEAK

Bright Peak is a privately held biotechnology company based in Basel and San Diego. We use our unique ability to chemically synthesize natively folded proteins to create a portfolio of designer immunotherapies for the treatment of cancer and autoimmune diseases. Our pipeline stretches from discovery to IND-enabling and encompasses half-life extended cytokines, antibody-cytokine conjugates and other novel formats.
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