1195 GENERATION OF A HIGHLY POTENT, CIS-SIGNALING PD1-IL2 IMMUNOCYTOKINE USING A NOVEL CHEMICAL SYNTHESIS AND CONJUGATION PLATFORM

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Background Immunocytokines (IC) provide the opportunity to simultaneously address distinct and complementary mechanisms of action and to deliver cytokine payloads to specific immune cells ("cis-signaling") resulting in enhanced antitumor activity. To generate ICs, we developed an entirely different approach based on the site-specific, chemical conjugation of cytokines to existing antibodies (Ab) taken "off-the-shelf". Using an anti PD-1 Ab that is in advanced stages of clinical development, we generated a PD1-IL2 IC to target an "alpha-dead" IL-2 to tumor resident PD-1^{high} effector T cells while simultaneously releasing PD-1-mediated immune suppression.

Methods Our novel protein engineering platform allows the generation of enhanced and conjugatable cytokines that can be used as "payloads" to create ICs. Enhanced cytokine payloads can be chemically conjugated to existing Abs in a site-specific manner without prior Ab engineering yielding ICs with a defined drug-Ab ratio. Using various IL-2 variants that lack IL-2Ra binding ("alpha-dead"), we created PD1-IL2 ICs with different payload potencies. PD1-IL2 ICs were characterized *in vitro* and subsequently *in vivo* determining PK/PD profiles and antitumor efficacy in tumor-bearing transgenic mice expressing human PD-1.

Results Chemical conjugation was found to have no impact on the potency and selectivity of the cytokine payload nor to alter Ab properties such as antigen recognition or binding to either Fc gamma receptors or the neonatal Fc receptor. PD1-IL2 IC shows superior potency *in vitro* due to *cis*-signaling on PD-1^{high} CD8+ T cells and, compared to a non-PD1 targeted control IL-2 IC, it exhibits increased tumor over plasma exposure levels. This translates into strong immune stimulation resulting in superior antitumor efficacy compared to the naked PD-1 Ab, the non-PD1 targeted IL-2 IC, and to the combination of both agents.

Conclusions Bright Peak has developed a unique, chemical conjugation process that enables the generation of ICs via rapid conjugation of synthetically engineered cytokines to the Fc domain of existing human Abs. We generated a PD1-IL2 IC with significantly enhanced potency in PD-1^{high}versus PD-1^{low} T cells. In mice, PD-1-targeting and *cis*-signaling results in high tumor exposure, potent immune stimulation, and strong antitumor efficacy. These results demonstrate the ability of our cytokine engineering platform to generate potent, multi-modal IC therapeutics that synergize complementary mechanisms of action.

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