1080 USING SITE-SPECIFIC CHEMICAL CONJUGATION TO GENERATE SUPERIOR HALF-LIFE EXTENDED OR PD-1-TARGETED FORMATS OF A POTENT IL-18 VARIANT RESISTANT TO IL-18 BINDING PROTEIN

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Background Interleukin-18 (IL-18) is a pro-inflammatory cytokine able to trigger both innate as well as adaptive immune responses. IL-18 is a potent amplifier of IFNg signaling which induces pleiotropic Th1 responses, making it an attractive cytokine for cancer immunotherapy. However, the clinical efficacy of wild-type (wt) IL-18 was limited, potentially due to the upregulation IL-18 binding protein (IL-18BP). IL-18BP is a secreted IFNg-induced antagonist that binds IL-18 with high affinity, prevents its interaction with the IL-18 receptor, and neutralizes IL-18 activity. Hence, we aimed to generate a potent IL-18BP-resistant IL-18 ”payload” that can be turned into optimal therapeutic formats with superior pharmacologic properties or leverage synergistic mechanisms of action (MOA).

Methods By introducing of a minimal set of mutations we generated an optimized human IL-18 that, compared to wt-IL-18, shows enhanced potency and significant resistance to IL-18BP. We further engineered the enhanced IL-18 variant to create a versatile “payload” that can be chemically conjugated in a site-specific manner. Conjugation to polyethylene glycol (PEG) resulted in a half-life extended agent, while conjugation to an anti-PD-1 antibody yielded a PD1-IL18 immunocytokine (IC) allowing exploitation of synergistic MOAs. We then characterized the pharmacologic and anti-tumor properties of these two therapeutic formats *in vitro* and *in vivo*.

Results Compared to wt-IL-18, the conjugatable payload is 300-fold more potent and >600-fold less sensitive to IL-18BP inhibition. Conjugation to a 30 kDa PEG yields a molecule with significantly improved PK properties in mice that is able to strongly activate CD8+ T and NK cells and to potently trigger the release of pro-inflammatory cytokines. PEGylated IL-18 showed strong anti-tumor efficacy which was enhanced in combination with an anti-PD-1 antibody. For the PD1-IL18 IC, the basic properties of the anti-PD-1 antibody and the cytokine payload were largely preserved after chemical conjugation. The PD1-IL18 IC exhibited potent antitumor activity *in vivo* inducing complete responses in the large majority of animals.

Conclusions We generated a potent and IL-18BP-resistant IL-18 payload that can be chemically conjugated to distinct chemical or biological entities in a site-specific manner to generate unique IL-18-based therapeutic formats with desirable pharmacological properties. Both the PEGylated half-life extended IL-18 and the PD1-IL18 IC are well tolerated, show encouraging efficacy in pre-clinical models, and have the potential to be next-generation enhanced IL-18 therapeutics.

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