1359 HALF-LIFE EXTENDED ENGINEERED IL18 VARIANTS THAT ESCAPE THE NEGATIVE REGULATION OF IL18BP

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Background Interleukin-18 (IL18) is a proinflammatory cytokine secreted by monocytes and macrophages to promote T and NK cells activation. While wild type IL18 was shown to be efficacious in inhibiting tumor growth in animal models, limited efficacy was observed for recombinant wild type IL18 in clinical trials. IL18 binding protein (IL18BP) is a decoy receptor for IL18 that binds to IL18 with much higher affinity than IL18 receptors (IL18Ra and IL18Rb). IL18BP is upregulated upon IL18 treatment and acts as a negative feedback for IL18 signaling pathway, which likely contributes to the limited efficacy observed for wild type IL18 in clinic.

Methods Engineered IL18 that escape the negative regulation of IL18BP could greatly improve the efficacy of IL18 in clinic. Sutro's ribosome display technology and XpressCF expression platform enabled fast engineering and testing cycles of IL18 variants. Ribosome display library was constructed based on site directed mutations and selected for IL18Ra binding in the presence of IL18BP. The resulting IL18 variants were further screened for IL18 pathway activation and thermostability. To increase the half-life of IL18 variants, a non-natural amino acid was introduced at an optimized site in the sequence to enable conjugation to a PEG molecule. Additional protein engineering was also employed to improve the expression, stability and PK profile of our IL18 variants.

Results IL18 variants that maintained binding to IL18Ra in the presence of IL18BP were identified from the ribosome display library. In biacore binding assay, the variants showed higher binding affinity to IL18 receptor (IL18Ra) compared to wild type IL18, while no binding observed to IL18BP. The identified IL18 variants induced potent IFNg secretion by human PBMCs from healthy donors, which was not affected by high concentration of IL18BP. Conjugation of PEGs to the nnAA inserted into an optimized site in the IL18 sequence enabled half-life-extension. Additional mutations were introduced on the IL18 variants to stabilize the molecule, which also improved the expression, thermal stability and serum clearance profile in mice. The final Sutro IL18 lead activates T and NK cells in the presence of high concentration of IL18BP and exhibited favorable PK profile in mice.

Conclusions Sutro's engineered IL18 variant induces potent T and NK cells activation without the negative regulation of IL18BP. The favorable immune stimulation, pharmacokinetics and adjustable half-life extension make it a compelling product concept for future cytokine therapy in broad oncology indications.

Ethics Approval All in vivo procedures were conducted in compliance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Sutro Biopharma

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