1060 OPTIMALLY ENGINEERED IL18 FC-FUSION PROTEINS BALANCE POTENCY AND PHARMACOKINETICS TO PROMOTE STRONG ANTI-TUMOR ACTIVITY

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Background Interleukin-18 (IL18) promotes expansion, survival, and cytotoxicity of T and NK cells. Tumor-experienced T cells upregulate the heterodimeric IL18 receptor as evidenced by gene expression correlation between immune checkpoints and IL18 receptor in the TCGA. Preclinical studies with wild-type IL18 have demonstrated anti-tumor activity in animal models, including synergy with immune checkpoint inhibitors and CAR-T therapy. However, IL18 induces a negative feedback loop with IL18 binding protein (IL18BP), a picomolar-affinity natural inhibitor. As IL18BP is also correlated with immune checkpoint and T cell genes in the TCGA, nullifying its inhibitory effects is important to the clinical success of IL18 treatment. Our previous experience with IL2 and IL15 has validated the approach of expanding therapeutic capability by balancing potency, pharmacokinetics, and tolerability; here, we apply this approach to IL18.

Methods We generated monovalent IL18-Fc fusions using our XmAb® heterodimeric Fc platform and introduced substitutions that improved cytokine stability and reduced affinity toward the IL18 receptor and IL18BP. Variants were evaluated for PD-L1 induction in KG-1 cells and in cynomolgus monkeys. Surrogate mouse IL18 (mIL18) heterodimeric mouse Fc fusions were generated and screened for substitutions with similarly modified properties. Several bispecific IL18 × checkpoint targeting molecules were also constructed and selected for improved activity in a mixed lymphocyte reaction. Anti-tumor effects of the surrogate variants with/without the bispecific were assessed in a CT26 syngeneic mouse model.

Results The native thermostability of wild-type IL18 was improved to 65 °C by introducing a disulfide bridge, providing benefits to expression yield, solution behavior, and mouse pharmacokinetics. Introducing substitutions along the receptor and IL18BP interfaces generated a series of potency variants exhibiting 10, 100, and >300-fold reduced PD-L1 induction in KG-1 cells versus wild-type with knocked-out IL18BP binding. A similar series of stabilized mIL18 surrogate variants was generated.

In a CT26 syngeneic mouse model, treatment with engineered mIL18-Fc fusions led to impressive tumor growth inhibition in a dose- and potency-dependent manner, significantly outperforming wild-type mIL18-Fc. Additionally, a bispecific targeting potency-reduced mIL18 to mPD1 demonstrated remarkable tumor growth inhibition, matching higher potency untargeted mIL18-Fc molecules at similar doses. In monkeys, reduced-potency variants demonstrated improved tolerability with similar pharmacodynamics compared to variants closer to wild-type potency. Overall, the variant with 100-fold potency reduction possessed an optimal balance of anti-tumor response, pharmacokinetics, and tolerability.

Conclusions Stabilized, potency-reduced, IL18BP-insensitive mono- and bispecific IL18-Fc fusions demonstrated robust anti-tumor activity in a surrogate mouse model and improved pharmacokinetics in cynomolgus monkeys compared to wild-type IL18.

Ethics Approval All mouse and non-human primate studies were approved by the respective Institutional Animal Care and Use Committees of the testing facilities.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1060