Interleukin-18 Engineered For Resistance to IL-18 Binding Protein (IL-18BP) and Half-Life Extension to Enhance Its Therapeutic Potential

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Take Away

- Half-life enhanced IL-18 variants with resistance to IL-18BP neutralization demonstrate a durable IFN-γ and T_H1 cytokine response in preclinical models, showing promise as a potential cancer immunotherapy
- A balance of potency and PK enhancement is being pursued to develop a best-in-class IL-18-based therapeutic

Conclusions

- IL-18 variants with resistance to high levels of IL-18BP have been generated, serving as the foundation of an immune-oncology approach for cancer therapy
- Fusion of IL-18BP resistant IL-18 variants to half-life enhancing scaffolds improved *in vivo* exposure in preclinical models
- IL-18BP resistant, half-life extended IL-18 variants stimulated durable increases in IFN-γ and T_H1 chemokines in preclinical *in vivo* models
- Because IFN-γ signatures correlate with clinical efficacy of Checkpoint Inhibitor (CPI) therapy,[1] an IL-18 therapeutic may complement CPI immunotherapy
- IL-18BP resistant, half-life enhanced IL-18 variants show promise for development as a cancer immunotherapy

Background

Interleukin-18 (IL-18) is a potent immune-stimulating cytokine that aligns with effective tumor immunotherapy

Discovered as IFN-γ inducing T_H1 polarizing cytokine, IL-18 has many immunological attributes associated with effective cancer immunotherapy.[2] Activation and expansion of antigen-experienced $CD8^+$ T-cells and NK cells promote key cellular mediators of direct tumor killing. Amplifying secretion of IFN-γ further supports development of anti-tumor immune responses. Additional effects, including re-invigoration of dysfunctional T-cells[2] and enhancement of dendritic cell antigen presentation indicate that IL-18 is poised to impact multiple nodes of the cancer-immunity cycle in a positive manner for cancer immunotherapy.

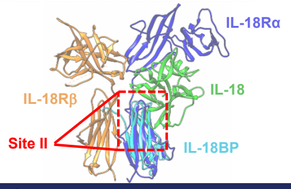
IL-18 clinical potential is limited by IL-18 binding protein (IL-18BP) – a secreted high-affinity IL-18 decoy receptor

IL-18BP binds to IL-18 and neutralizes interaction with IL-18Rα (Figure 1), thereby down-regulating the immunological activity of IL-18. Indeed, rapid induction of IL-18BP limited the efficacy of recombinant wild-type IL-18 (rIL-18) in clinical trials.[3] Thus, we sought to overcome the limitations of rIL-18 by applying protein engineering solutions.

Protein engineering aims to unleash the therapeutic potential of IL-18 for cancer immunotherapy

- Overcome IL-18 neutralization from IL-18BP by introducing mutations into IL-18 (Figure 1) which negate binding to IL-18BP and retain full IL-18 activity
- Fusion to pharmacokinetic (PK)-enhancing protein scaffolds for half-life extension

Figure 1. ***Overlay of ribbon diagrams depicting interaction of IL-18 (green) with IL-18Rα (blue) and IL-18Rβ (orange) with the insertion of IL-18BP (cyan).*** **Protein engineering for resistance to IL-18BP focused on site II of IL-18 (red box) which interacts with IL-18BP and IL-18Rα.**



Methods

Protein Engineering

1. *IL-18BP resistance (Figure 1)*

By utilizing rational design (computational modeling) and directed evolution (yeast display), we introduced mutations into IL-18 and screened for the best mutation combinations based on binding assays (IL-18BP) and biological activity (IL-18 reporter assays and primary cell IFN-γ secretion assays).

2. *Half-life extension*

To further enhance the pharmacological properties, IL-18BP-resistant IL-18 variants were fused to half-life enhancing protein scaffolds, i.e., Fc and serum albumin (SA) to enhance *in vivo* half-life and exposure.

IL-18BP Binding and *In Vitro* Bioassays with Primary Cultures of Human or Mouse Immune Cells

- ***IL-18BP binding by Octet Biolayer Interferometry***
- ***Human variants:*** **IFN-γ secreted from IL-18-stimulated human PBMC cultures ± 300nM recombinant human IL-18BP**
- ***Mouse variants:*** **IFN-γ secreted from IL-18-stimulated mouse splenocyte cultures ± 300nM recombinant mouse IL-18BP**

In Vivo Pharmacokinetics of Mouse and Human Variants Fused to Fc and Serum Albumin Scaffolds

- ***Single subcutaneous injection of mouse orthologs in C57BL/6 mice***
- ***Single subcutaneous injection of human variants in immunocompromised mice "humanized" with human immunocytes (CD34+ stem cells)***

Longitudinal Th1/Th2 Serum Cytokine Response to Mouse Orthologs

- ***Single subcutaneous injection of mouse orthologs in C57BL/6 mice followed by longitudinal collection of plasma for cytokine measurement using a mouse T_H1/T_H2 MSD cytokine panel***

Longitudinal Th1/Th2 Serum Cytokine Response to Human Variants

- ***Single subcutaneous injection of human variants in "humanized" mice followed by longitudinal collection of serum for cytokine measurement using a human T_H1/T_H2 MSD cytokine panel***

Results

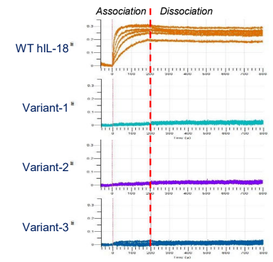
Human variants do not bind IL-18BP, show varied potency, and are resistant to IL-18BP suppression

- Human variants do not bind human IL-18BP (Figure 2A – Octet)
- Human variants show a range of potencies and are resistant to suppression by 300 nM IL-18BP (Figure 2B – PBMC Assay)

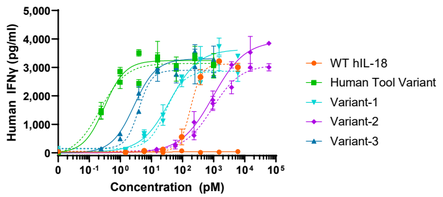
Figure 2 (A-B). ***Human variant binding to IL-18BP (A) and potency in the presence or absence of 300nM IL-18BP (B)***

A. Binding to IL-18BP by Octet-BLI

Note: hIL18BP tested up to 1μM.



B. Concentration-response curves for human IL-18 variant stimulated IFN-γ secretion by human PBMCs cultured in the presence (solid line) or absence (dashed line) of 300nM IL-18BP.



Mouse orthologs are resistant to IL-18BP and show varied potency as "naked" molecules and Fc- or MSA-fusion proteins

- Mouse orthologs are maximally resistant to suppression by mouse IL-18BP
- Mouse orthologs show varied potency when fused to half-life enhancing scaffolds

Figure 3. ***Mouse orthologs are resistant to IL-18BP suppression (A) and have varied potency (B)***

A. IFN-γ secretion from splenocytes stimulated with wild-type IL-18 and IL-18 mouse orthologs in the absence (dark blue) and presence (light blue) of a high IL-18BP concentration

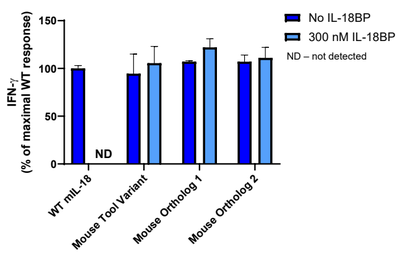
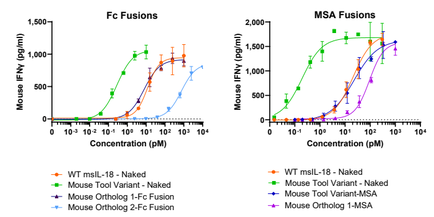


Figure 3B. Concentration-response curves for mouse ortholog IL-18 stimulated IFN-γ secretion from cultured splenocytes

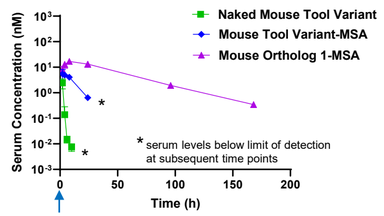


Pharmacokinetics of IL-18BP-resistant IL-18 variants fused to serum albumin and Fc half-life enhancing scaffolds

- IL-18BP resistant IL-18 variants fused to half-life enhancing scaffolds display increased peripheral blood exposure relative to high potency naked IL-18BP-resistant tool variants (Figures 4-5).
- Mouse variants with different potency fused to the same half-life enhancing scaffolds display distinct peripheral blood exposure (Figure 4A-B).
- Half-life extension might be the result of both the scaffolds and the affinity between IL-18 variants and their receptors

Figure 4. ***Pharmacokinetics of mouse orthologs fused to MSA (A) and Fc (B) scaffolds in wild-type C57BL/6 mice***

A. Mouse Tool Variant (mTV, blue diamond) and Mouse Ortholog 1 fused to MSA scaffold (purple triangle) relative to "naked" mTV (green square)



B. Mouse Ortholog 1 (blue triangle) and Mouse Ortholog 2 (cyan circle) fused to Fc scaffold relative to "naked" mTV (green square)

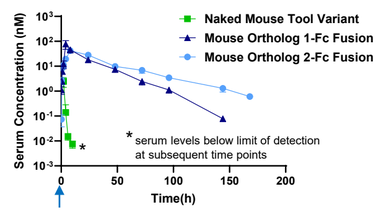
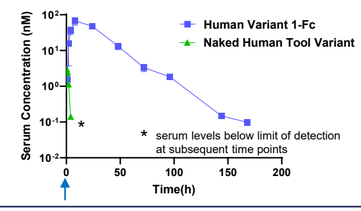


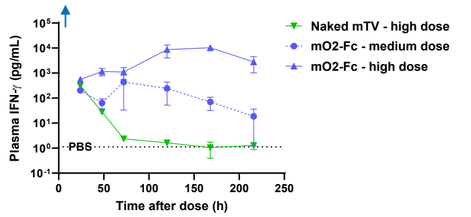
Figure 5. ***Pharmacokinetics of human variants in humanized mice.***
PK of Naked IL-18BP resistant Human Tool Variant (green triangle) and IL-18BP resistant Human Variant 1 fused to human Fc scaffold (blue square) following subcutaneous injection of humanized mice.



Plasma cytokine response after subcutaneous injection of half-life enhanced IL-18BP resistant mouse IL-18 variant

- Half-life enhanced mouse variants exhibited more durable plasma IFN-γ (T_H1 cytokine) responses relative to a naked mouse variant (Figure 6) following subcutaneous administration.

Figure 6. ***Longitudinal plasma IFN-γ response to mouse ortholog 2 fused to Fc scaffold (mO2-Fc, blue) relative to a naked IL-18BP resistant mouse tool variant (mTV, green)***

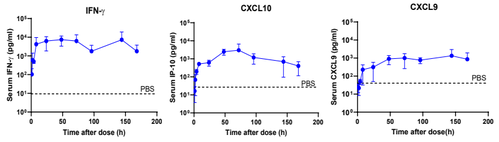


Serum cytokine response after subcutaneous injection of half-life enhanced IL-18BP resistant human IL-18 variant

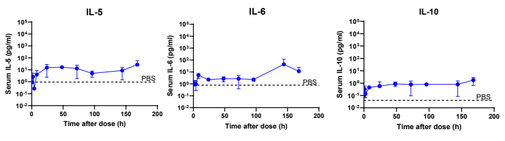
- Human variants stimulated a durable T_H1 dominant cytokine response after subcutaneous injection in humanized mice

Figure 7. ***Longitudinal T_H1 (A) and T_H2 (B) serum cytokine levels after a single subcutaneous injection of human Variant 1-Fc fusion in humanized mice***

A. Durable T_H1 cytokine response after subcutaneous injection of human Variant 1-Fc fusion in humanized mice



B. Minimal serum T_H2 cytokine response after subcutaneous injection of human Variant 1-Fc fusion in humanized mice



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2. Zhou et al. (2020) Nature 583:609-14.
3. Robertson et al (2008). Clinical Cancer Research, 14(11), 3462-3469.