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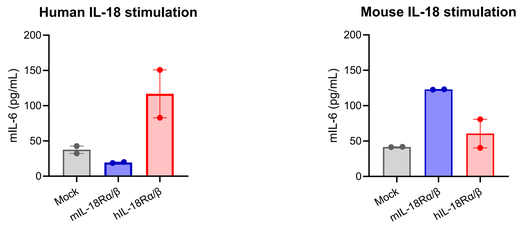
Novel preclinical mouse models to investigate IL-18-targeting therapies

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Abstract #618

Background: Interleukin-18 (IL-18) is an immunomodulatory cytokine with growing relevance in immuno-oncology due to its capacity to potentiate both innate and adaptive immune responses. It promotes IFN-γ production in T and natural killer (NK) cells, thereby amplifying anti-tumor immunity. Recent evidence suggests that IL-18–based therapies may help overcome resistance in immunologically "cold" tumors[1] and could be combined with checkpoint inhibitors, such as PD-1 blocking antibodies to overcome IL-18BP natural inhibition. Robust preclinical evaluation of human targeted IL-18 therapeutics requires models that faithfully recapitulate the human IL-18/IL-18R axis and enable assessment of combination strategies with PD-1–targeting therapies. Therefore, we generated and characterized humanized mouse models expressing human IL-18, human IL-18 receptor, and human PD-1 (genO-hIL-18/hIL-18R and genO-hIL-18/hIL-18R/hPD-1).

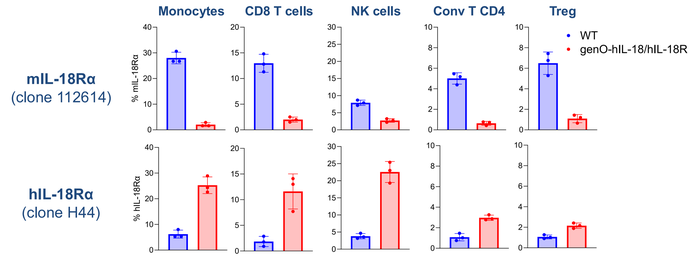
1. MOUSE IL-18 PARTIALLY INDUCES SIGNALING THROUGH HUMAN IL-18Rα/β, REQUIRING HUMANIZATION OF BOTH IL-18 AND IL-18Rα/β TO MAINTAIN FUNCTIONAL IL-18/IL-18R AXIS



B16F10 cells stably expressing mouse or human IL-18Rα/β were stimulated with recombinant mouse or human IL-18 (10 ng/mL) for 24h. Mouse IL-6 secretion in the supernatant was then assessed by ELISA.

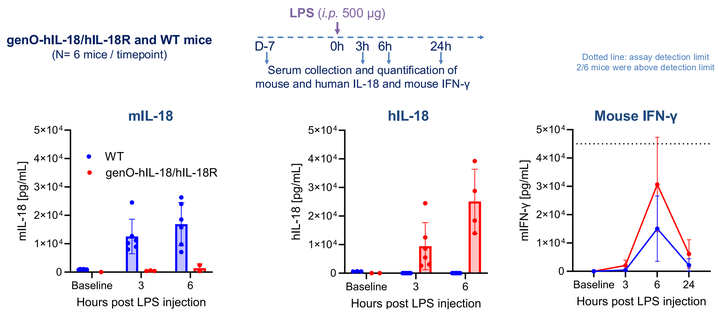
✓ **A double humanized genO-hIL-18/hIL-18R model has been generated to maintain functional IL-18/IL-18R axis**

2. HUMAN IL-18Rα EXPRESSION ON IMMUNE CELLS FROM genO-hIL-18/hIL-18R MICE



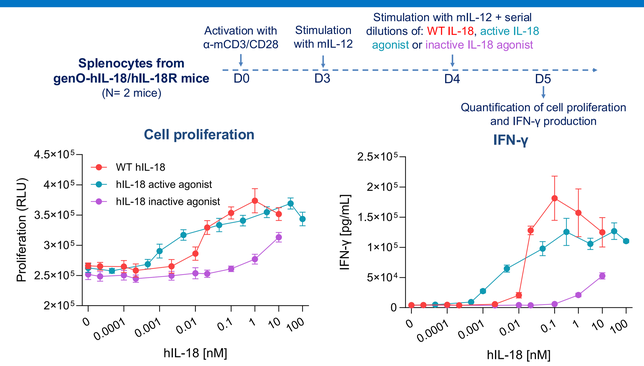
Freshly isolated splenocytes, gated on single live CD3⁻CD19⁻NKp46⁻CD317⁻Ly-6G⁻Ly-6C⁺CD11b⁺ (Monocytes/macrophages), CD3⁻CD19⁻NKp46⁺ (NK cells), CD3⁺TCRβ⁺TCRγδ⁻CD4⁻CD8⁺ (CD8 T cells), CD3⁺TCRβ⁺TCRγδ⁻CD4⁺CD25⁻FoxP3⁻(convCD4), CD3⁺TCRβ⁺TCRγδ⁻CD4⁺CD25⁺FoxP3⁺ (Treg).

3. *IN VIVO* LPS-INDUCED IL-18 AND IFN-γ RELEASE



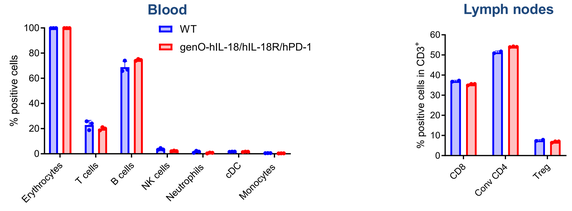
✓ **LPS-induced hIL-18 secretion in genO-hIL-18/hIL-18R mice mirrors mIL-18 secretion in WT mice**
✓ **IFN-γ is secreted in a similar kinetic in genO-hIL-18/hIL-18R and WT mice**
✓ **mIL-18BP expression is increased by LPS similarly in genO-hIL-18/hIL-18R and WT mice** *(data not shown)*

4. *EX VIVO* EFFICACY OF IL-18 AGONISTS



✓ **Splenocytes from genO-hIL-18/hIL-18R mice treated with engineered hIL-18 exhibit enhanced cell proliferation and increased IFN-γ secretion compared to inactive hIL-18**

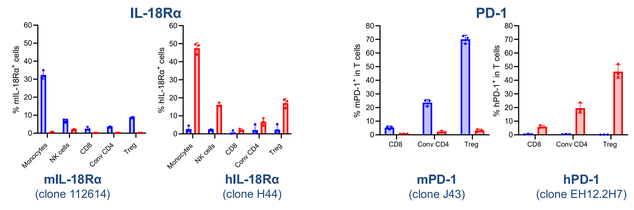
5. GENERATION OF genO-hIL-18/hIL-18R/hPD-1 MODEL TO ENABLE ASSESSMENT OF COMBOTHERAPIES



Gated on single live cells, Erythrocytes (TER119⁺), T cells (CD45⁺CD3⁺CD19⁻), B cells (CD45⁺CD3⁻CD19⁺), NK cells (CD45⁺CD3⁻CD19⁻NKp46⁺), Neutrophils (CD45⁺CD3⁻CD19⁻Ly-6G⁺), cDC (CD45⁺CD3⁻CD19⁻Ly-6G⁻CD11c⁺) and Monocytes (CD45⁺CD3⁻CD19⁻Ly-6G⁻Ly-6C⁺CD11c⁻CD11b⁺). T cell subsets: CD8 (CD3⁺CD4⁻CD8⁺), Conv CD4 (CD3⁺CD4⁺CD25⁻FoxP3⁻) and Treg (CD3⁺CD4⁺CD25⁺ FoxP3⁺).

✓ **Humanization of IL-18, IL-18R and PD-1 does not alter immune cell distribution**
✓ **Similar results in spleen and bone marrow** *(data not shown)*

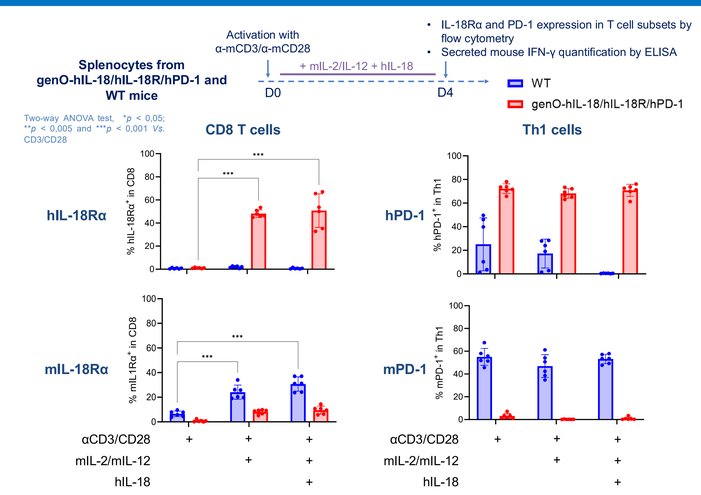
6. IL-18Rα AND PD-1 EXPRESSION ON IMMUNE CELLS FROM genO-hIL-18/hIL-18R/hPD-1 MICE



Freshly isolated splenocytes, gated on single live CD3⁻CD19⁻NKp46⁻CD317⁻Ly-6G⁻Ly-6C⁺Ly-6C⁺CD11b⁺ (Monocytes/macrophages), CD3⁻CD19⁻NKp46⁺ (NK cells), CD3⁺TCRβ⁺TCRγδ⁻CD4⁻CD8⁺ (CD8 T cells), CD3⁺TCRβ⁺TCRγδ⁻CD4⁺CD25⁻FoxP3⁻(convCD4), CD3⁺TCRβ⁺TCRγδ⁻CD4⁺CD25⁺FoxP3⁺ (Treg).

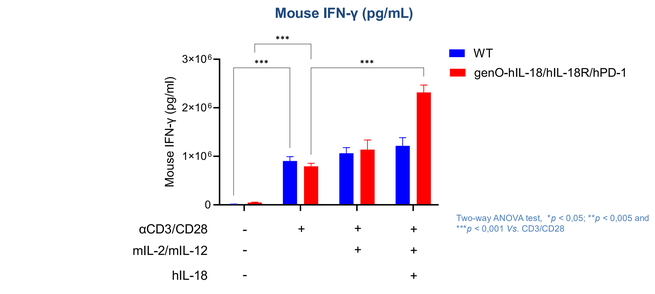
✓ **Human IL-18Rα and PD-1 are detected at similar levels than their murine counterparts, indicating preserved immune homeostasis**

7. HUMAN IL-18Rα AND PD-1 REGULATION AND FUNCTIONALITY ARE UNALTERED IN genO-hIL-18/hIL-18R/hPD-1 MICE



Gated on single live CD45⁺CD3⁺ cells, Th1 (CD8⁻CD4⁺CD25⁺T-bet⁺FoxP3⁻) and CD8 T cells (CD8⁺CD4⁻CD25⁺).

✓ **Both IL-18Rα and PD-1 expression are modulated upon activation with anti-CD3/CD28, suggesting that humanization do not alter the regulation of their expression**



✓ **hIL-18R is functional**
✓ **More potent effect of hIL-18 on humanized mice is likely due to the higher expression of IL-18Rα observed on CD8 and Th1 cells upon treatment with IL-2/IL-12**

Conclusion: Altogether, these results support the suitability of the genO-hIL-18/hIL-18R/hPD-1 mouse model for assessment of IL-18-based immunotherapies and their synergy with PD-1 blockade.

Reference: 1. Kessel C, Rossig C, Abken H. Weal and woe of interleukin-18 in the T cell therapy of cancer. Journal for ImmunoTherapy of Cancer. 2025;13:e010545. https://doi.org/10.1136/jitc-2024-010545