IL-18 Surrogate Cytokine Agonists (SCAs): Overcoming Limitations of IL-18 Cancer Immunotherapy

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Synthekine

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Overview

Cytokines are key regulators of the immune system and important targets for both immuno-oncology as well as autoimmune diseases, but their therapeutic use has been limited due to dose-limiting toxicities[1]. IL-18 is a pro-inflammatory cytokine capable of activating a broad spectrum of immune cells including innate myeloid and adaptive lymphoid compartment resulting in interferon gamma secretion and type I response amplification[2]. Recombinant IL-18 has been evaluated for the treatment of cancer in both preclinical studies and clinical trials[3,4]. In clinical trials IL-18 has shown good tolerability but modest efficacy possibly due to inhibition by IL-18 binding protein (BP)[3-5]. Here we describe bispecific, VHH-based surrogate cytokine agonists (SCAs) capable of signaling through the IL-18 receptor while bypassing the IL-18BP inhibition mechanism and overcoming IL-18's notoriously poor drug-like properties. We believe IL-18 SCAs show potential for development of cytokine therapeutics with improved efficacy and reduced toxicity.

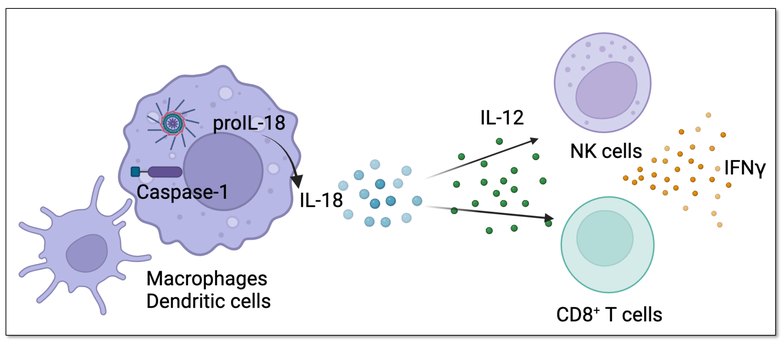

Figure 1: IL-18 is a pro-inflammatory cytokine produced by various cell types. Together with IL-12, IL-18 triggers the release of IFN-γ from NK- and CD8⁺ T-cells and thereby boosts both innate and adaptive anti-cancer immune responses.

SCA-mediated IL-18 Receptor Dimerization as an Approach to Escape Inhibition via IL-18BP

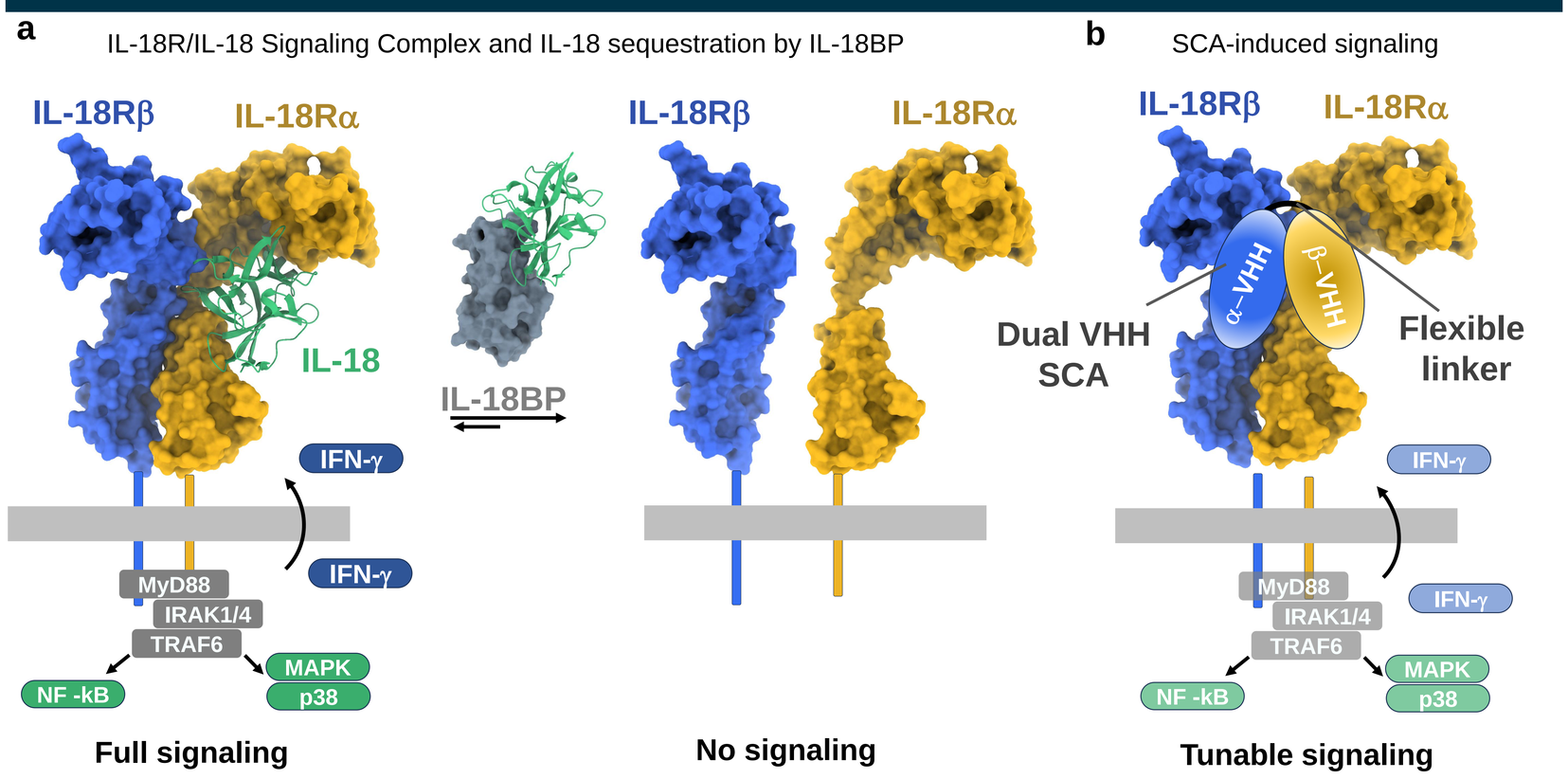

Figure 2: (a) IL-18 induces the formation of an active ternary complex with IL-18 Receptor α and Receptor β (IL-18Rα and IL-18Rβ)[6]. Activation causes secretion of the IL-18 Binding Protein (IL-18BP), a decoy-receptor that prevents signaling through the IL-18R through IL-18 sequestration[5,7]. **(b)** Our Surrogate Cytokine Agonists (SCAs) are bispecific, dual-VHHs connected by a flexible linker and can induce active receptor dimers while bypassing inhibition through IL-18BP. PDB codes: 3WO4, 7AI7

"Med Chem" Approach to Discovery of SCAs at Synthekine

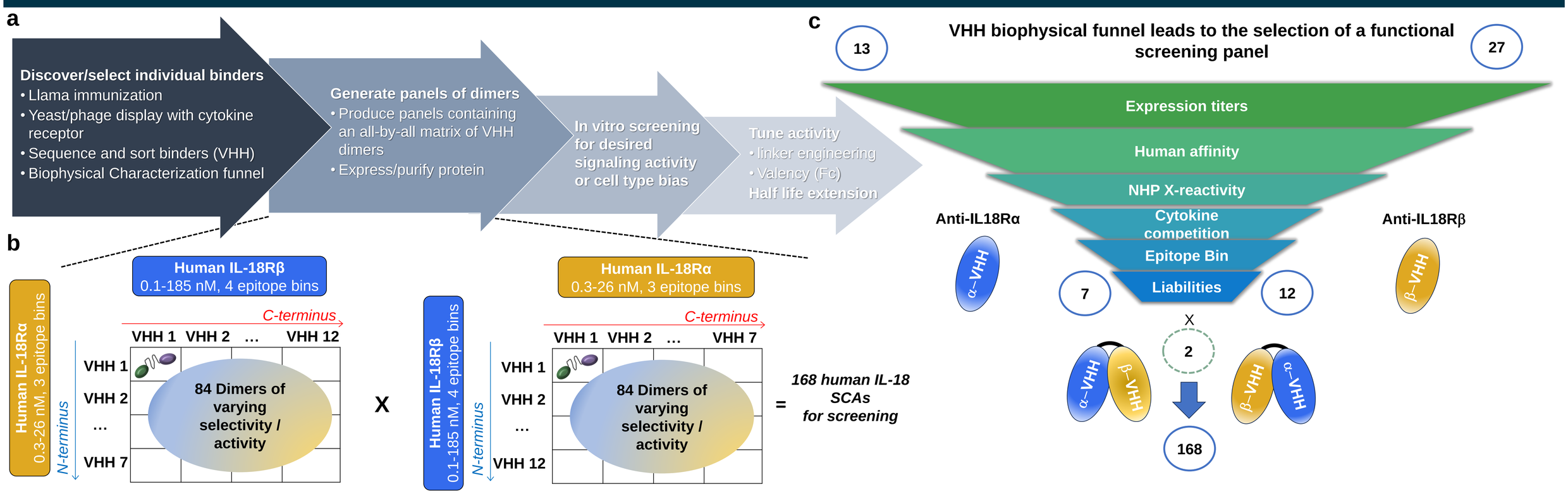

Figure 3: (a) "Med Chem" approach overview to discovery of SCAs at Synthekine from Llama immunization to in vitro screening and tuning. **(b)** Overview of dual-VHH SCA-panel generation from individual VHHs. **(c)** Biophysical funnel that guides selection of VHHs for a functional screening panel of SCAs.

IL-18 SCAs Exhibit Tunable Biological Activity *in vitro*

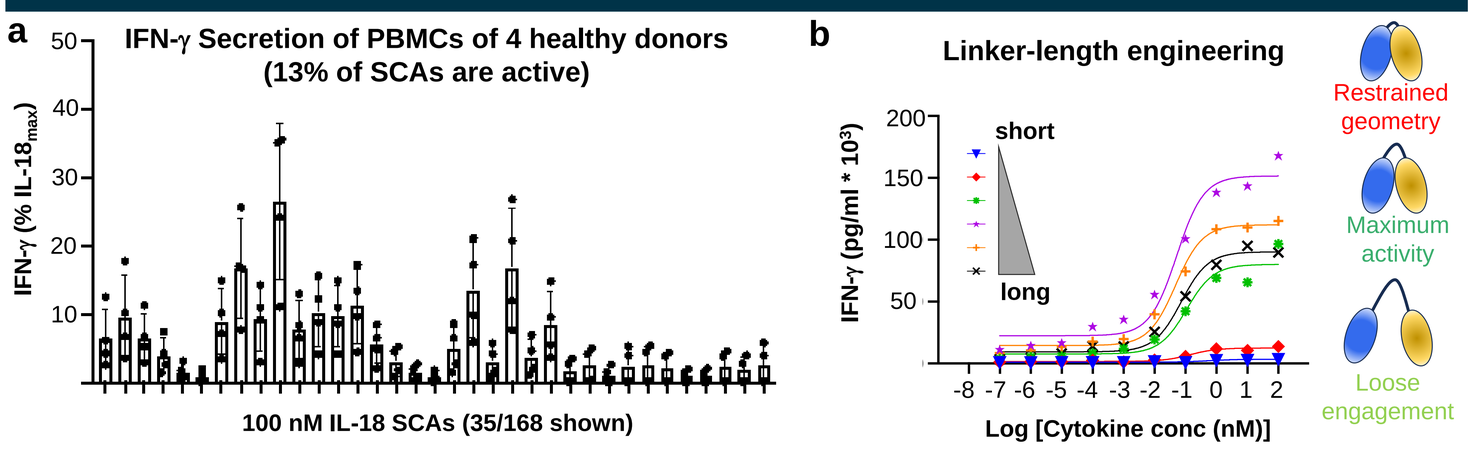

Figure 4: (a) 168 IL-18 SCAs with intermediate VHH linker length were screened for activity in an IFN-γ release assay using human PBMCs isolated from healthy donors. PBMCs were incubated for 24 hours with IL-18/IL-18 SCA in the presence of 10 ng/ml IL-12. IFN-γ concentration in the supernatant was measured by MSD. **(b)** Four selected SCAs with various inter-VHH linkers were tested for activity in the same IFN-γ release assay on human PBMCs.

IL-18 SCAs Evade IL-18BP Inhibition *in vitro*

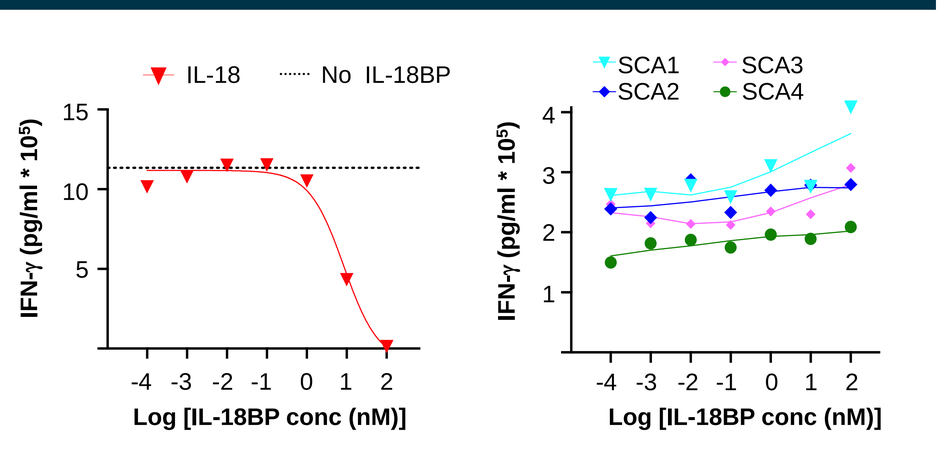

Figure 5: Human PBMCs were incubated for 48 hours with 10 nM IL-18 or 100 nM of selected IL-18 SCAs in the presence of 10 ng/mL IL-12 and variable concentrations of IL-18BP. IFN-γ concentrations were measured by MSD.

IL-18 SCA Format Engineering Reveals Tunable Biological Activity *in vitro*

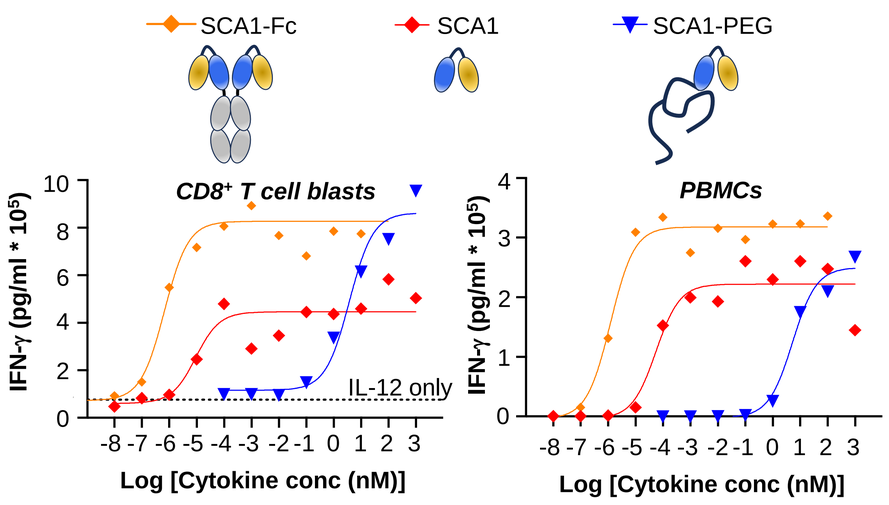

Figure 6: A selected IL-18 SCA with intermediate linker-length was fused to an antibody Fc fragment (SCA1-Fc) or PEGylated (SCA1-PEG) and activity was compared to unmodified SCA1 via IFN-γ release assay using human CD8⁺ T-cell blasts and PBMCs as described in Figure 4.

IL-18 SCAs Activate Human NK and T Cells in NSG Mice and Accelerate GvHD

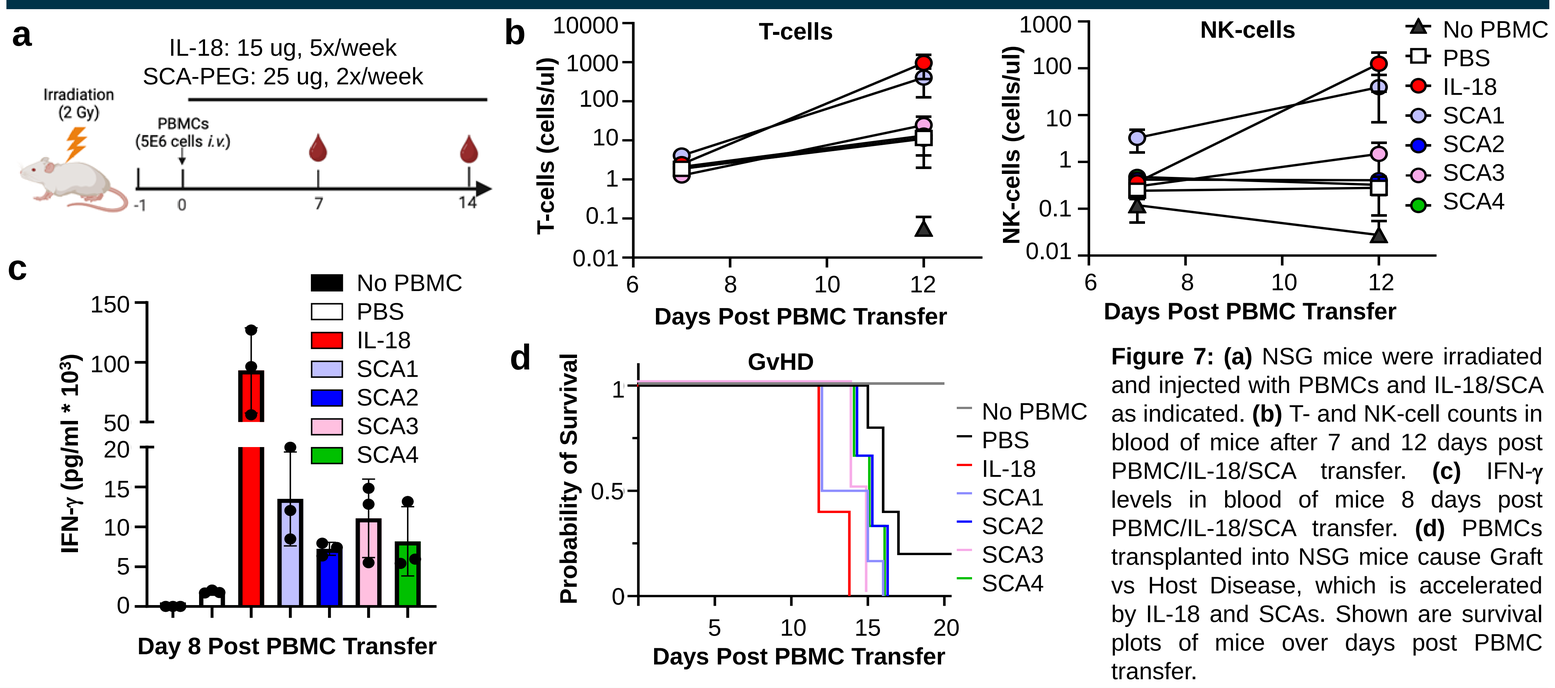

Figure 7: (a) NSG mice were irradiated and injected with PBMCs and IL-18/SCA as indicated. **(b)** T- and NK-cell counts in blood of mice after 7 and 12 days post PBMC/IL-18/SCA transfer. **(c)** IFN-γ levels in blood of mice 8 days post PBMC/IL-18/SCA transfer. **(d)** PBMCs transplanted into NSG mice cause Graft vs Host Disease, which is accelerated by IL-18 and SCAs. Shown are survival plots of mice over days post PBMC transfer.

References:
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