Potency-reduced and extended half-life IL12 heterodimeric Fc-fusions exhibit strong anti-tumor activity with potentially improved therapeutic index compared to native IL12 agents

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Introduction

- Interleukin-12 (IL12) is a heterodimeric proinflammatory cytokine produced by activated antigen-presenting cells that induces differentiation of Th1 cells and increased proliferation and cytotoxicity of T and NK cells.
- Stimulation of these cells by IL12 leads to production of high levels of IFNγ. These immune-stimulating aspects of IL12 are promising for cancer treatment and may help to convert immunologically suppressed "cold" tumors into inflamed "hot" tumors.
- Preclinical studies in mice revealed that IL12 can have a dramatic effect on shrinking syngeneic tumors; however, clinical studies in humans have resulted in severe toxicity and a small therapeutic window, limiting response rates.
- Prior work at Xencor demonstrated that reduced-potency IL15/IL15Rα-Fc fusion proteins exhibited superior pharmacokinetics, pharmacodynamics, and safety in non-human primates through reduction of receptor-mediated clearance. Applying similar principles to IL12, we created IL12 heterodimeric Fc-fusions (IL12-Fc) with reduced potency in order to improve tolerability, slow receptor-mediated clearance, and prolong half-life compared to native IL12 agents.

Potency-reduced IL12-Fc are engineered for optimal activity and extended serum half-life

Mechanism and anti-tumor effects of IL12

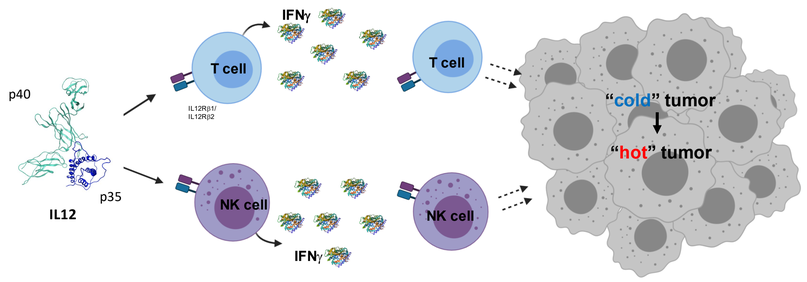


Figure 1. IL12 is a heterodimer consisting of p40 and p35 subunits that signals through the STAT4 pathway. IL12 may help to turn non-inflamed, cold tumors into inflamed, hot tumors that are amenable to checkpoint inhibitor therapy by inducing proliferation of NK and T cells and by production of IFNγ.

Design and analytical characterization of IL12-Fc heterodimers

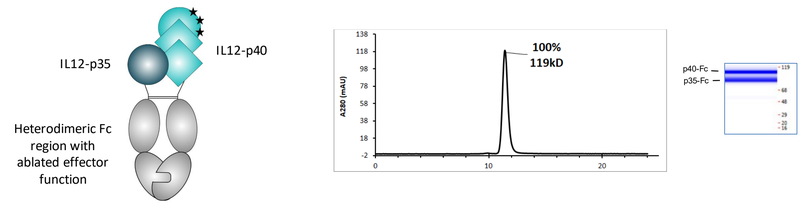


Figure 2. Schematic of IL12-Fc heterodimers and analytical characterization. Monovalent IL12 p35/p40 is attached to Xencor's well-validated heterodimeric Fc domain. The IL12 has been engineered for decreased binding to its receptors in order to reduce potency and the Fc domain is modified to eliminate FcγR interactions. The Fc domain may also be modified with Xtend™ Fc technology to promote longer half-life. IL12-Fc heterodimers can be produced in high yields and are purified using standard methods (protein A and IEX chromatography).

IL12-Fc with reduced in vitro potency were engineered in order to improve therapeutic index

Engineering potency-reduced IL12-Fc and in vitro activity

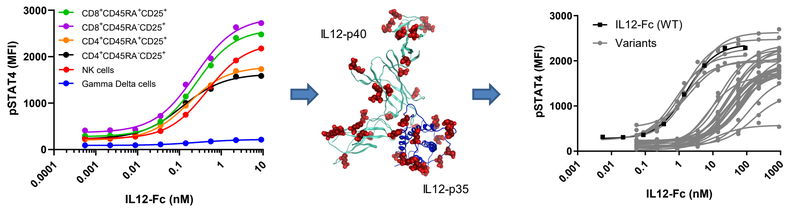


Figure 3. Left: In vitro activity of WT IL12-Fc was assessed on activated human PBMCs by measuring intracellular pSTAT4 by flow cytometry. Middle: A library of amino acid substitutions at putative IL12-receptor-interface positions (in red) was created. Right: The library was screened for reductions of in vitro potency by pSTAT4 (each curve is an IL12 variant).

In vitro pSTAT4 and MLR activity of lead IL12-Fc variants

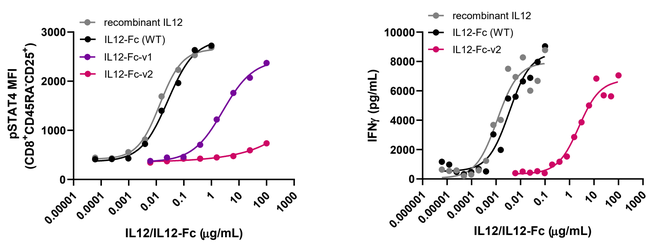
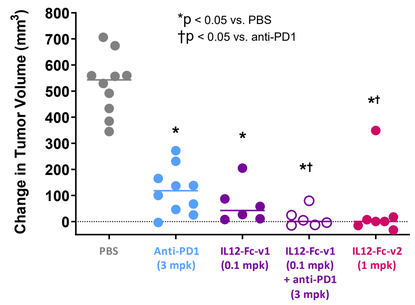


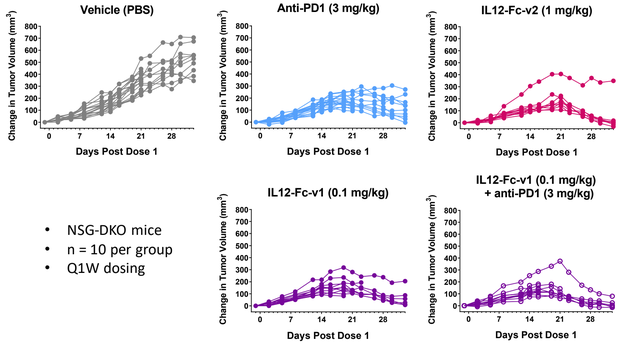
Figure 4. In vitro activity of rIL12, WT IL12-Fc, and lead potency-reduced IL12-Fc were assessed on activated human PBMCs by measuring intracellular pSTAT4 by flow cytometry (left) and IFNγ production in a mixed-lymphocyte reaction (MLR) (right).

Potency-reduced IL12-Fc show strong anti-tumor activity and PD response as single-agent and in combination with anti-PD1

Anti-tumor efficacy at day 33



Tumor volume - Spider plots



- NSG-DKO mice
- n = 10 per group
- Q1W dosing

In vivo pharmacodynamics (PD)

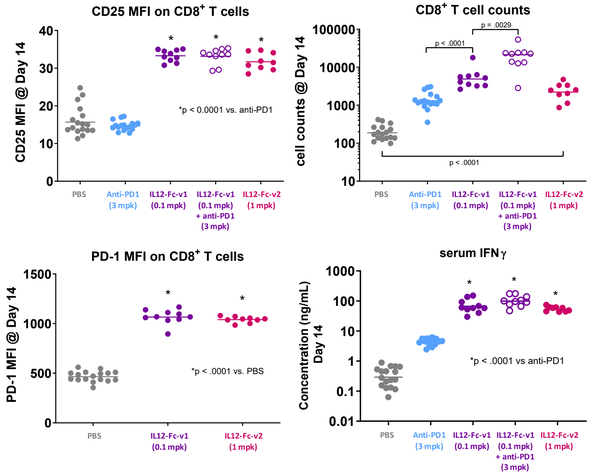


Figure 5. Human IL12 is inactive in mice, so in vivo anti-tumor activity was assessed by engrafting pp65-MCF-7 cancer cells into human PBMC engrafted NSG-DKO mice. Mice were dosed weekly (IP) with the indicated dose of test article. Tumor volume, lymphocyte activation/proliferation, and serum IFNγ production were measured over time. Left and middle panels: Potency-reduced IL12-Fc-v1 demonstrated significant anti-tumor activity as a single-agent at 0.1 mg/kg and stronger activity when combined with anti-PD1. Potency-reduced IL12-Fc-v2 demonstrated significant anti-tumor activity at a 10x higher dose level of 1 mg/kg. Right panel: Treatment with potency-reduced IL12-Fc results in activation and proliferation of CD8+ T cells, increased PD1 expression, and >200-fold increases in serum IFNγ.

Potency-reduced IL12-Fc have antibody-like PK in mice

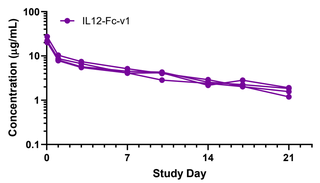


Figure 6. The pharmacokinetics (PK) of potency-reduced IL12-Fc-v1 were evaluated in C57BL6/J mice. N = 4 mice were injected IV with 2 mg/kg IL12-Fc-v1 on Day 0 and drug concentration in serum was measured over time. The estimated half-life ($t_{1/2}$) is approximately 10 days and similar to that of monoclonal antibodies, indicating that IL12-Fc-v1 has a long half-life and favorable stability in the absence of TMDD.

Summary

- IL12 heterodimeric Fc-fusions were engineered with a potency-reduced IL12 in order to improve tolerability, slow receptor-mediated clearance, and prolong half-life in vivo compared to therapeutics using native IL12
- Potency-reduced IL12-Fc demonstrate significant anti-tumor activity concurrent with activation and proliferation of CD8+ T cells, increased PD1 expression, and increased serum IFNγ in mice.
- These results support further testing of potency-reduced IL12-Fc as a potential novel cytokine therapy in cancer patients.

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