Background Interleukin-12 (IL12) is a 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 may help to establish an inflammatory tumor microenvironment critical for anti-tumor responses. Preclinical studies in mice revealed that native IL12 can dramatically shrink syngeneic tumors, however clinical studies in humans 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 to improve tolerability, slow receptor-mediated clearance, and extend half-life.

Methods IL12 is a heterodimeric protein consisting of two subunits, so we engineered IL12-Fc fusions by fusing the IL12p35 subunit to one side of a heterodimeric (inactive) Fc domain, and the IL12p40 subunit to the other side. These Fc-fusions were tuned for optimal activity by introducing amino acid substitutions at putative receptor-interface positions and screening for reductions of in vitro potency. In vitro activity was assessed on human PBMCs by measuring signaling in a STAT4 phosphorylation assay and IFNγ production in a mixed-lymphocyte reaction (MLR). In vivo anti-tumor activity was assessed by engrafting MCF-7 cells into PBMC-engrafted NSG MHC class I and II double-knockout mice and by measuring tumor volume, lymphocyte activation/proliferation, and IFNγ production over time.

Results IL12-Fc were produced with good yield and purity. An IL12-Fc potency series was created, and variants had up to a 10,000-fold reduction in STAT4 signaling potency and IFNγ production in an MLR assay compared to native IL12-Fc. Anti-tumor activity in the huPBMC-MCF7 model was achieved with potency-reduced IL12-Fc as a single-agent and in combination with anti-PD1, with weaker variants maintaining anti-tumor activity at higher dose levels. Analysis of peripheral lymphocytes indicated increased numbers of T and NK cells as well as activation of CD8+ T cells, as evidenced by upregulation of CD25. Increased expression of immune checkpoints including PD1 was also observed. Analysis of serum indicated up to 200-fold increases in IFNγ levels.

Conclusions Combined, these data indicate that potency-reduced IL12-Fc retain strong anti-tumor activity, while potentially overcoming safety and tolerability issues related to small therapeutic index associated with recombinant native IL12 or IL12-Fc agents.