707 IL12 FC-FUSIONS ENGINEERED FOR REDUCED POTENCY AND EXTENDED HALF-LIFE EXHIBIT STRONG ANTI-TUMOR ACTIVITY AND IMPROVED THERAPEUTIC INDEX COMPARED TO WILD-TYPE IL12 AGENTS

[1]Matthew Bernett*, [1]Ke Liu, [1]Christine Bonzon, [1]Michael Hackett, [1]Katrina Bykova, [1]Rumana Rashid, [1]Nicole Rodriguez, [1]Nargess Hassanzadeh-Kiabi, [1]Connie Ardila, [1]Norman Barlow, [1]Irene Leung, [1]Hanh Nguyen, [1]Araz Eivazi, [1]Kendra Avery, [2]Rajat Varma, [1]Umesh Muchhal, [1]John Desjarlais. [1]*Xencor, Inc., MONROVIA, CA, United States*; [2]*Independent Consultant, Monrovia, CA, United States*

Background Interleukin-12 (IL12) is a proinflammatory cytokine that induces differentiation of Th1 cells and increased cytotoxicity of T and NK cells. Stimulation by IL12 leads to production of IFNγ and an inflammatory tumor microenvironment critical for anti-tumor responses. Studies in mice revealed IL12 can dramatically shrink syngeneic tumors, however human clinical studies 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 therapeutic index (TI) in non-human primates (NHP) by reducing receptor-mediated clearance. Applying similar principles to IL12, we created IL12 heterodimeric Fc-fusions (IL12-Fc) with reduced potency to improve TI.

Methods IL12 is a heterodimer of two subunits, so we engineered IL12-Fc fusions by fusing the IL12p35 subunit to one side of a heterodimeric (and inactive) Fc domain, and IL12p40 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 of human IL12-Fc was assessed in huPBMC-NSG-DKO and huCD34+ MCF7 xenograft models. Surrogate mouse potency-reduced IL12-Fc were evaluated in syngeneic tumor models. Tolerability and pharmacodynamic activity were assessed in NHP.

Results An IL12-Fc potency series was created, and variants had up to a 10,000-fold reduction in STAT4 signaling and IFNγ production in an MLR assay compared to wild-type IL12-Fc. Anti-tumor activity was achieved with potency-reduced IL12-Fc as single-agents 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. Increased expression of immune checkpoints including PD1 was also observed. Analysis of serum indicated up to 200-fold increases in IFNγ levels. Surrogate potency-reduced IL12-Fc had improved tolerability and greater selectivity of IFNγ production in tumors compared to spleen and less production of IL10 compared to wild-type IL12-Fc. In NHP, potency-reduced IL12-Fc had superior exposure with slower, more sustained accumulation of IFNγ and IP10, and a more gradual dose-dependent peak response, as well as more sustained margination of T and NK cells compared to wild-type IL12-Fc.

Conclusions Potency-reduced IL12-Fc retain strong anti-tumor activity, while potentially overcoming safety and tolerability issues related to narrow TI associated with wild-type IL12 or IL12-Fc agents.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.707