IL-12 as a Cancer Immunotherapy

Xilio Therapeutics is pioneering an innovative platform to progress a pipeline of novel, tumor-activated immunotherapies that have the potential to significantly improve outcomes for patients living with cancer. Xilio is focused on developing tumor-activated cytokine immunotherapies with exemplary clinical activity and tolerability.

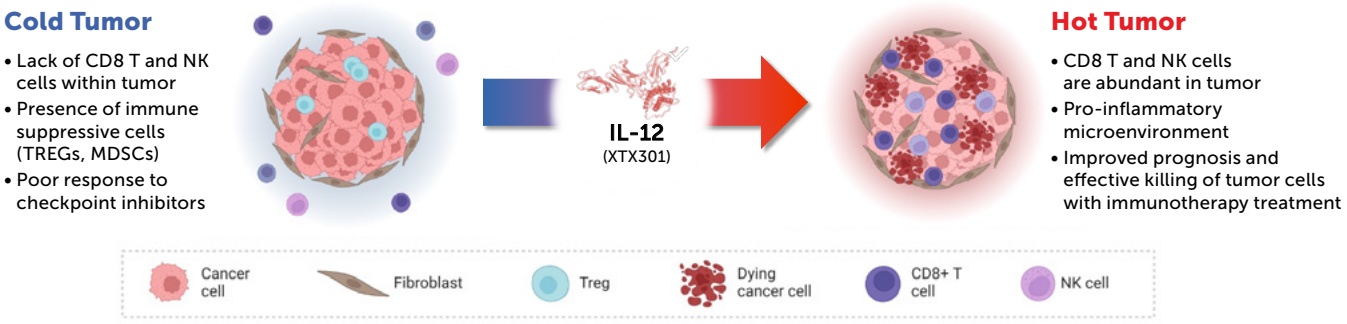
What is IL-12?

Cytokines are small proteins that carry messages between cells and serve as master regulators of the immune system.[1] Interleukins are groups of cytokines expressed and secreted by immune cells that regulate their activity.[2] Interleukin-12 (or IL-12) has emerged as one of the most potent cytokine mediators of antitumor activity because of its multiple effects on different immune cells in the tumor microenvironment.[3] IL-12 is able to activate and bridge both innate (natural killer or NK cell) and adaptive (cytotoxic CD8+ T and CD4+ T cells) immunity to promote tumor cell killing while further coordinating additional anti-cancer defenses such as regulatory T-cell suppression and anti-angiogenic effects.[4]

IL-12: The 'Holy Grail' of Immuno-Oncology

Not all tumors are created equal. Some are immunologically "cold" and lack immune cell infiltrates such as NK or T cells or may contain immune suppressive cells (e.g., ovarian, prostate, pancreatic, MSS colorectal). Others are "hot," abundant with NK and CD8+ T cells and a pro-inflammatory microenvironment that is more conducive for an immunotherapy agent to kill tumor cells (e.g., melanoma, head & neck, non-small cell lung, triple-negative breast, MSI high colorectal, endometrial).

Traditional immuno-oncology (I-O) therapies have been most effective at targeting hot tumors. IL-12, however, 'remodels' the tumor microenvironment, potentially shifting cold tumors to warm or hot. Furthermore, IL-12 can synergize with other cytokines like IL-2 to potently stimulate anti-tumor immunity.[5] There are presently no approved IL-12 therapies due to severe systemic toxicity with unmodified IL-12. An I-O candidate that can harnesses the potential of IL-12 and direct its potency selectively toward the tumor could deliver a transformative range of therapeutic opportunity.

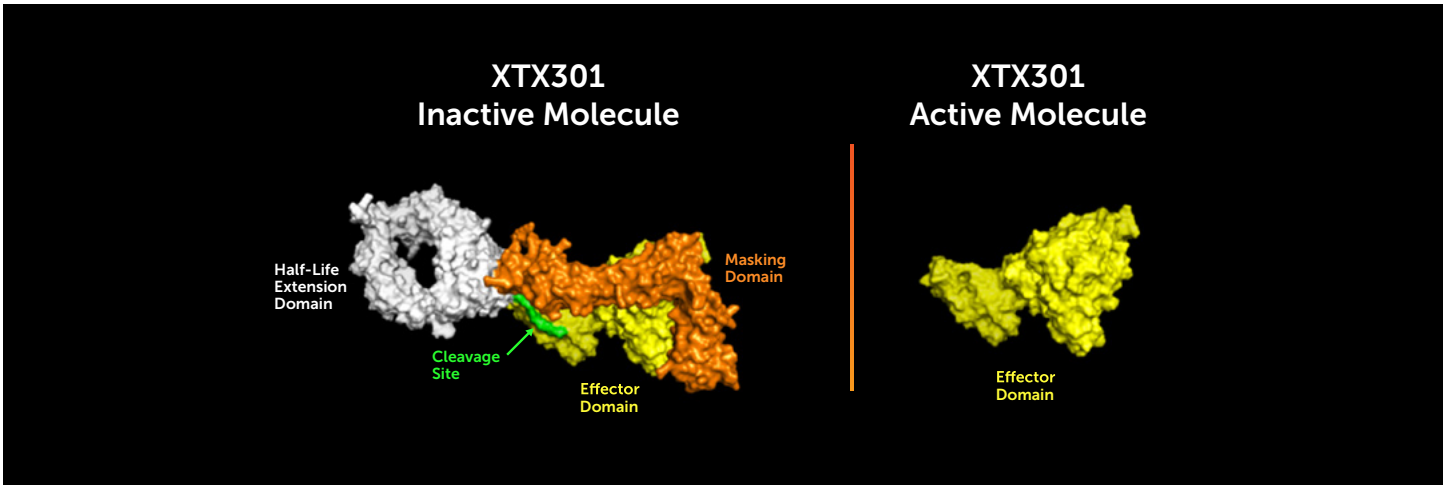


[1] "NCI Dictionary of Cancer Terms." *National Cancer Institute*. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cytokine.
[2] *Brocker C, Thompson D, Matsumoto A, Nebert DW, Vasiliou V (Oct 2010). "Evolutionary divergence and functions of the human interleukin (IL) gene family". Human Genomics. 5 (1): 30–55. doi:10.1186/1479-7364-5-1-30. PMC 3390169. PMID 21106488.*
[3] S Tugues, et al., "New insights into IL-12-mediated tumor suppression," *Cell Death and Differentiation* 22 (2015): 237-246.
[4] W Lasek, et al., "Interleukin 12: Still a promising candidate for tumor immunotherapy?," *Cancer Immunology and Immunotherapy* 63 (2014): 419-435.
[5] Wigginton, et al., Administration of Interleukin 12 with Pulse Interleukin 2 and the Rapid and Complete Eradication of Murine Renal Carcinoma," *Journal of the National Cancer Institute* 2 (1996): 38-43.

XTX301: Xilio's Tumor-Activated IL-12 Candidate

Xilio engineered XTX301 as a highly potent, extended half-life, tumor-activated IL-12 that has the potential to deliver a therapeutic dose with low systemic toxicity for a broad therapeutic index.

When XTX301 enters the body, the protein-engineered masking domain prevents it from binding to IL-12 receptors on cells and activates them in the blood stream. Once XTX301 reaches the tumor microenvironment, enzymes present in the tumor called matrix metalloproteinases (MMPs) activate a switch in XTX301 and release the active IL-12 cytokine. Once activated, XTX301 can unleash its potent immune stimulatory effects selectively in the tumor with the goal of promoting an anti-tumor response. In addition to single-agent application, XTX301 has significant potential as a combination agent with other I-O agents, including IL-2 (XTX202) and checkpoint inhibitors including anti-CTLA-4 (XTX101) and anti-PD-1.



XTX301 In Pre-Clinical Studies

A murine version of XTX301 (mXTX301) potently inhibited tumor growth in MC38 (hot) and B16F10 (cold) syngeneic tumor mouse models without toxicity. Furthermore, XTX301 was well-tolerated in non-human primates with repeated dosing.

XTX301 demonstrated robust activation in human tumor samples but was minimally activated *in vitro* in human patient plasma, suggesting tumor-selective activation.

XTX301 is designed to achieve potent anti-tumor activity while widening the potential therapeutic index of IL-12 treatment.

XTX301 In the Clinic

The U.S. Food and Drug Administration cleared an investigational new drug (IND) application for XTX301 in November 2022. Xilio anticipates initiating a Phase 1 dose-escalation trial to characterize the safety and tolerability profile of XTX301 in patients with advanced solid tumors in the first quarter of 2023.