

Next-Gen Cytokine Therapeutics Summit



**ENGINEERING GEOGRAPHICALLY PRECISE ACTIVATION OF CYTOKINES TO
ACHIEVE IMPROVED THERAPEUTIC BENEFIT IN ONCOLOGY AND BEYOND**

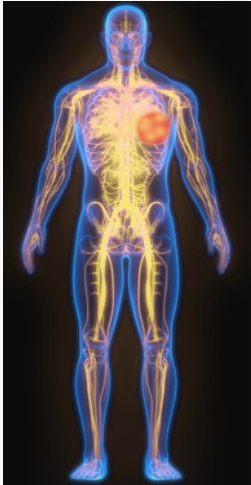
SEPTEMBER 22, 2021

Disclosure of Interest

Natalia Malkova is a full-time employee and equity holder of Xilio Therapeutics Inc (“Xilio”)

XTX202 and XTX301 are investigational agents discovered and in development by Xilio

Today's I-O agents offer curative potential but are limited due to systemic toxicity



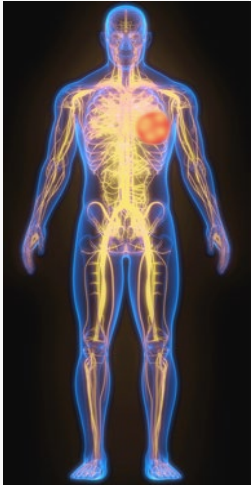
Compelling Efficacy

- Improved survival achieved with high dose ipilimumab (anti-CTLA-4) at 10 mg/kg
- 10-year durable CRs achieved in melanoma with high dose IL-2 as a monotherapy
- Tumor shrinkage observed in patients with IL-12

Dose-limiting Toxicity

- Multi-organ AEs and peripheral side effects of potent I-O therapy can be lethal
- Often results in dose reductions, interruptions or discontinuations for many patients
- Many I-O agents remain completely untapped (IL-12)

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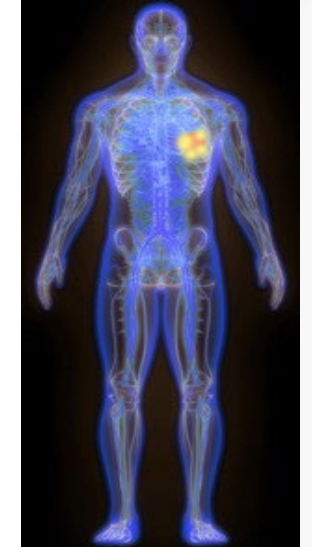
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Geographically precise solutions (GPS) are designed to solve this problem by localizing the desired I-O effect in the tumor



Xilio's GPS Platform:

- ✓ Engineer highly potent I-O molecules
- ✓ Designed to be systemically inactive
- ✓ Activated locally in the tumor microenvironment



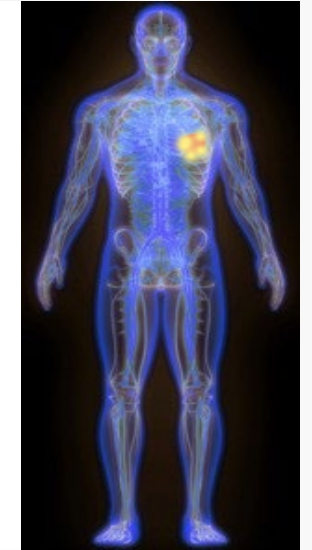
GPS-enabled therapies are designed to unlock the full potential of immuno-oncology

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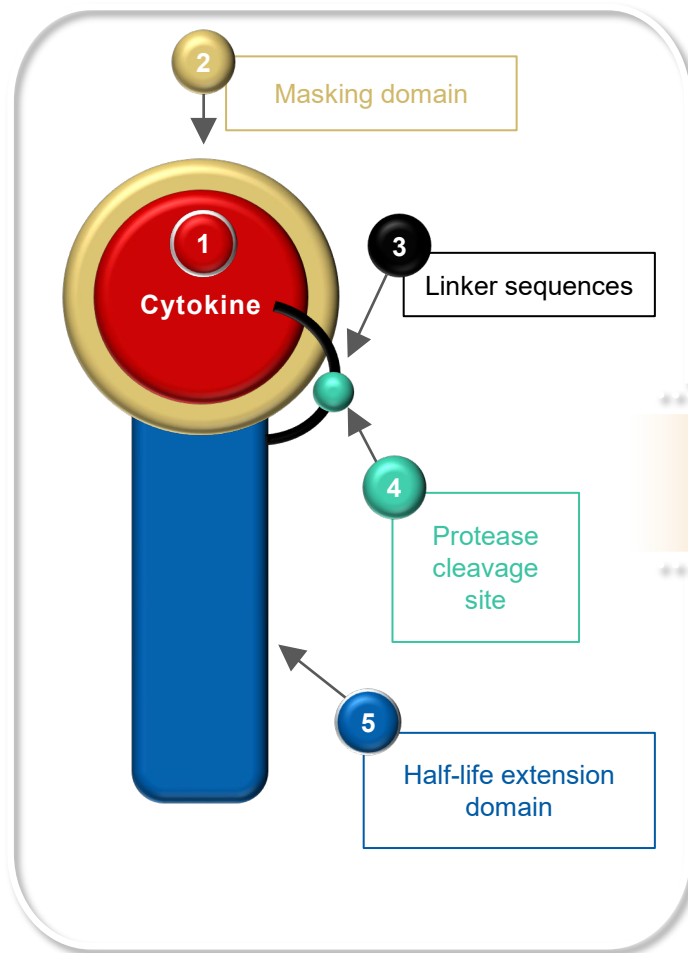
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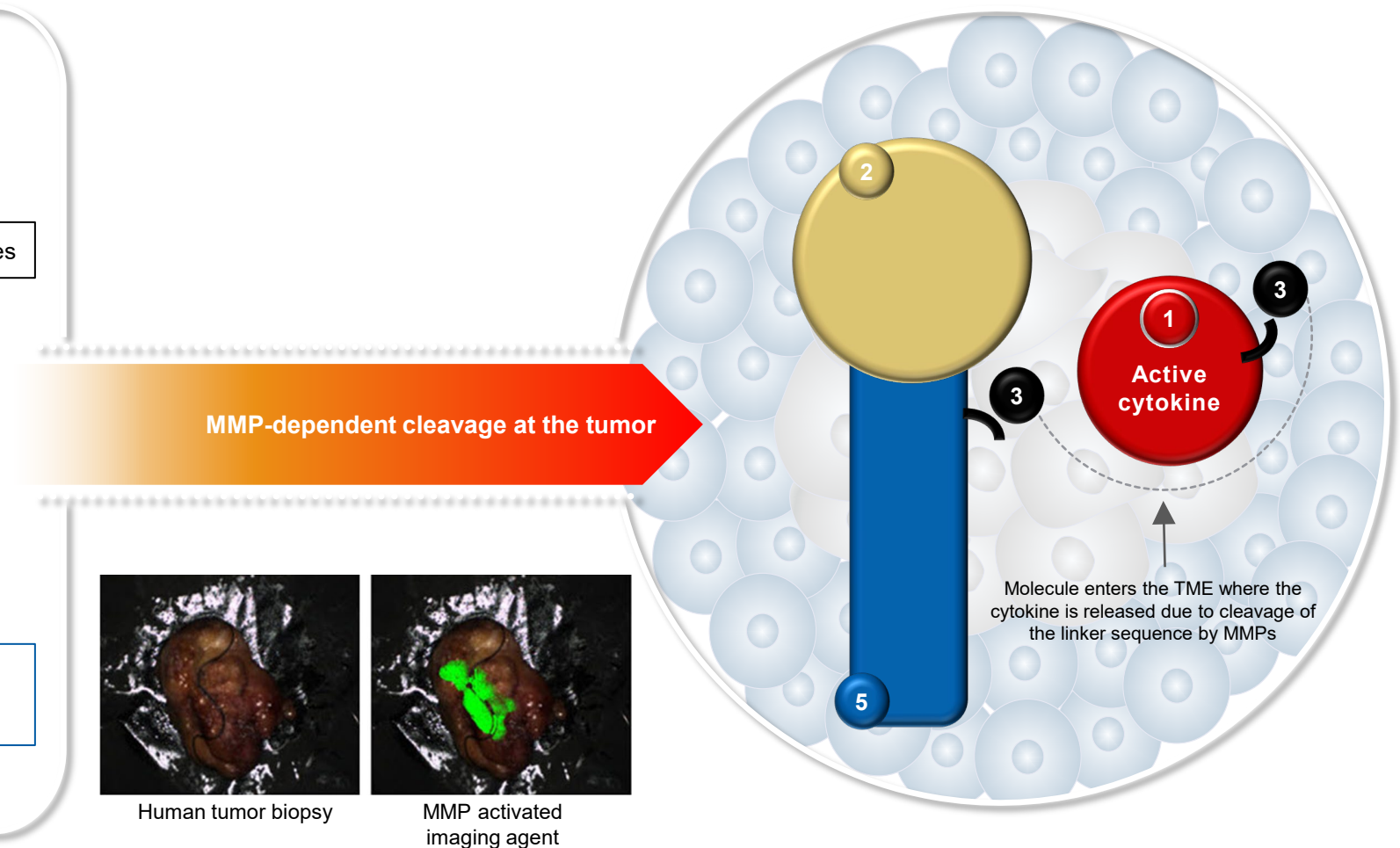
	1 st generation cytokines (aldesleukin)	2 nd generation systemically active engineered cytokines	Opportunity for GPS-enabled cytokines
Efficacy	✓	—	✓
Tolerability	—	✓	✓
Therapeutic Index	—	—	✓

GPS platform components designed to work synergistically to improve I-O therapeutic index

GPS-enabled Cytokine

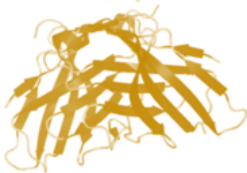


Tumor Microenvironment-Activated Cytokine

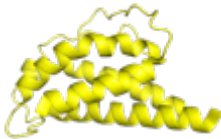


Building a robust pipeline of tumor-selective cytokine and checkpoint inhibitor programs

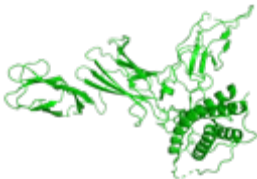
Advancing two lead programs into clinic



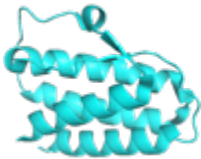
CTLA-4



IL-2



IL-12



IL-15

Tumor-Selective Program	Mechanism of Action	Discovery	IND-enabling	Phase 1	Phase 2	Phase 3
XTX101	Anti-CTLA-4					
XTX202	IL-2					
XTX301	IL-12					
XTX401	IL-15					
Targeted Cytokine Program	Undisclosed					

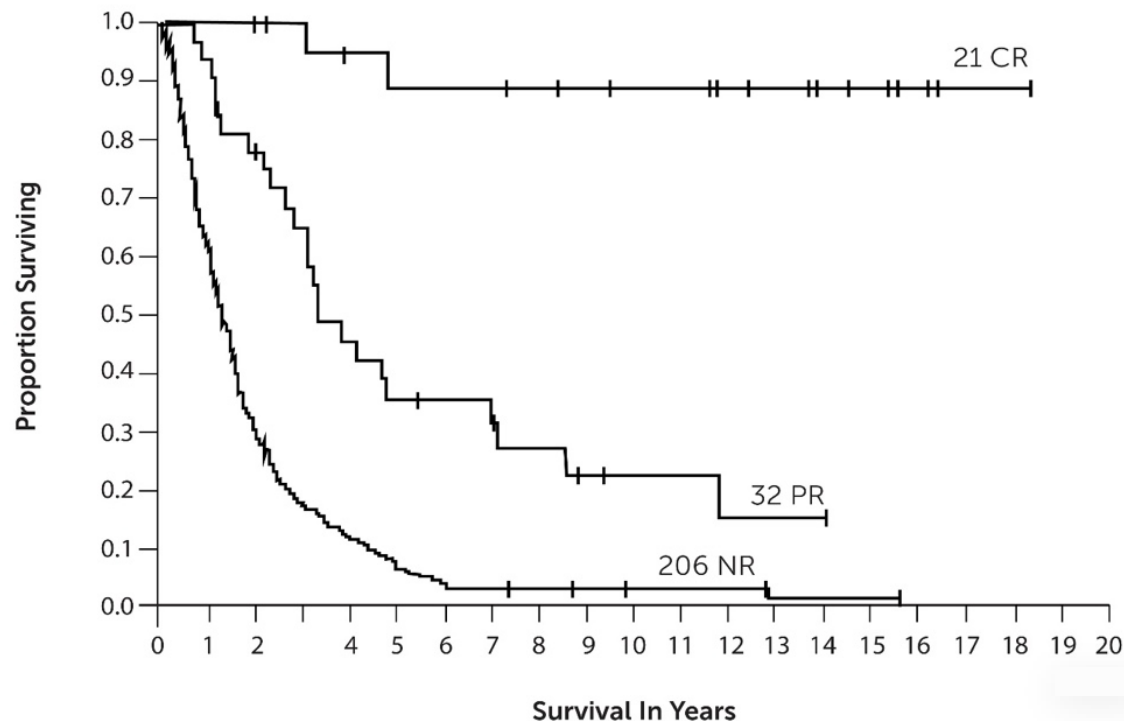
A microscopic image of a cell cluster, likely a tumor, with a red and orange color scheme. The cells are densely packed and have a granular texture. A semi-transparent purple overlay is on the right side of the image.

XTX202

TUMOR-SELECTIVE IL-2 PRODUCT CANDIDATE

High-dose IL-2 offered curative potential, but usage limited in patients due to life-threatening vascular leak syndrome

High-dose IL-2 resulted in overall survival >10 years, mostly in patients who achieved a CR*



Data represents all patients that received IL-2 (no control group)

Limited use of high-dose IL-2 in patients with cancer

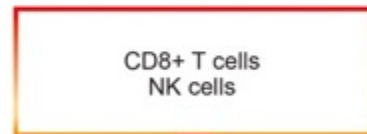
- Must have normal cardiac and pulmonary function
- Most develop hypotension, despite strict requirements
- Unable to receive full dose due to life-threatening toxicity
- Treatment restricted to use at specialized centers

Avoiding IL-2 receptor α reduces immune suppression and supports immune activation

Intermediate-affinity
 $\beta\gamma$ receptor



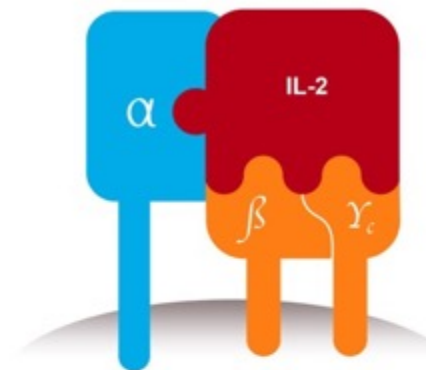
Immune Activating



Stimulates immune response

Inhibits tumor growth

High-affinity
 $\alpha\beta\gamma$ receptor

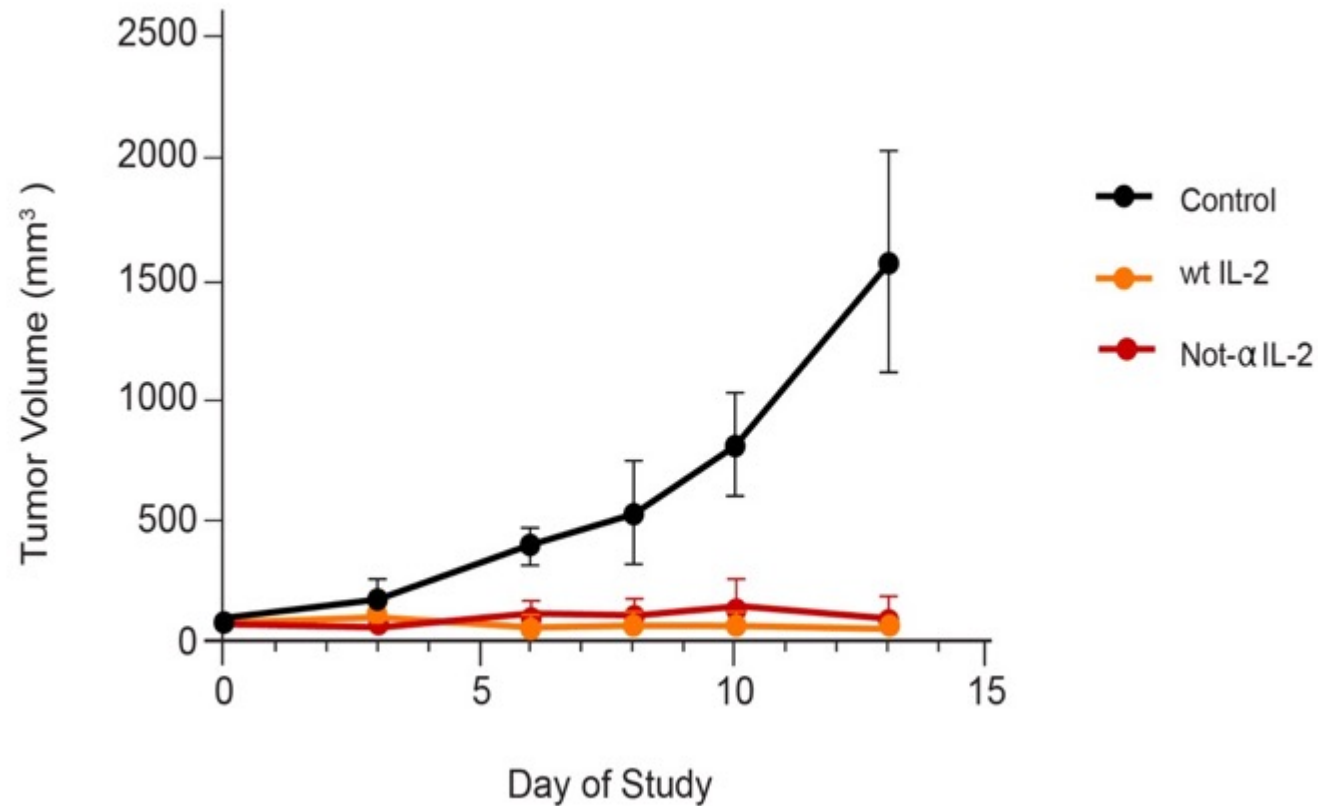


Immune Suppressive



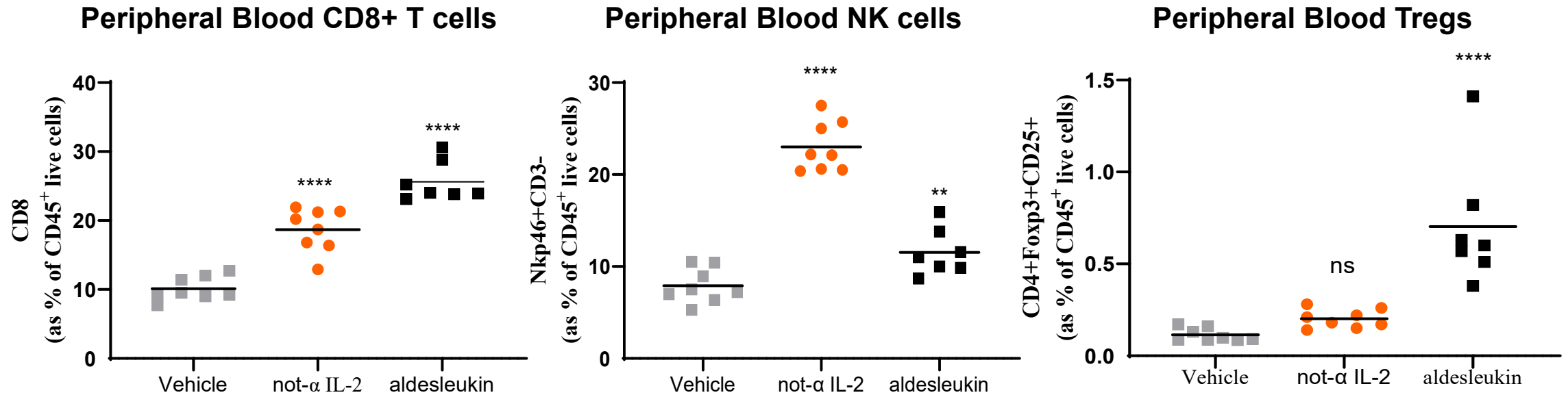
Suppresses immune response

Not- α IL-2 has potential to increase potency in preclinical models and cancer patients



- Not- α IL-2 matches efficacy of wild-type IL-2 at the same dose and schedule in mice
- Not- α IL-2 reduces activation of immune suppressive Treg cells compared to wild-type IL-2
- XTX202 is engineered to avoid binding to IL-2 receptor α

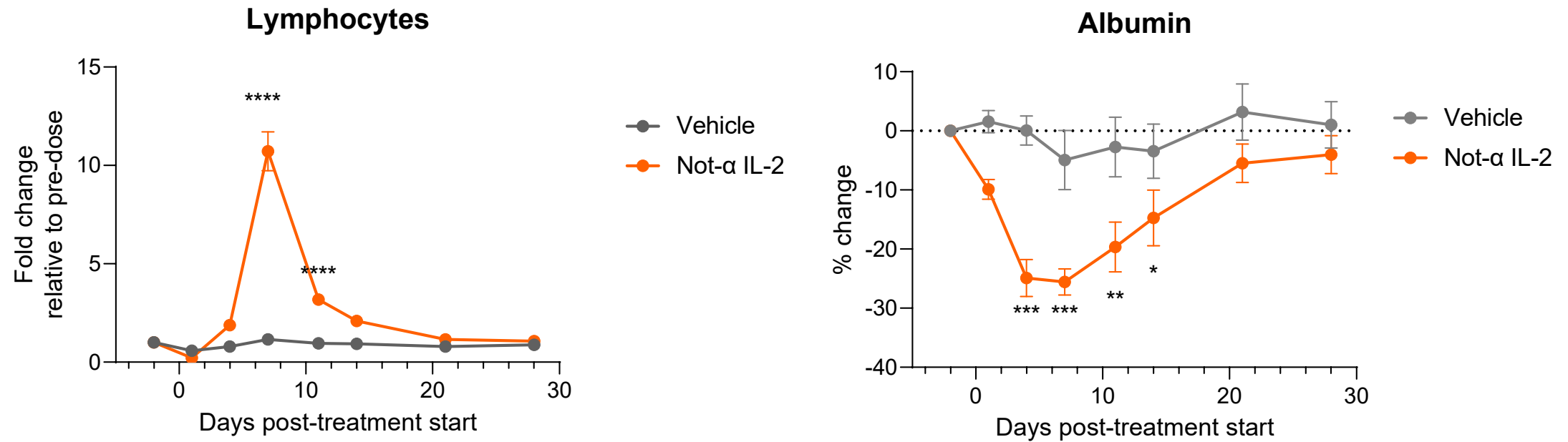
Not- α IL-2 did not affect regulatory T cells in periphery *in vivo*



O'Neill *et al.*, ASCO 2021

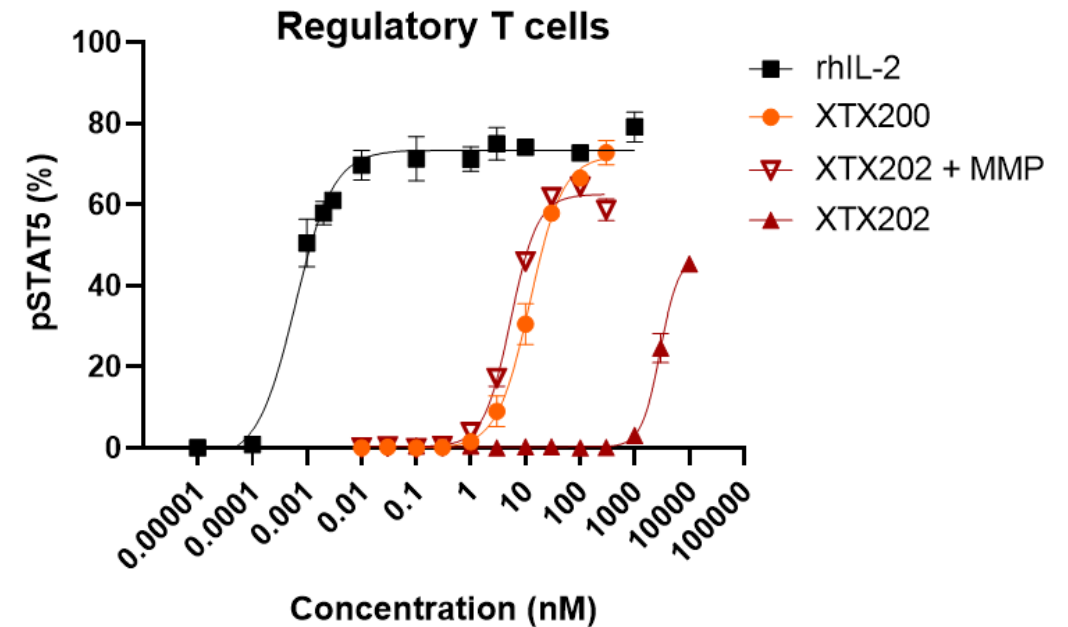
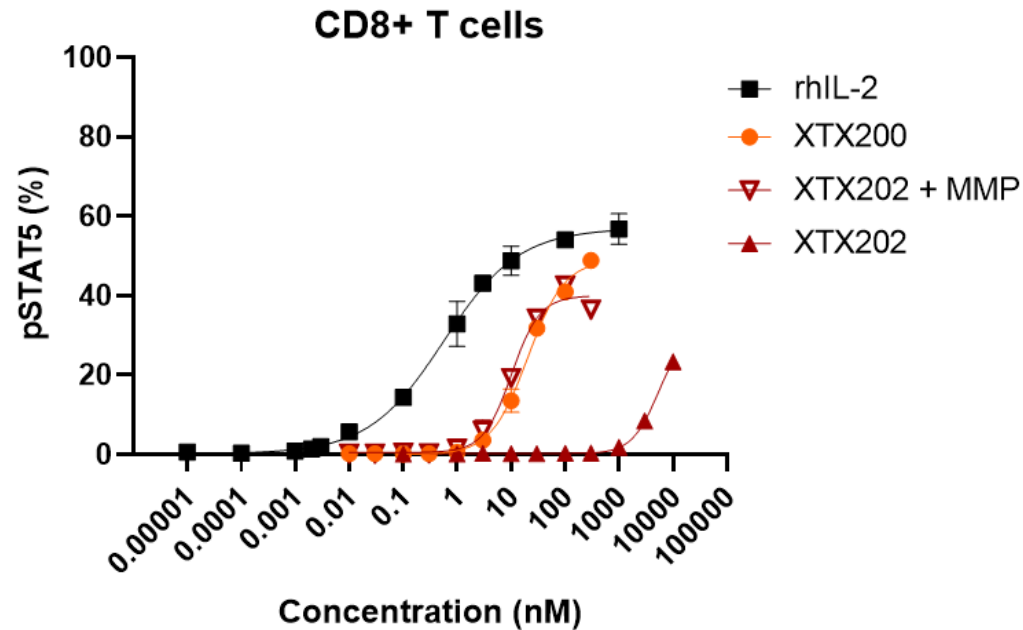
- Not- α IL-2 therapy resulted in an increase in circulating CD8⁺ T cells and NK cells
- Compared to aldesleukin, not- α IL-2 did not affect regulatory T cells

Not-alpha IL-2 induced peripheral activity consistent with IL-2 Activity in non-human primates



- Not- α IL-2 therapy resulted in an increase in peripheral lymphocytes in NHPs
- Compared to vehicle control, not- α IL-2 induced capillary leakage in NHPs

XTX202 provided protease-dependent control of IL-2 activity *in vitro*

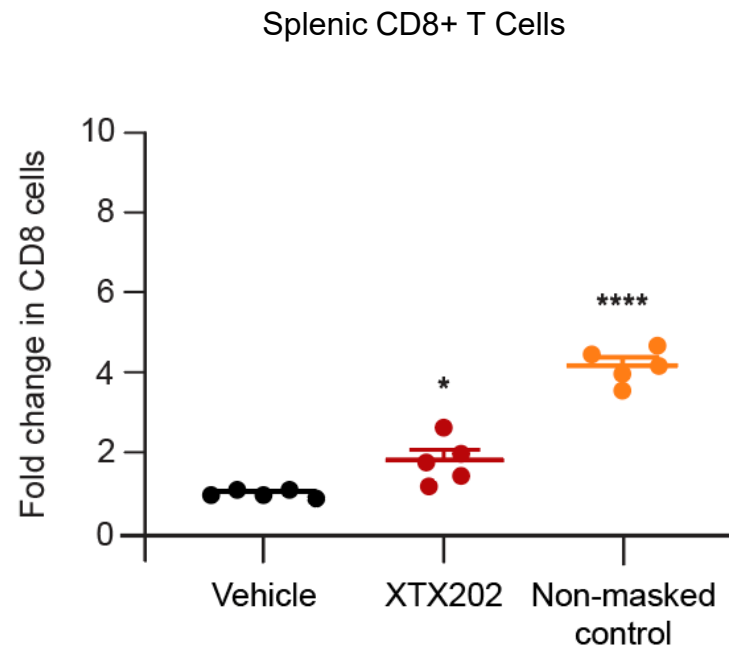
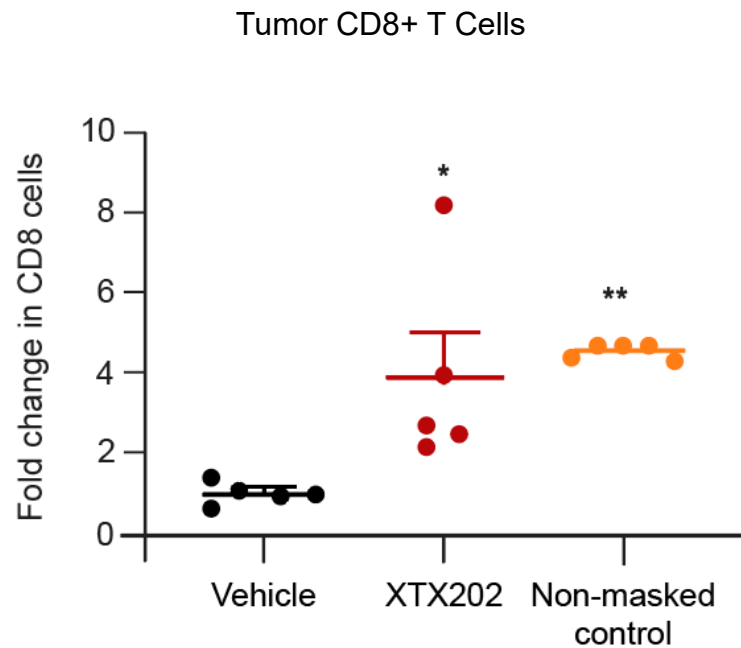


- XTX202 was effectively masked and its signal through IL-2 receptor was highly attenuated in CD8+ T and regulatory T cells compared to its unmasked control XTX200.
- MMP cleavage restored XTX202 activity to the level of unmasked control XTX200 in CD8+ T and regulatory T cells.
- In contrast to rhIL-2 that was more active against Treg cells, XTX200 and proteolytically cleaved XTX202 demonstrated similar activity in primary human CD8+ T cells and regulatory T cells

XTX202 activated intratumoral immune response in syngeneic B16F10 melanoma mouse model

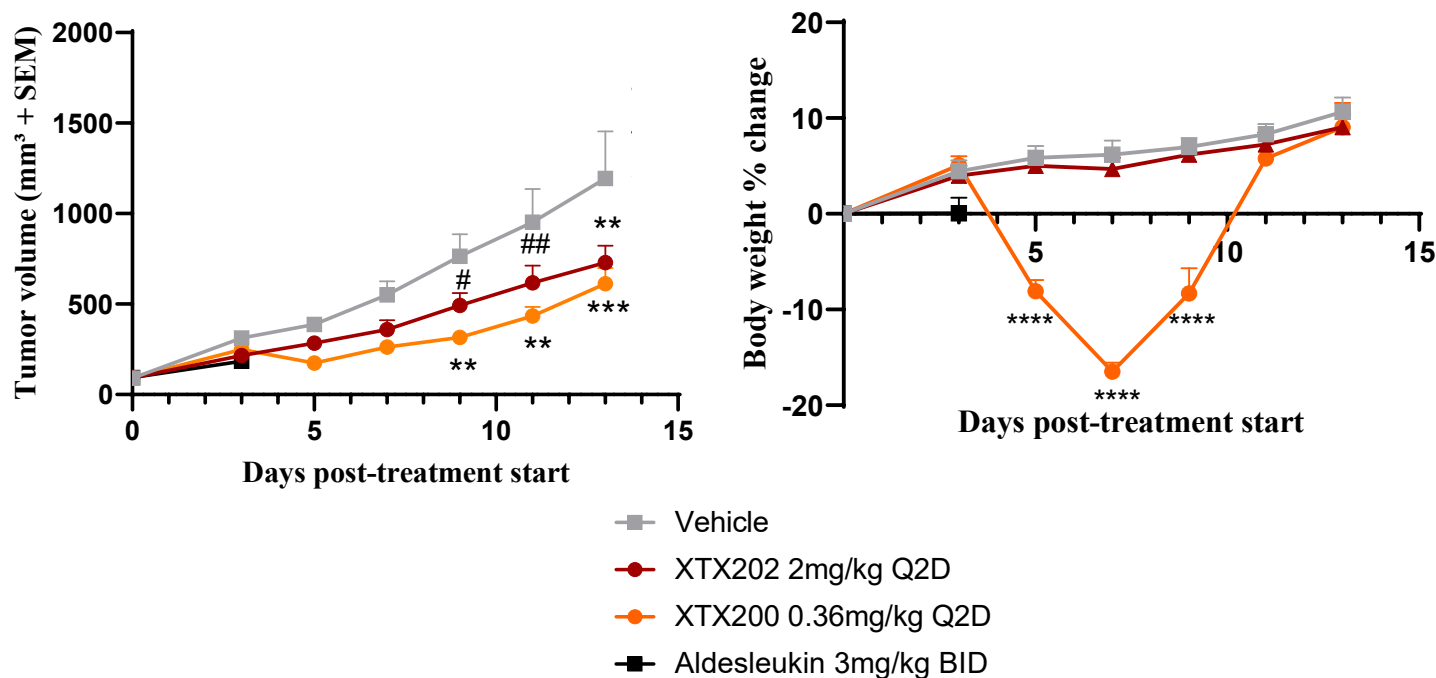
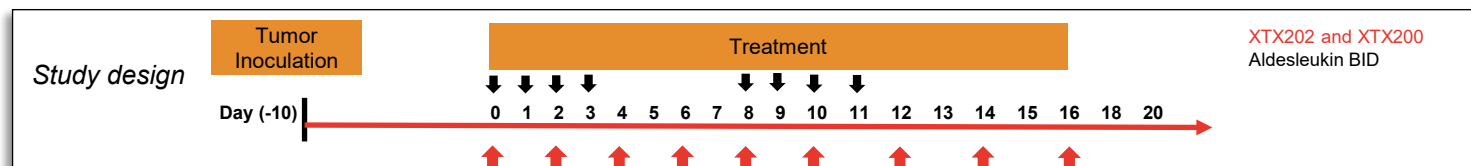
XTX202: active in tumor

XTX202: not active systemically



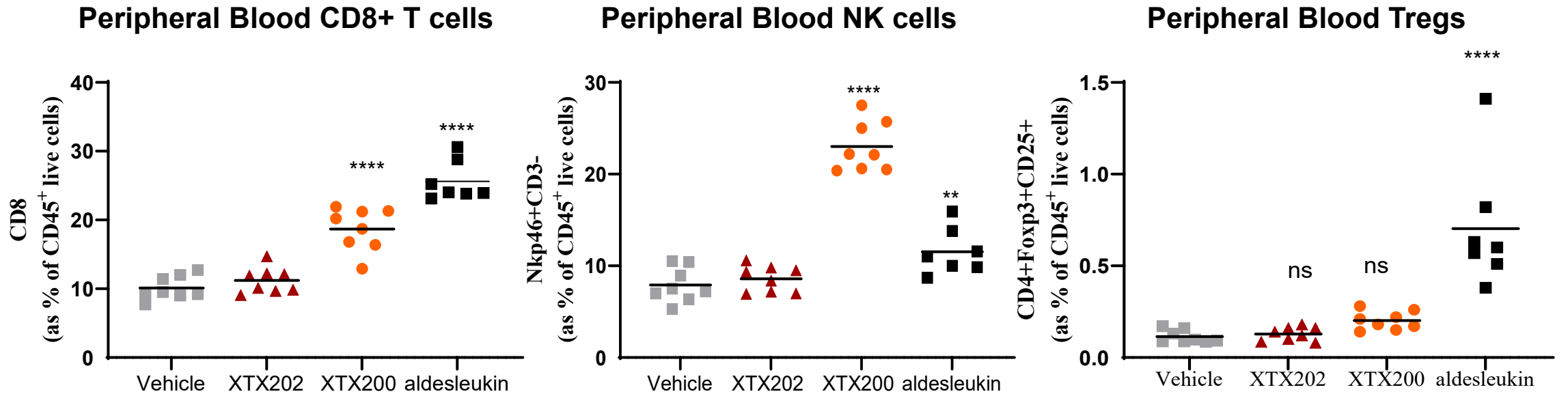
- XTX202 activated immune response in tumor, but not in spleen
- Data support tumor-selective pharmacodynamic activity

XTX202 elicits tumor growth inhibition at well-tolerated doses in syngeneic bladder cancer MB49 model



- XTX200 at 0.36 mg/kg Q2D and aldesleukin at 3 mg/kg BID for five days were not well-tolerated
- XTX202 elicited tumor growth inhibition at the well-tolerated dose of 2 mg/kg Q2D

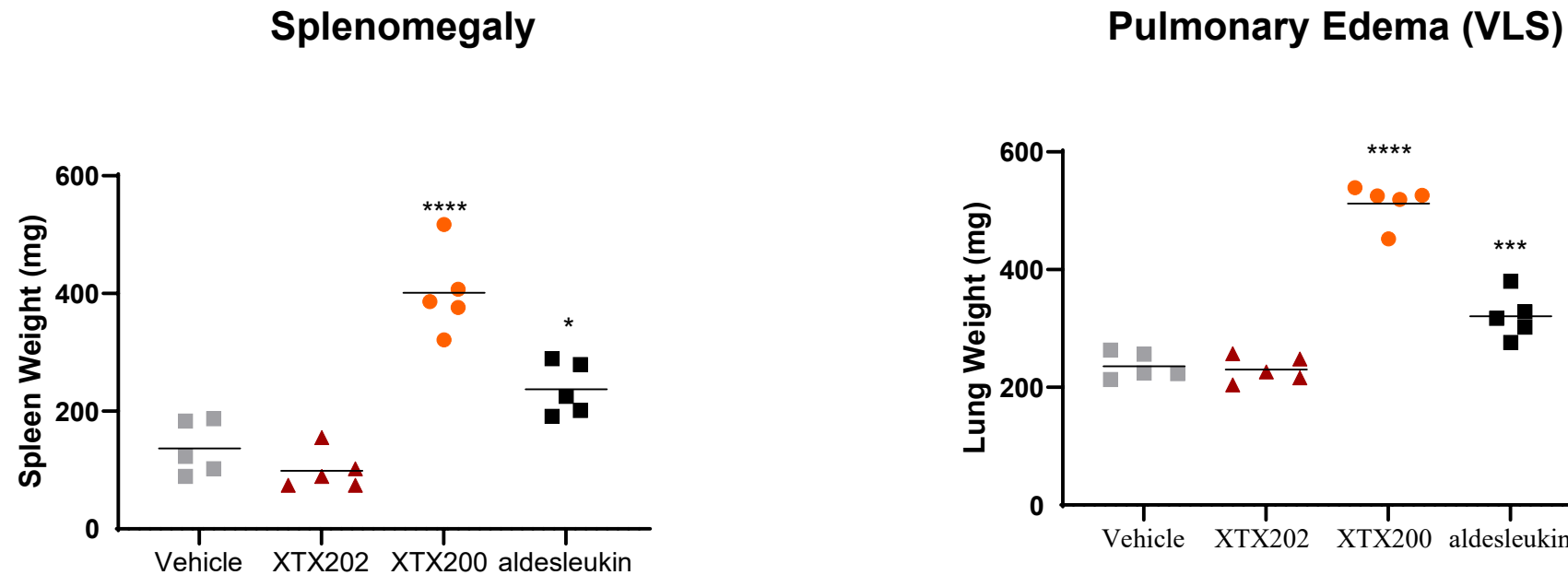
XTX202 did not affect immune cells in periphery in syngeneic bladder cancer MB49 model



O'Neill et al., ASCO 2021

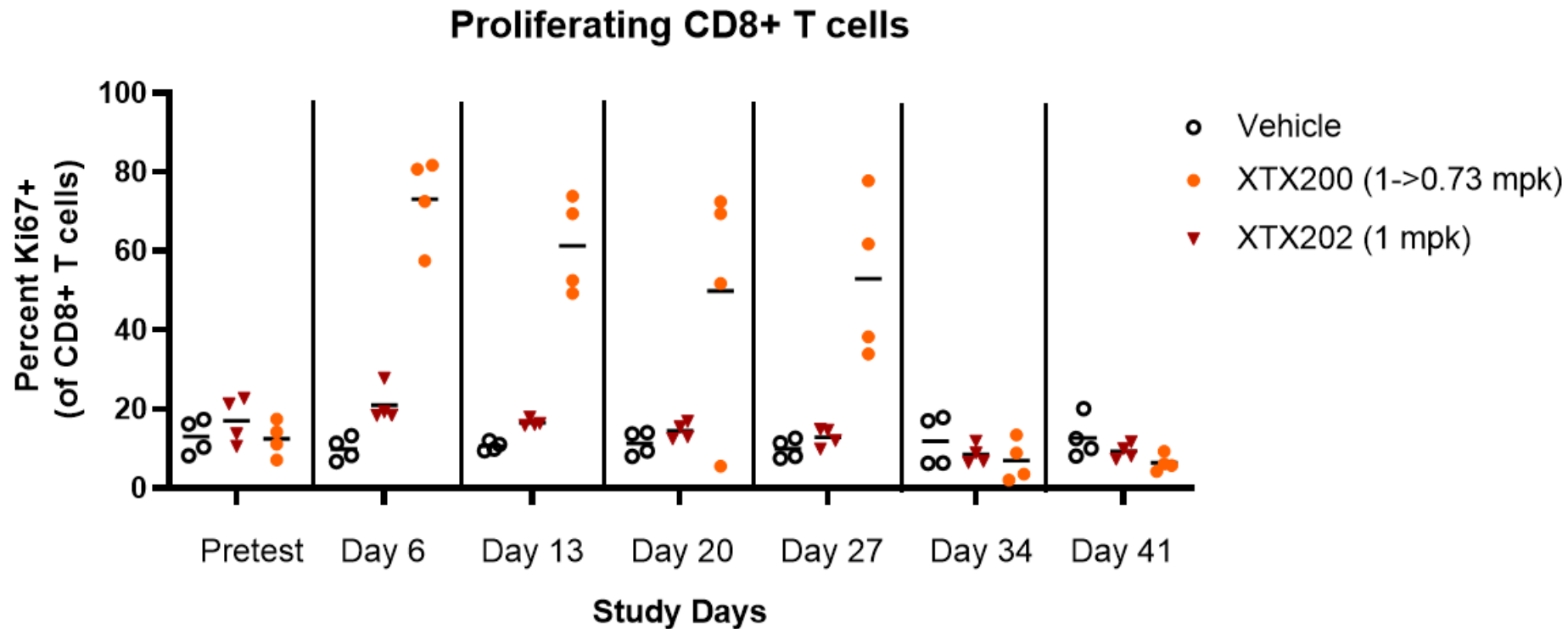
- XTX202 did not cause an increase in circulating CD8+ T cells, NK cells or CD4+ Tregs

XTX202 was well-tolerated *in vivo* and overcame toxicity observed with aldesleukin



- XTX202 did not induce splenomegaly or VLS, evidenced by the lack of pulmonary edema

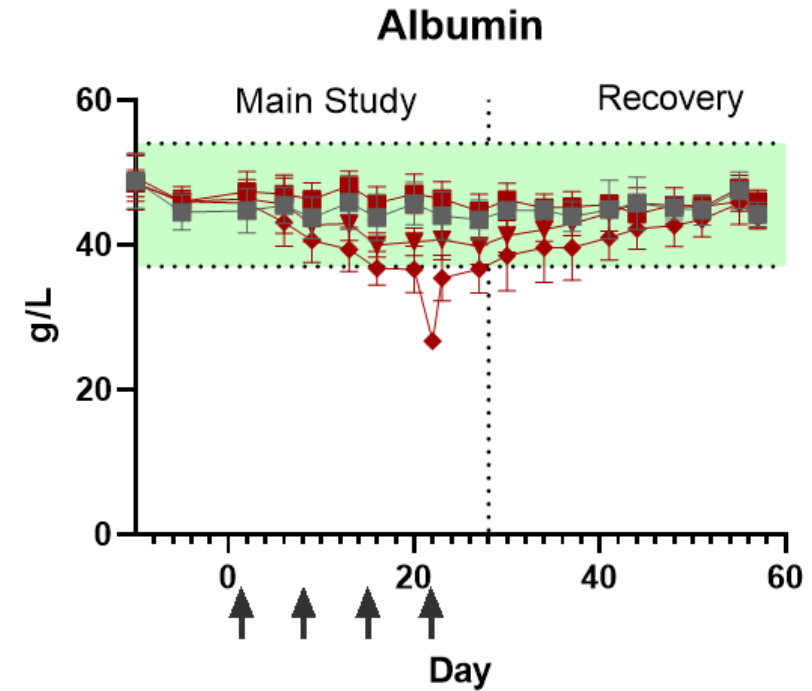
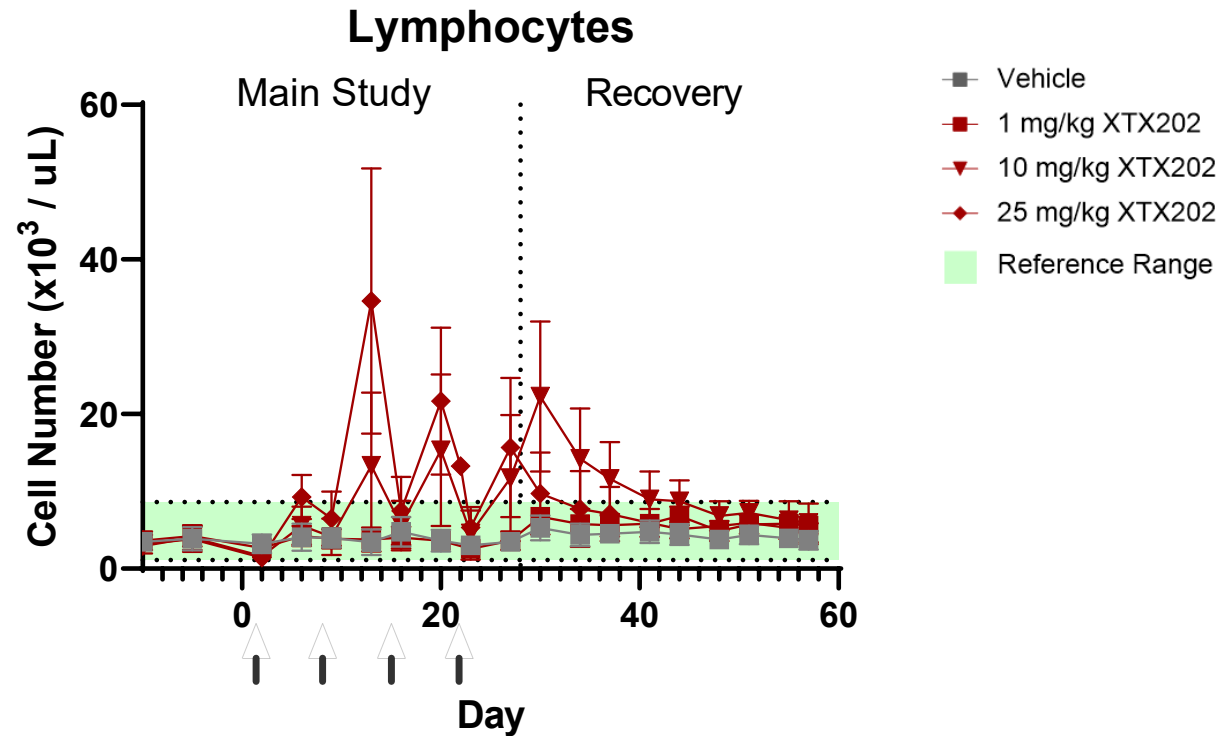
XTX202 does not induce peripheral immune cell activation in NHPs



- Cynomolgus monkeys in a 4-week repeat dose study
- Dosing on study days 1, 8, 15, and 21

- XTX200 treatment resulted in a 3 to 4-fold increase in the level of proliferating CD8+ cells in NHPs over the repeat dose course (weekly x4).
- XTX202 at a 1 mg/kg dose did not cause peripheral CD8+ T cell expansion.

XTX202 was well-tolerated in NHPs after weekly doses up to 10 mg/kg



- XTX202 did not cause peripheral lymphocyte expansion or capillary leakage at repeat (weekly x4) doses up to 10 mg/kg in a GLP toxicology study.
- XTX202 half-life in NHP was 4-6 days that support plans for Q3W dosing in patients.

XTX202 (GPS-enabled IL-2) Summary

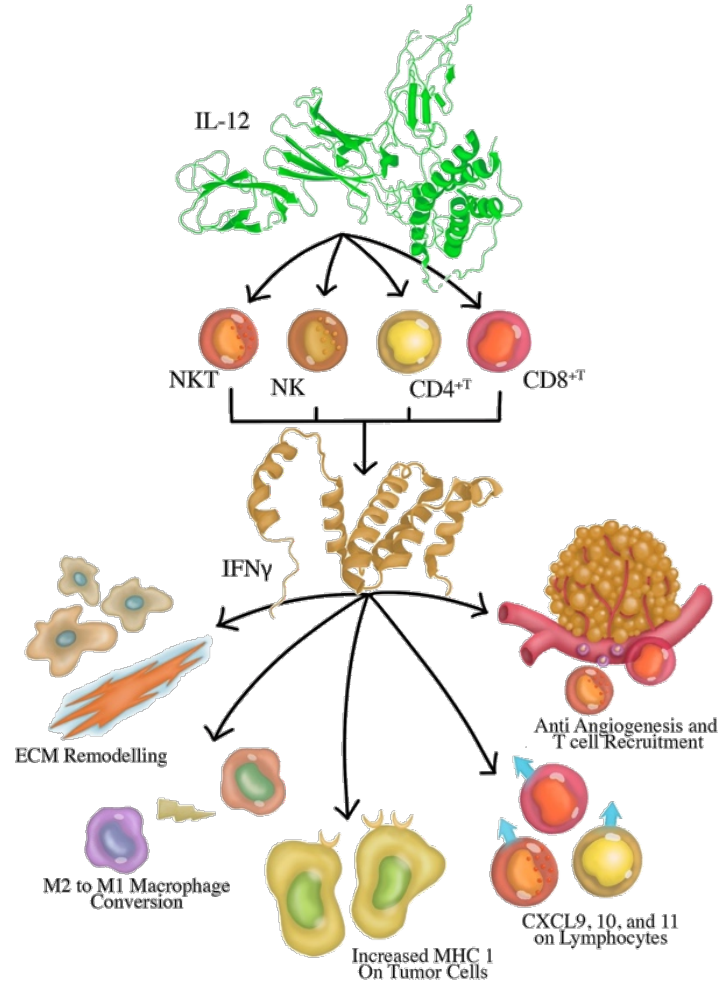
- XTX202 demonstrated protease dependent activity in human PBMC-based assays.
- XTX202 exhibited significant tumor growth inhibition in syngeneic mouse bladder MB49 model as a single agent with no evidence of toxicity or peripheral immune activation, thus demonstrating tumor-selective activity.
- XTX202 was well-tolerated up to 10 mg/kg in non-human primates in 4-week repeat dose studies.
- IND filing is anticipated in September 2021.

A microscopic image of a tumor cell cluster, showing a dense arrangement of cells with prominent nuclei and irregular shapes. The image is overlaid with a semi-transparent dark red/purple gradient that covers the right side and bottom of the frame.

XTX301

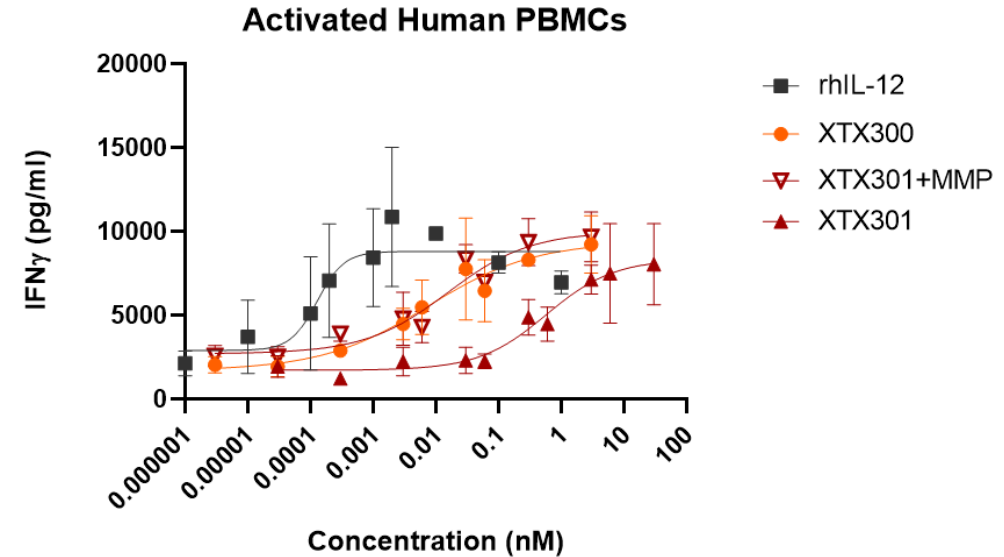
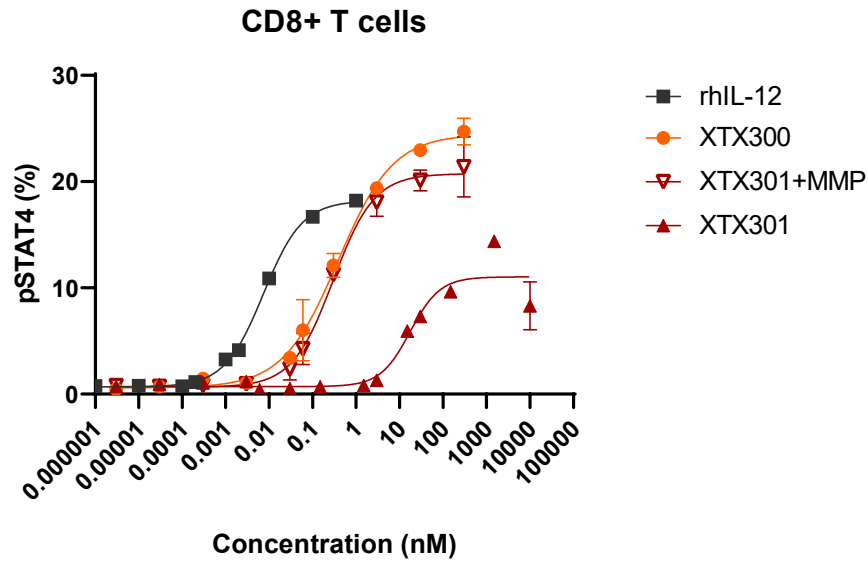
TUMOR-SELECTIVE IL-12 CANDIDATE

IL-12 induced objective responses in patients with cancer



- IL-12 is a key pro-inflammatory cytokine produced by antigen presenting cells in response to pathogen infection.
- IL-12 has demonstrated anti-tumor effects against a range of malignancies in preclinical studies. These effects are largely dependent on CD8⁺ T cells, NK cells, and NK T cells.
- Systemic IL-12 therapy causes severe and non-severe AEs in cancer patients, including life-threatening hepatotoxicity.
- Lack of meaningful anti-tumor activity in cancer patients generally attributable to dose limiting toxicity.

XTX301 provided protease-dependent control of IL-12 activity *in vitro*



- Compared to unmasked control XTX300, XTX301 was effectively masked and its signal through IL-12 receptor was highly reduced in human immune cells.
- MMP activation restored pSTAT4 signaling in human CD8+ T cells and functional IFN γ secretion by activated PBMCs back to XTX300 levels.

XTX301 (GPS-enabled IL-12) Summary

- Compared to unmasked control XTX300, XTX301 demonstrated attenuated pSTAT4 signaling and functional IFN γ secretion in primary human immune cells.
- MMP activation restored potency of XTX301 signaling back to XTX300 levels.
- XTX301 anti-tumor activity was evaluated in vivo. In vivo efficacy and NHP tolerability data will be presented at a future date.

Conclusions

- Tumor-selective XTX202 (IL-2) and XTX301 (IL-12) drug candidates exhibited strong protease-dependent control *in vitro*.
- XTX202, demonstrated tumor-selective activity that resulted in significant tumor growth inhibition in syngeneic mouse bladder MB49 model and showed minimal to no evidence of toxicity or peripheral immune activation in mouse preclinical models and non-human primates.
- These data support the broad application of our approach to develop tumor-selective cytokine therapies for oncology.
- Xilio's protein engineering platform can be broadly applied to confer geographical selectivity of cytokines allowing us to explore therapeutic applications in other disease areas including autoimmune and inflammatory diseases.

