XTX202, a tumor-selective protein-engineered IL-2, exhibited enhanced anti-tumor activity in combination with checkpoint inhibition in mice

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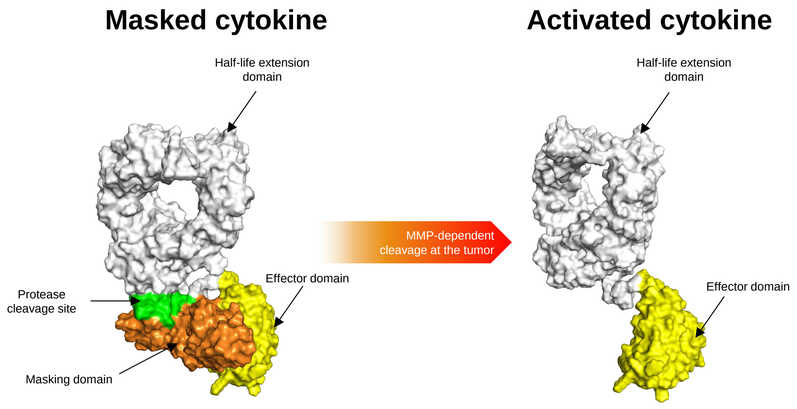
Background

High-dose recombinant human interleukin-2 (IL-2, aldesleukin) is approved for the treatment of renal cell carcinoma (RCC) and melanoma based on durable responses. However, use of aldesleukin is limited due to treatment-related life-threatening toxicities. With the goal of overcoming these toxicities and improving the therapeutic index of IL-2, we employed protein engineering to generate XTX202, a masked, non-alpha, tumor-selective IL-2. XTX202 is designed as a beta/gamma biased IL-2 that is pharmacologically inactive until unmasked by proteases that are enriched in the tumor microenvironment (TME), resulting in activation and IL-2 signaling.

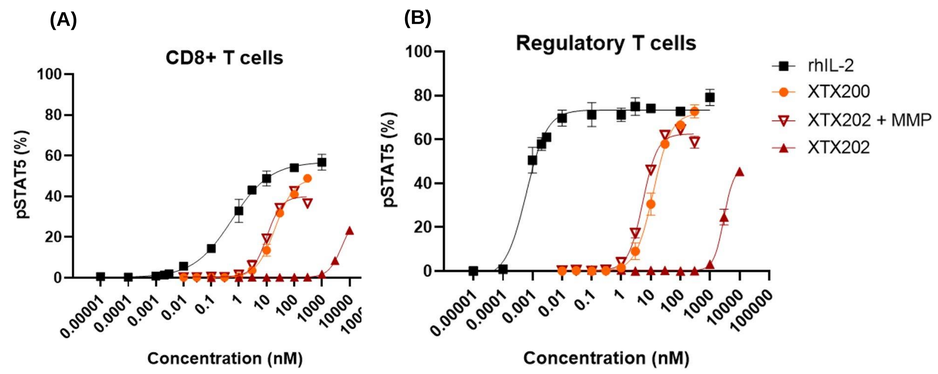
XTX202 design

Tumor-selective activation of XTX202



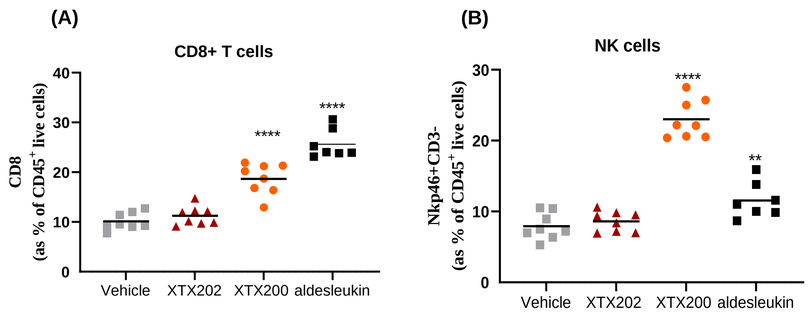
XTX202 is a masked half-life extended tumor-selective IL-2. It is pharmacologically inactive until activation by matrix metalloproteinases (MMPs) in the TME.

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



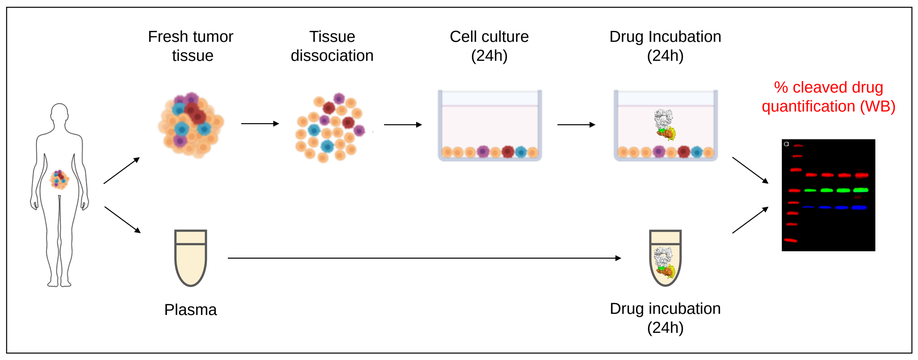
Primary human peripheral blood mononuclear cells were incubated with varying doses of recombinant human IL-2 (rhIL-2) or test articles for 20 min and evaluated for STAT5 phosphorylation by flow cytometry. Relative to rhIL-2 and XTX200 (unmasked molecule), STAT5 phosphorylation was reduced in XTX202 treated cells. Upon MMP activation, XTX202 resulted in a similar STAT5 phosphorylation as unmasked XTX200 in CD8 T cells (A) and Treg cells (B). In contrast to rhIL-2 that was more active against Treg cells, XTX200 and proteolytically activated XTX202 demonstrated similar activity in primary human CD8+ T cells and Treg cells.

XTX202 showed no effect on immune cells in periphery in the mouse bladder MB49 tumor model



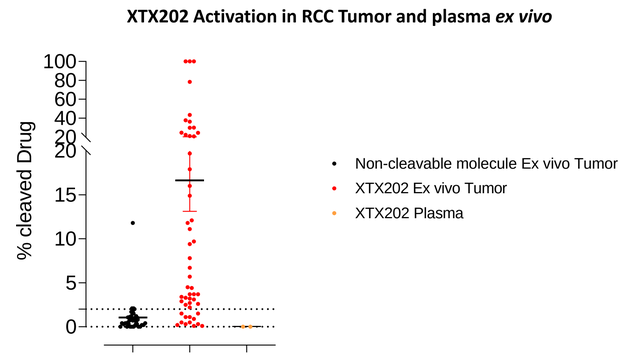
Systemic response to XTX202, unmasked XTX200 and aldesleukin was evaluated in huFcRn mice bearing MB49 tumors. Peripheral blood mononuclear cells (PBMCs) were collected on D5 after treatment with XTX202 (2 mg/kg, Q2D) and XTX200 (0.36 mg/kg, Q2D); and aldesleukin (3 mg/kg, 4 doses, twice a day) (One-way ANOVA Dunnet's multiple comparison post-test was performed to determine the statistical significance of treatment vs vehicle, **P < 0.01; ****P < 0.0001).

XTX202 was efficiently activated by human tumors ex vivo but was not activated by human plasma



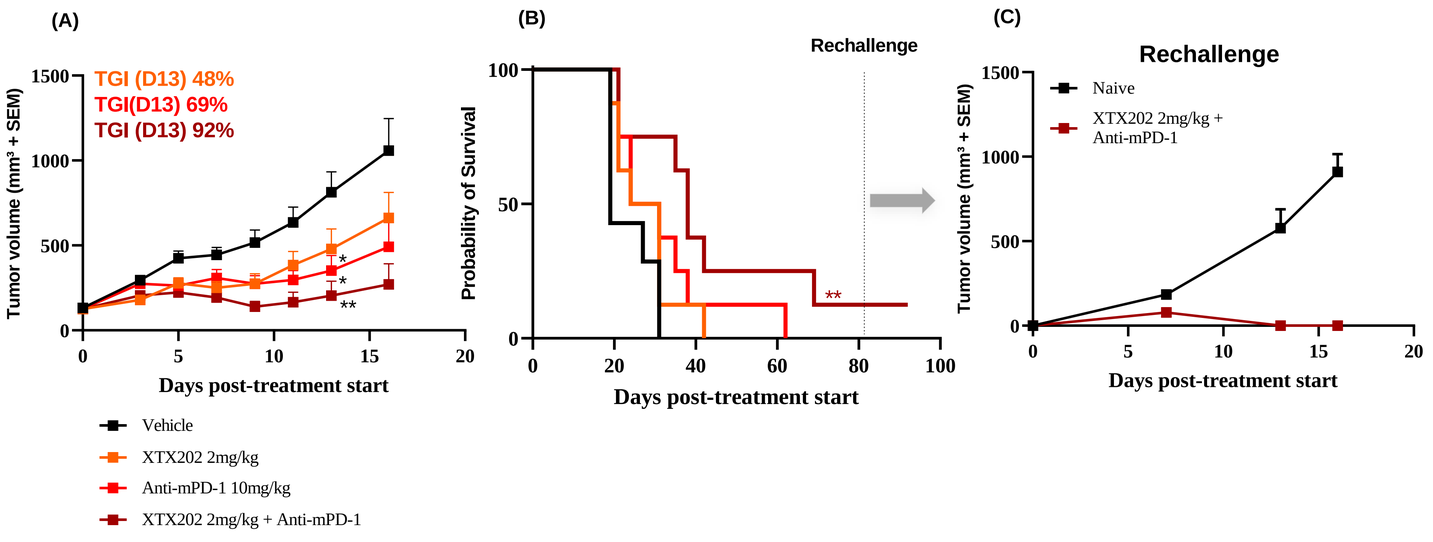
Tumor indication	Number of Tumor Samples	% of tumor samples activating XTX202
Colon	9	100
H&N	14	86
Prostate	5	80
RCC	52	77
Lung	12	67
Melanoma	8	25

Plasma Indication	Number of Plasma samples	% of plasma samples activating XTX202
Healthy Control Donor	10	0%
RCC	2	0%



Proteolytic activation of XTX202 (or the non-cleavable control molecule, lacking protease substrate) in human tumors was determined by incubating drug with dissociated cells from primary tumor tissues from colorectal, head and neck (H&N), prostate, RCC, lung, or melanoma cancer patients. Proteolytic activation (cleavage) of XTX202 in circulation was determined by incubating XTX202 with plasma from healthy human control donors (HCD) and RCC cancer patients. Samples were collected after a 24-hour incubation period. The percentage (%) of cleaved XTX202 was determined by fluorescent triplex western blot (WB) and used to calculate the % of tumor or plasma samples activating XTX202. Samples from all tumor types evaluated in this study were able to activate XTX202, and the % of tumors in each tumor type that activated XTX202 ranged from 25% to 100%. No activation of XTX202 was observed in human plasma after 24-hour incubation. The horizontal bar in the graph represents the mean of % of cleaved drug.

The combination of XTX202 with anti-PD-1 blockade demonstrated anti-tumor activity in the mouse bladder MB49 tumor model



Anti-tumor activity of XTX202 as a single agent and in combination with anti-mPD-1 was evaluated in hFcRn Tg32 transgenic mice bearing the murine MB49 bladder carcinoma model. Compared with vehicle, XTX202 administered at 2 mg/kg every 3 days (three doses total) significantly inhibited tumor growth, achieving 48% tumor growth inhibition (TGI) on Day 13 (**A**). The combination of XTX202 with anti-mPD-1 further improved anti-tumor activity with TGI 92% on Day 13 (Data presented as mean ±SEM, two-way ANOVA followed by post hoc Dunnett's test, *P < 0.05; **P < 0.005) (**A**). The treatment with XTX202 alone or in combination with anti-mPD-1 improved animal survival from 19 days to 27.5 and 38 days, respectively (**B**) (Geham-Breslow-Wilcoxon test, **P < 0.01). A mouse with complete regression of MB49 tumor after combination therapy with XTX202 and anti-PD-1 was resistant to tumor rechallenge with autologous MB49 tumor (**C**).

Conclusions

- XTX202, a tumor-selective IL-2, was proteolytically activated by a broad range of solid primary human tumors while not activated by human plasma.
- In the syngeneic mouse bladder MB49 model, XTX202 as a single agent demonstrated significant tumor-growth inhibition, and showed no effect on peripheral immune activation, thus indicating tumor-selective activity in this model.
- The combination of XTX202 with immune checkpoint blockade demonstrated further enhancement in the anti-tumor activity in MB49 tumor bearing mice.
- XTX202 is being evaluated in an ongoing Phase 1 trial (NCT05052268).