XTX301, a tumor-activated, half-life extended IL-12 promoted potent anti-tumor immunity and activity across multiple syngeneic tumor models

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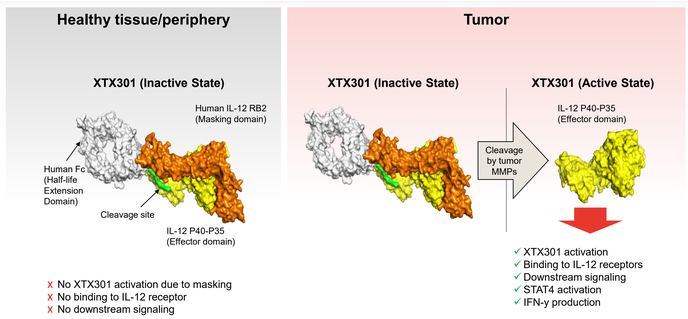
Background

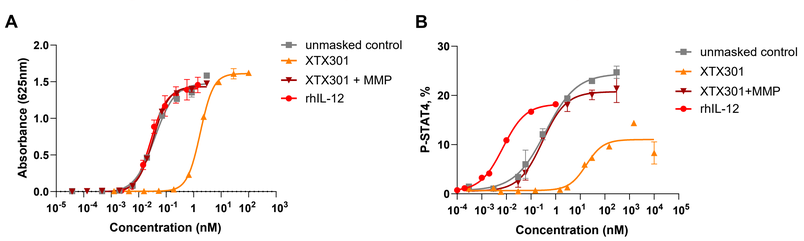
Interleukin-12 (IL-12) is a proinflammatory cytokine that bridges innate and adaptive immunity by stimulating the differentiation of T helper 1 cells and enhancing the cytolytic activity of natural killer (NK) and T cells. Historically, IL-12 has demonstrated remodeling of the tumor microenvironment (TME) from a cold to a hot state, potent anti-tumor activity in syngeneic mouse models, and promising efficacy in humans. However, development of IL-12-based agents has been limited by severe systemic toxicities in the clinical setting. XTX301 is an investigational tumor-activated, half-life extended engineered IL-12 designed to potentially reduce toxicity and improve the therapeutic index of IL-12 when administered systemically. The XTX301 masking domain is designed to pharmacologically inactivate IL-12 in non-tumor tissue, while enabling generation of an active IL-12 moiety upon cleavage by proteases enriched in the TME. mXTX301 is a murine surrogate for XTX301 that was made for in vivo studies since human IL-12 does not bind to mouse IL-12 receptors.

Design of XTX301, a tumor-activated, half-life extended engineered IL-12



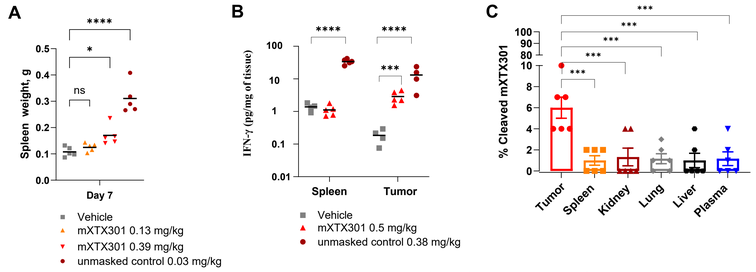
XTX301 is a tumor-activated, half-life extended engineered IL-12 which is designed to be pharmacologically inactive until activation by matrix metalloproteinases (MMPs) in the TME.

Masking of XTX301 activity and reactivation by MMPs *in vitro*



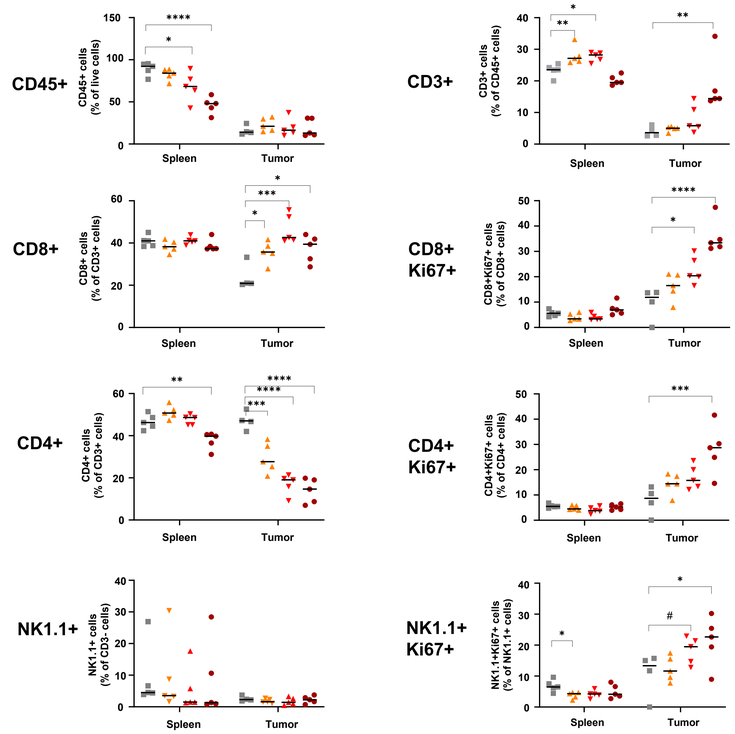
(A) IL-12 HEK-Blue reporter gene cells were incubated with either rhIL-12, unmasked control, or XTX301 at varying doses, and the reporter activity was measured. The data represents mean ± SD. **(B)** Primary human peripheral blood mononuclear cells (PBMCs) were incubated with varying doses of rhIL-12 or test articles and evaluated for STAT4 phosphorylation by flow cytometry. Relative to rhIL-12 and unmasked molecule, STAT4 phosphorylation was reduced in XTX301 treated cells. Upon MMP activation, XTX301 resulted in a similar STAT4 phosphorylation as unmasked control in PBMCs.

mXTX301 demonstrated effective peripheral masking and tumor-selective activation in the MC38 syngeneic tumor model



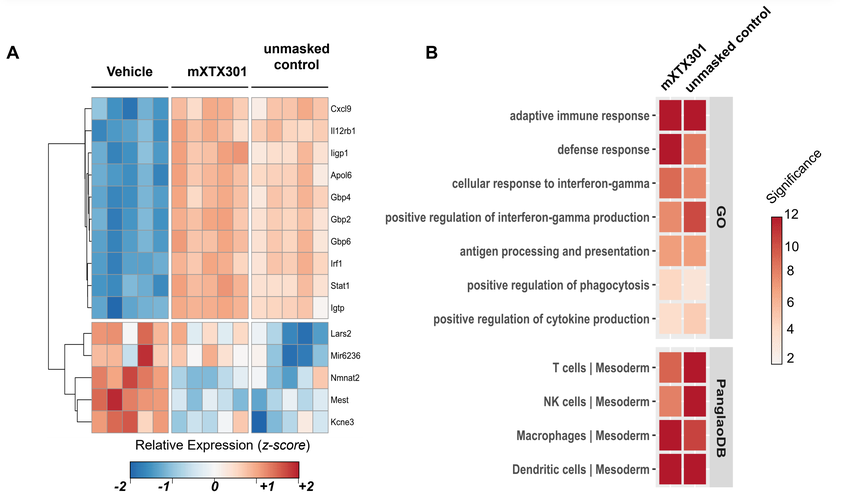
Systemic response to mXTX301 and unmasked control was evaluated in C57BL/6J mice bearing MC38 tumors. **(A)** Spleen were collected on D7 after treatment with mXTX301 (0.13 and 0.39 mg/kg, single IV dose) and unmasked control (0.03 mg/kg, single IV). One-way ANOVA Dunnett's multiple comparison post-test was performed to determine the statistical significance of treatment vs vehicle, *P < 0.05; ****P < 0.0001. **(B)** IFN-γ was measured in tumors and spleens collected on D7 after treatment with mXTX301 (0.5 mg/kg, single IV) or unmasked control (0.38 mg/kg, single IV). One-way ANOVA Dunnett's multiple comparison post-test was performed to determine the statistical significance of treatment vs vehicle, ***P < 0.0005; ****P < 0.0001. **(C)** Measurement of percentage of cleaved mXTX301 in the tumor, peripheral organs, and plasma in the MC38 syngeneic tumor model. The percentage of cleaved mXTX301 was quantified by fluorescent triplex Western blot. The data represents mean + SEM. Statistical comparisons were performed using Dunnett ordinary one-way ANOVA test versus tumor, where ***P < 0.001.

mXTX301 induced tumor-selective pharmacodynamic effects in the MC38 syngeneic tumor model



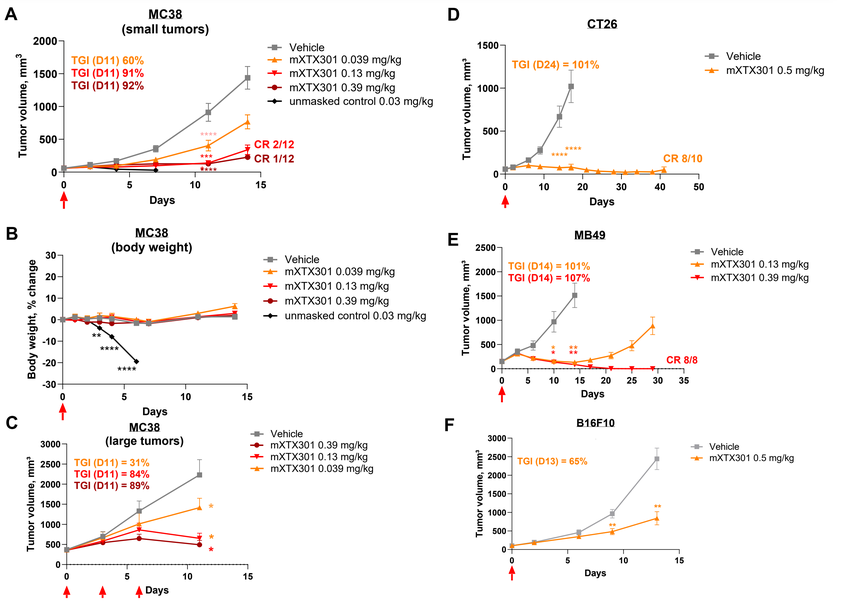
Effect of mXTX301 therapy on immune cell infiltration was evaluated in MC38 tumor bearing mice. The animals were sacrificed on day 7 (n=5 mice per group), and immune cells isolated from spleen and tumor were characterized via flow cytometry. The data are presented as mean ± SEM. Statistical significance was determined by one-way ANOVA test followed by Dunnett's multiple comparisons test. #P = 0.05, *P < 0.05, **P < 0.01, ***P < 0.0005, and ****P < 0.0001

mXTX301 induced pro-inflammatory gene programs and broad remodeling of the TME



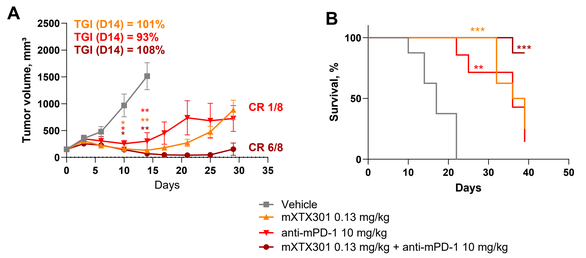
Comprehensive evaluation of mXTX301 induced gene expression changes in MC38 tumors by bulk RNA sequencing. **(A)** Heatmap showing relative expression of top mXTX301 differentially expressed genes (N = 10 upregulated and N = 5 downregulated; by P-value). Rows (genes) were clustered according to Euclidean distance. Keys above each column (sample) indicate treatment group (vehicle, mXTX301 [0.39 mg/kg], or unmasked control [0.03 mg/kg]). Box color tracks with z-score-transformed relative expression of each gene across samples (blue, under-expression compared to the mean; red, over-expression compared to the mean). **(B)** GO: Gene ontology; NK: natural killer cells. Heatmap summarizing results of a pathway enrichment analysis where each box indicates the enrichment significance (-log10 Fisher P-value) of a gene set of interest (rows) in the top 250 most significantly upregulated genes (by P-value) computed by comparing either mXTX301 or unmasked control to vehicle at Day 7 (columns). Color intensity tracks with significance.

mXTX301 demonstrated anti-tumor activity in syngeneic tumor models with hot and cold immune phenotype



mXTX301 demonstrated anti-tumor activity in hot (MC38, CT26, MB49) and cold (B16-F10) tumor models. **(A-C)** C57BL/6 mice were implanted subcutaneously (SC) with MC38 tumor cells and received 1-3 doses of mXTX301, unmasked control, or vehicle (PBS) at indicated dose levels (N=10-12). Tumor and body weight measurements were taken 2-3 times a week. Data represent mean ± SEM (standard error of the mean). **(B)** Body weight data are displayed until Day 14 when 75% of the vehicle-treated group were alive (N=9). **(C)** TGI was assessed in mice bearing large (~360mm³) MC38 tumors. Tumor growth data are presented as mean for tumor volume + SEM. **(D-F)** Animals were implanted SC with CT26, MB49, or B16F10 tumor cells and received a single dose of mXTX301 at indicated dose levels. Tumor measurements were taken 2-3 times a week (N=8 per group). Tumor growth data are presented as mean for tumor volume + SEM. For all studies, two-way ANOVA followed by Dunnett's post-hoc test (*P<0.05; **P<0.005; ****P<0.0001). TGI: tumor growth inhibition; CR: complete regression.

Enhancement of mXTX301 *in vivo* activity in combination with anti-mPD1 in the MB49 syngeneic tumor model



(A) Anti-tumor activity of mXTX301 as a single agent and in combination with anti-mPD-1 was evaluated in C57BL/6J mice bearing the murine MB49 bladder carcinoma model. Compared with vehicle, mXTX301 (IV, single) significantly inhibited tumor growth, achieving 101% TGI on Day 14. The combination of mXTX301 with anti-mPD-1 further improved anti-tumor activity with TGI 108% on Day 14 and resulted in CR in 75% of the mice (Data presented as mean ±SEM, two-way ANOVA followed by post hoc Dunnett's test on Days 10 and 14, *P < 0.05; **P < 0.005). **(B)** The treatment with mXTX301 alone or in combination with anti-mPD-1 improved median for animal survival from 17 days to >40 days (Gehan-Breslow-Wilcoxon test, **P < 0.005; ***P < 0.0005). TGI: tumor growth inhibition; CR: complete regression.

Conclusions

- mXTX301, a tumor-activated, half-life extended engineered IL-12, demonstrated tumor-selective cleavage and minimal peripheral activation in the MC38 mouse model.
- In preclinical models, mXTX301 demonstrated remodeling of the TME and improved tolerability compared to a systemically active IL-12.
- mXTX301 as a single agent demonstrated significant tumor growth inhibition in a range of syngeneic mouse models with hot and cold immune phenotypes.
- mXTX301 demonstrated anti-tumor activity in mice with large MC38 tumors that have highly immunosuppressive TME and typically respond poorly to treatment.
- The combination of mXTX301 with anti-PD-1 demonstrated further enhancement in the anti-tumor activity in MB49 tumor bearing mice.
- XTX301 is being evaluated in a Phase 1 clinical trial (NCT05684965).