# XTX501, a tumor-activated PD1/IL2 bispecific molecule, designed to overcome IL-2 receptor-mediated clearance, improve tolerability and stimulate antigen-experienced CD8+ T cells in the tumor microenvironment of murine models

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## Introduction

PD-1/PD-L1 therapies have shown significant activity across a range of tumor types, however, 70-90% of patients do not derive durable benefit. In preclinical settings, delivery of PD-1 blockade and IL-2 agonism in cis via a bispecific molecule, comprising a PD-1 antibody fused to IL-2, has demonstrated superior activity compared to delivery of individual components or their combination. Targeting of IL-2 to PD-1+ cells in cis has been shown to drive a unique differentiation path endowing CD8+ T cells with enhanced functionality. However, we show that the IL-2 component in an unmasked PD1/IL2 bispecific molecule dominates its pharmacology, driving marked peripheral activation of immune cells, rapid IL-2 receptor-mediated clearance and systemic toxicity. To address these challenges and enable tumor-specific activity, we designed XTX501, a PD1/IL2 bispecific molecule, that incorporates a single domain antibody-based mask to bind to the IL-2 component and maintain it in a quiescent state until activated by prevalent and dysregulated proteases in the tumor microenvironment (TME). In preclinical models, XTX501 demonstrated anti-tumor activity in both PD1-sensitive and insensitive settings. XTX501 had better activity than IL-2 + PD1 combination. The affinity-optimized mask prevented peripheral IL-2 receptor binding, associated toxicity and receptor-mediated clearance. Improved pharmacokinetics (PK) of XTX501 enabled PD1 antibody-like exposures and is projected to achieve complete blockade of PD1 signaling, and simultaneously directing immunostimulatory IL-2 to antigen-experienced effector cell populations in the TME. Taken together, these preclinical data suggest XTX501 has the potential to improve upon existing PD-1/PD-L1 immunotherapies by inhibiting the PD-1/PD-L1 axis and simultaneously directing immunostimulatory IL-2 to antigen-experienced effector cell populations in the TME. XTX501 is ready for IND enabling studies.

### XTX501 is a PD1/IL2 bispecific; a PD1/PD-L1 blocker with a tumor activated engineered IL-2 agonist

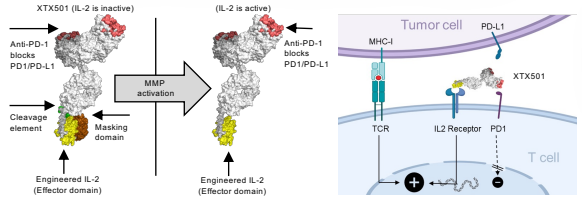


Figure 1: The anti-PD1 component of XTX501 blocks the PD1/PD-L1 interaction. The masking domain keeps IL-2 pharmacologically inactive in non-tumor tissue. Upon proteolytic cleavage by proteases that are enriched in the TME, the IL-2 moiety becomes activated and provides IL-2 signaling selectively in the tumor microenvironment in the tumor. The cis-targeting of PD1 and IL-2 receptor (IL2R) enables effective delivery of IL-2 to PD1+ immune cells.

### XTX501 was efficiently masked and PD1 targeting enhanced potency of IL-2 in vitro

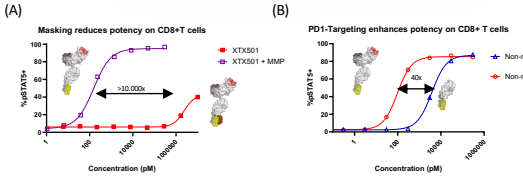


Figure 2: (A) Primary human peripheral blood mononuclear cells (PBMCs) were preactivated to upregulate PD1 expression. Preactivated PBMCs were incubated with serial dilutions of XTX501 or MMP-activated XTX501 for 12 minutes followed by evaluation for STAT5 phosphorylation. XTX501 exhibited minimal activity on hPBMCs while MMP-activated XTX501 exhibited potent signaling on CD8+ T cells. (B) Cells were incubated with varying doses of test articles including non-masked PD1/IL2 and evaluated for STAT5 phosphorylation by flow cytometry. PD1-targeting enhanced the potency in comparison to a non-masked Fc-IL2 version of this molecule. *

## Masking of the IL-2 component improved the tolerability of XTX501 in vivo

### XTX501 was effectively masked in the periphery of MC38 murine tumor model

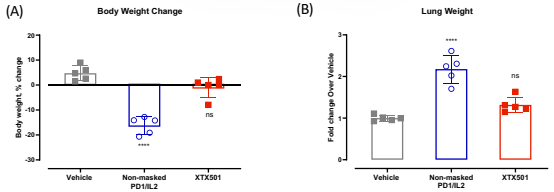


Figure 3: Evaluation of peripheral activity of PD1/IL2 bispecific in MC38 syngeneic murine tumor model. (A) XTX501 (8mg/kg) resulted in no body weight change. Non-masked PD1/IL2 (0.75mg/kg) resulted in significant body weight change. (B) XTX501 resulted in no pulmonary edema. Non-masked PD1/IL2 resulted in significant pulmonary edema. Statistical comparisons by a one-way ANOVA with Bonferroni's post-hoc pairwise comparison test compared to vehicle.**** P <0.0001 compared to vehicle-treated animals on Day 6. *

## The affinity-optimized mask and engineered IL-2 improved pharmacokinetics, enabling antibody-like exposures

### IL-2 component in non-masked XTX501 drive PK, resulting in rapid clearance. XTX501 with non-masked IL-2 was not well tolerated

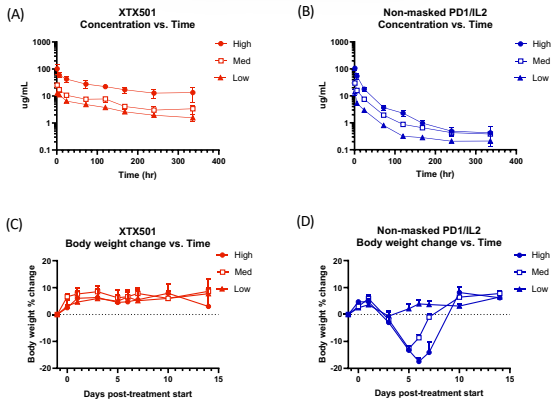


Figure 4: XTX501 exposure and tolerability in non-tumor bearing mouse model. (A) XTX501 exposure after a single 10 (High), 3 (Med) or 1 (Low) mg/kg intravenous (I.V.) injection in non-tumor bearing C57BL/6-hFcRn mice. (B) Non-masked PD1/IL2 bispecific exposure after a single equal molar dose of 9.25 (High), 2.75 (Med) or 0.92 (Low) mg/kg I.V. injection in non-tumor bearing C57BL/6-hFcRn mice. (C-D) Body weight data are displayed until day 14 the last time point measured.

## Anti-tumor activity and tumor-selective pharmacodynamic effects in pre-clinical mouse tumor model

### XTX501 demonstrated anti-tumor activity in anti-PD1 insensitive setting at well-tolerated exposures

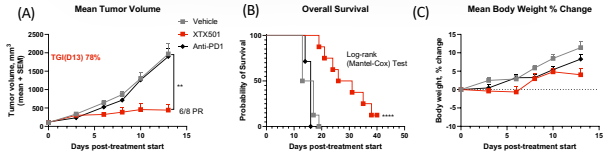


Figure 5: XTX501 anti-tumor activity in an anti-PD1 insensitive MC38 murine tumor model. C57BL/6-B-hPD1 mice were implanted subcutaneously with MC38 tumor cells and received two intravenous (i.v) injections of XTX501 (N=8), an equimolar concentration of anti-PD1 (N=7), or vehicle (N=8). Tumor and body weight measurements were taken two or three times a week. Data represent mean ± SEM (Standard error of the mean). (A) Tumor volume was assessed by a two-way ANOVA with Bonferroni's multiple comparisons test compared to vehicle treated animals.** P=0.0019 compared to vehicle-treated animals on Day 13. (B) Kaplan-Meier survival curves; animals were sacrificed when the ethical burden limit was reached (2000 mm³);(Log-rank (Mantel-Cox) test, ****P< 0.0001. (C) Body weight data are displayed until Day 13 when 100% of the vehicle-treated group were alive (N=8). PR: Partial Regression. *

### XTX501 treatment results in tumor-specific pharmacology with peripheral effects limited to increases in antigen-specific cells and memory cells in vivo

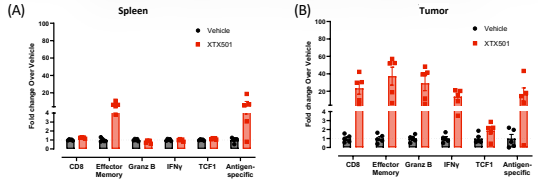


Figure 6: C57BL/6-B-hPD1 mice were implanted subcutaneously with MC38 tumor cells and received two (i.v) injections of XTX501 or vehicle control. (A-B) Immune cells were phenotyped. The percentage of cells for each immune phenotype was calculated as percentage of live CD45+ cells and the ratio of percent cells after XTX501 treatment to vehicle treatment is presented as mean ± SEM.. Effector memory (CD44+CD62L-), Antigen-Specific (p15E-Pentamer). *

### XTX501 demonstrated better activity than IL-2 + PD1 combination in pre-clinical mouse model

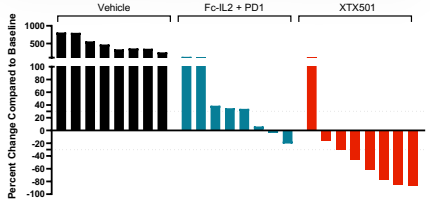


Figure 7: Female C57BL/6-B-hPD-1 mice (n=8 in each treatment group) were inoculated with MB49 tumor cells and received two (i.v) injections of vehicle or equimolar doses of anti-PD1 antibody (pembrolizumab) plus Fc-IL-2, or XTX501. Percent change in tumor volume on day 12 post treatment is shown as a waterfall plot. *

## XTX501 PK and safety profile in non-human primates (NHP)

### XTX501 demonstrated dose-proportional PK and was well-tolerated following a single IV dose in NHP

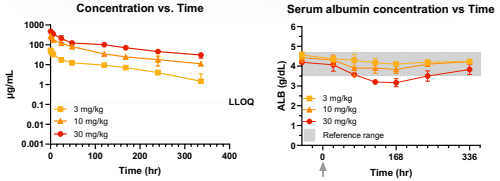


Figure 8: Female cynomolgus monkeys were given a single 30-minute I.V. infusion of XTX501 at 3, 10, and 30 mg/kg and samples were collected for PK and clinical pathology analysis. (A) PK analysis demonstrated dose-proportional exposure and linear elimination across all doses tested. (B) Albumin remained within normal ranges in animals receiving 3 and 10 mg/kg XTX501 and was transiently decreased in animals receiving 30 mg/kg PD1/IL2 bispecific. There were no adverse clinical observations, and transaminase levels remained within normal ranges for all animals (data not shown). *

### XTX501 is predicted to maintain >95% PD-1 receptor occupancy (RO); PD1/IL2 with non-masked IL-2 requires high and frequent dosing for sustained RO

| Species | Molecule | T1/2 (days) | Predicted PD-1 % Trough RO @Q3W dosing |
|---|---|---|---|
| NHP* | XTX501 | 3.4 | 95% |
| hFcRn mouse | XTX501 | 6 | >95% |
| hFcRn mouse | PD1/IL2 with non-masked IL-2 | 1.3 | <10% |

Figure 9: Trough Receptor Occupancy was modeled for PD-1 engagement of XTX501 at different dosing schemes in cynomolgus monkey and human. Calculated $t_{1/2}$ and predicted PD-1 Trough Receptor Occupancy of XTX501 given at 3 mg/kg in cynomolgus monkey and hFcRn mouse, and the equivalent dosage of PD1/IL2 with non-masked IL-2 in hFcRn mouse.

## Summary

Designed using a knowledge-based approach informed by Xilio's extensive clinical experience in tumor activated molecules[1,2], XTX501:

- Was better tolerated than non-masked PD1/IL2 in mouse models.
- Showed superior PK profile compared to non-masked PD1/IL2 in mice, akin to PD1 antibodies, projected to achieve >95% PD-1 RO with once every 3 weeks (Q3W) dosing.
- Exhibited anti-tumor activity in both PD1-sensitive and resistant preclinical models as a single agent, and showed enhanced efficacy compared to IL-2+PD1 combination therapy.
- Is ready for IND enabling studies

These data suggest that XTX501, a a tumor-activated PD1/IL2 bispecific, has the potential to improve upon the anti-tumor activity of the existing PD-1/PD-L1 immunotherapies while maintaining a favorable therapeutic index.

* Data generated with analog of XTX501 with minimal variance in amino acid sequence.

References: [1] Davar, D. ESMO GI 2023, [2] Hanna, D. SITC 2023