Tumor-activated Fc-engineered Anti-CTLA-4 Monoclonal Antibody, XTX101, Demonstrates Tumor-selective PD and Efficacy in Preclinical Models

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Disclosure Slide

- Financial relationships: Presenter is an employee of Xilio Therapeutics, Inc.

Introduction

- The clinical benefit of CTLA-4 blockade to cancer patients has been well established
- However, efficacy of current therapies is impaired by dose limiting toxicity arising from systemic immune activation

- **XTX101** is a tumor-selective anti-CTLA-4 mAb that is designed to improve upon the therapeutic index of existing anti-CTLA-4 therapies by overcoming potency and tolerability limitations
- **XTX101** has improved potency
  - Higher affinity binding to the target CTLA-4
  - Enhanced Fc effector function
- **XTX101** has reduced peripheral immune activity
  - Inactive while in circulation in the periphery due to masking of the CDR sequences
  - Activated by protease-dependent release of the masks
  - Active selectively in the tumor microenvironment and avoids toxicity associated with systemic immune activation

Ipilimumab data strongly validate potential for improved α-CTLA4 mAb

Ipilimumab Melanoma Randomized Phase 3 Study[1]

	Dose	Median OS	Adverse Events: gr 3/4 irAEs/discont.
Standard Approved Dose	3 mg/kg	11.5 mo	14% / 19%
	10 mg/kg	15.7 mo	30% / 31%

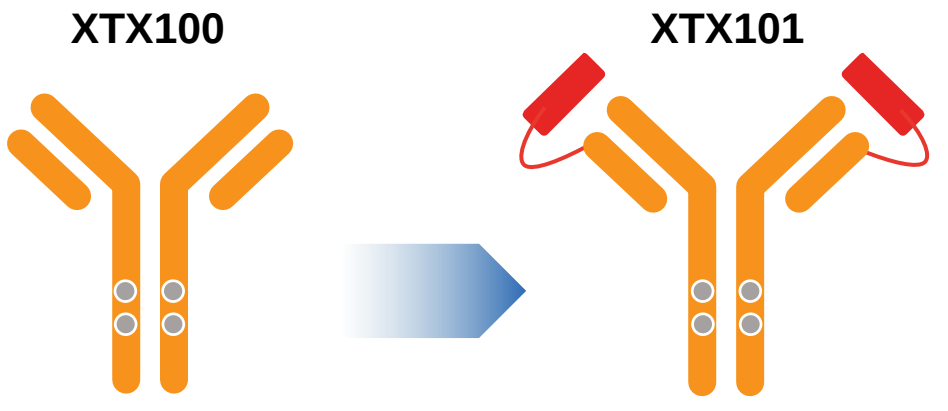
Conclusion: Treatment with higher dose resulted in increased OS but also increased AEs and discontinuations

- Ipilimumab shows preliminary evidence of promising antitumor activity in a range of tumor types, outside of the approved indications[2,3,4]
- Patients with high affinity FcγR polymorphisms have shown improved clinical responses to ipilimumab[5,6,7]
- Ipilimumab is more active when combined with nivolumab, including increased rate of irAEs[8,9,10]
- The therapeutic potential of ipilimumab mono-therapy or in combination with anti-PD-1 is curtailed by dose limiting systemic immune toxicities

- Xilio's approach is to combine tumor selectivity and enhanced potency of anti-CTLA4 treatment to achieve an improved therapeutic index (TI) with XTX101

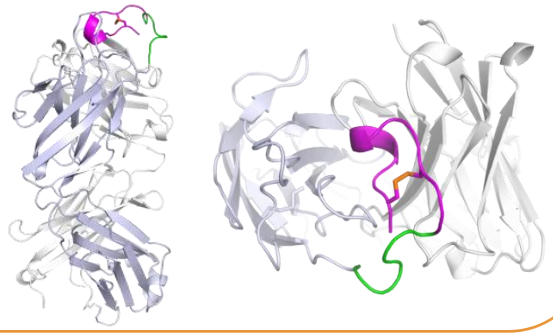
Sources: [1]Ascierto PA, *Lancet Oncol.* (2017); [2]Beer TM, *J. Clin. Oncol.* (2017), [3]Hellmann MD, *NEJM* (2019); [4]Kao HF, *Head Neck. (2019);* [5]Arce-Vargas F, *Cancer Cell* (2018); [6]Quezada SA *Clin. Cancer Res.* (2019); [7]Snyder A, *NEJM* (2014); [8]Wolchok JD, *Lancet Oncol.* (2010); [9]Hamid O., *J. Trans. Med* (2011); [10]Lebbé C, *J. Clin. Onc* (2019)

XTX101: Affinity and Fc enhancements combined with tumor selectivity for optimized TI

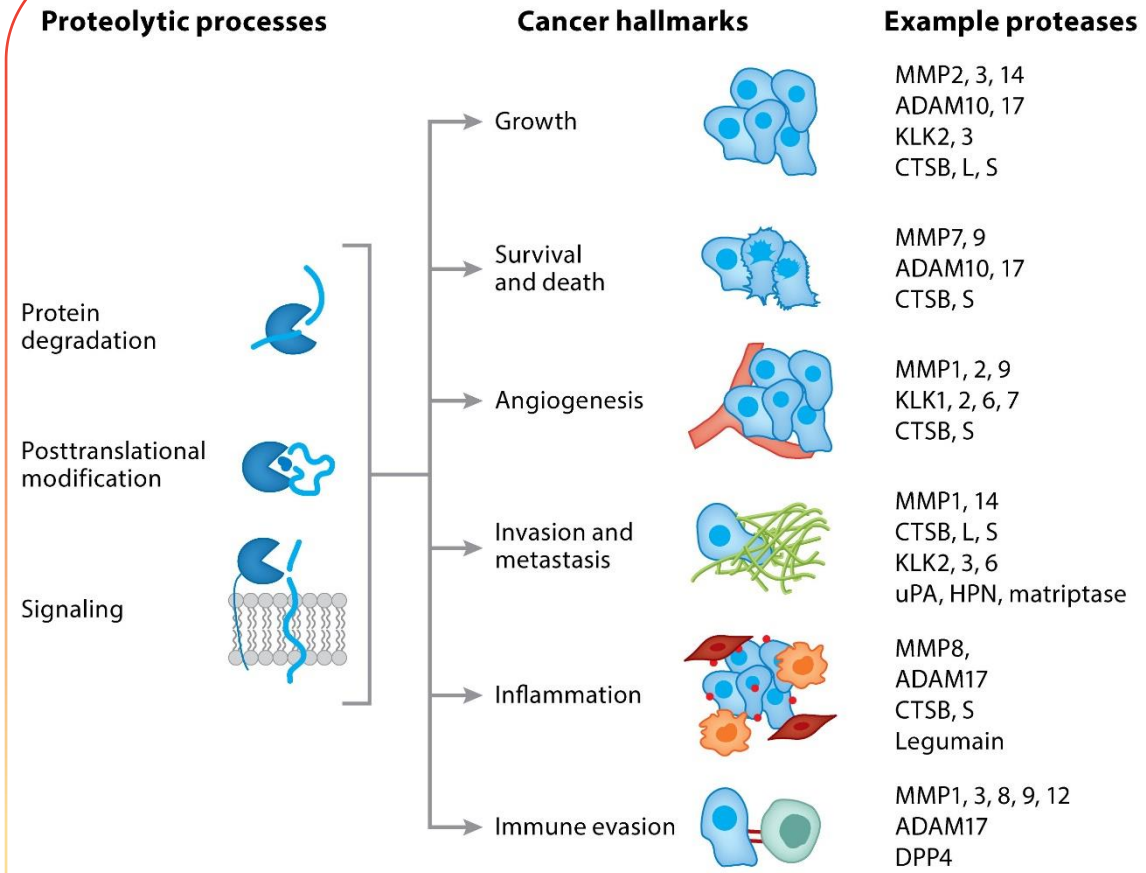


- α-CTLA4 mAb
- Improved affinity over ipilimumab
- Fc mutations for enhanced FcγR binding and ADCC

- Tumor-selectivity



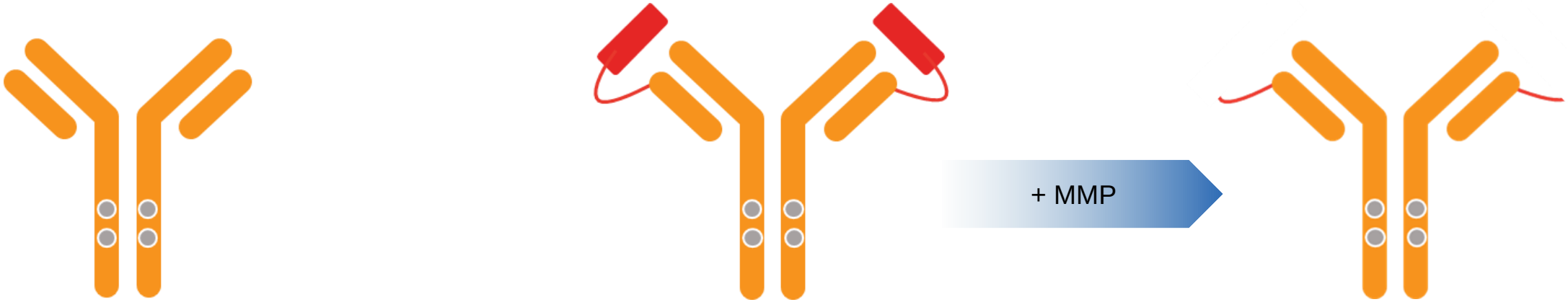
- Increased potency through improved affinity and enhanced ADCC to deplete Tregs
- Improved tolerability by adding tumor-selectivity
- Combining increased potency and improved tolerability to maximize opportunity for improved TI



- Protease dysregulation within the tumor microenvironment (TME) enable tumorigenesis.

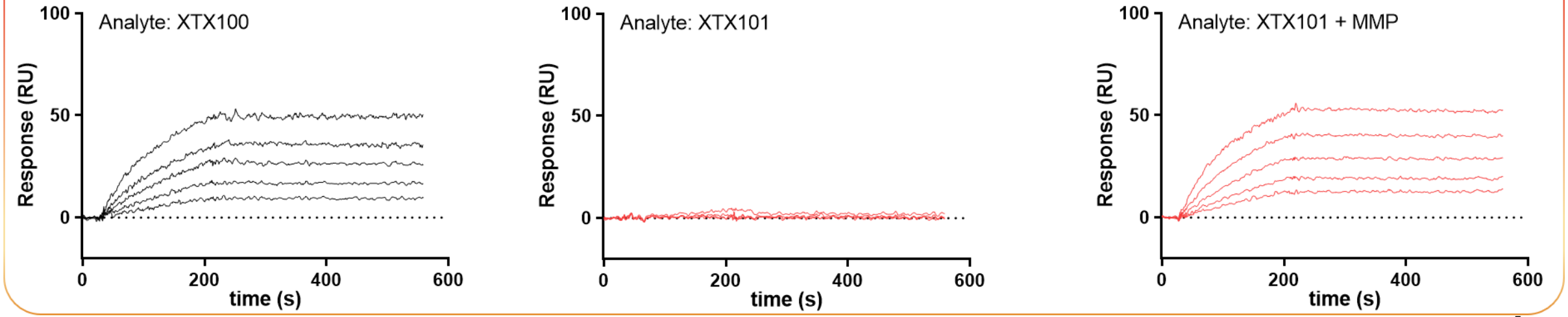
Modified from Dudani JS, et al. Annu Rev Cancer Bio *(2018)*

After proteolytic activation, full binding is restored to XTX101



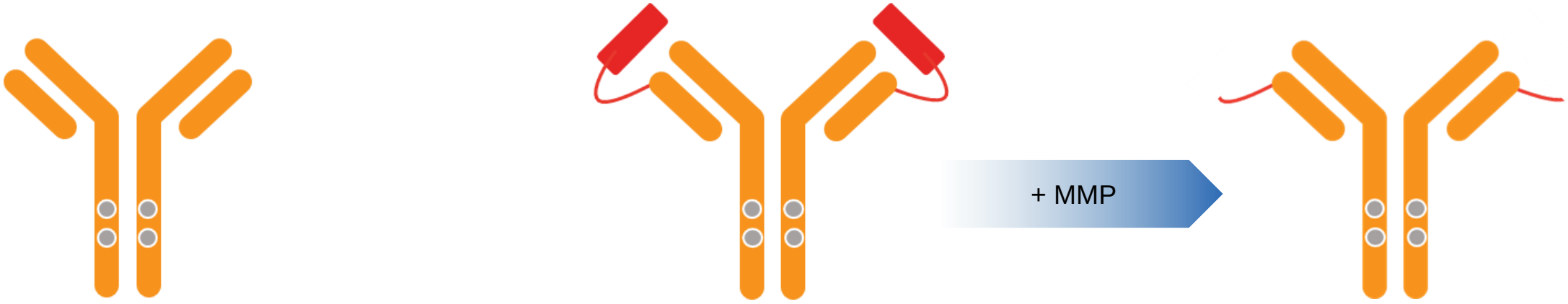
XTX100 **XTX101** **XTX101 + MMP**

Surface Plasmon Resonance (SPR) analysis; Ligand: hCTLA4

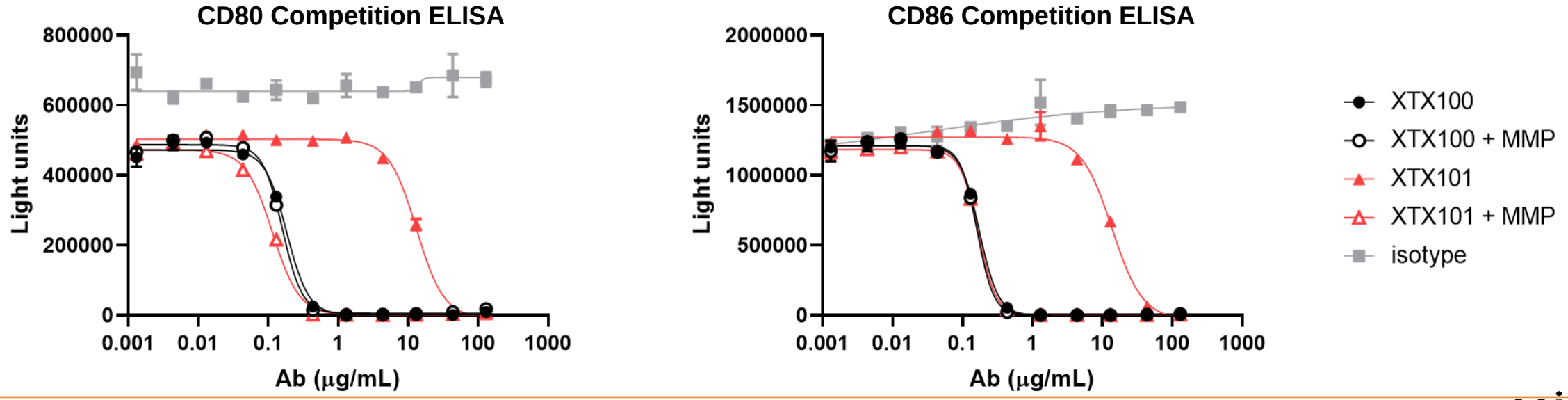


- Protease-dependent activation of XTX101 *in vitro*: biophysical assay

After proteolytic activation, XTX101 inhibits the binding of CTLA4 to its cognate ligands CD80 and CD86

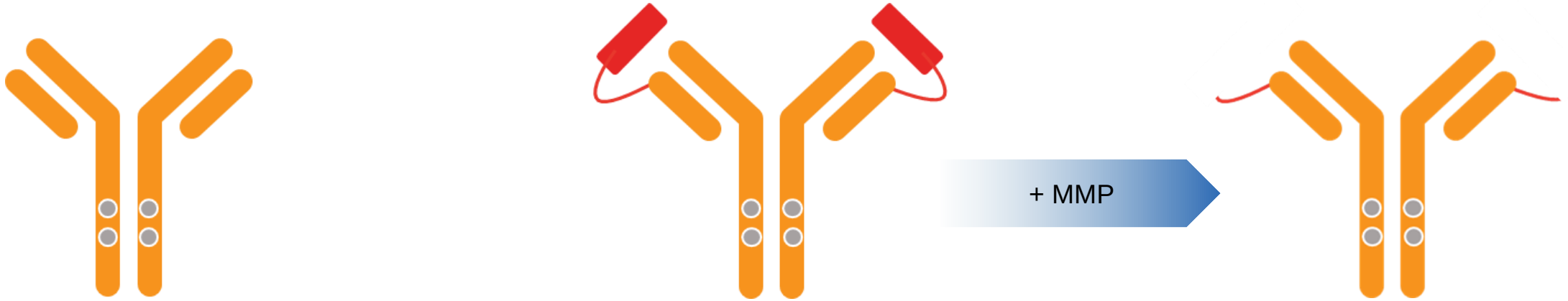


XTX100 **XTX101** **XTX101 + MMP**

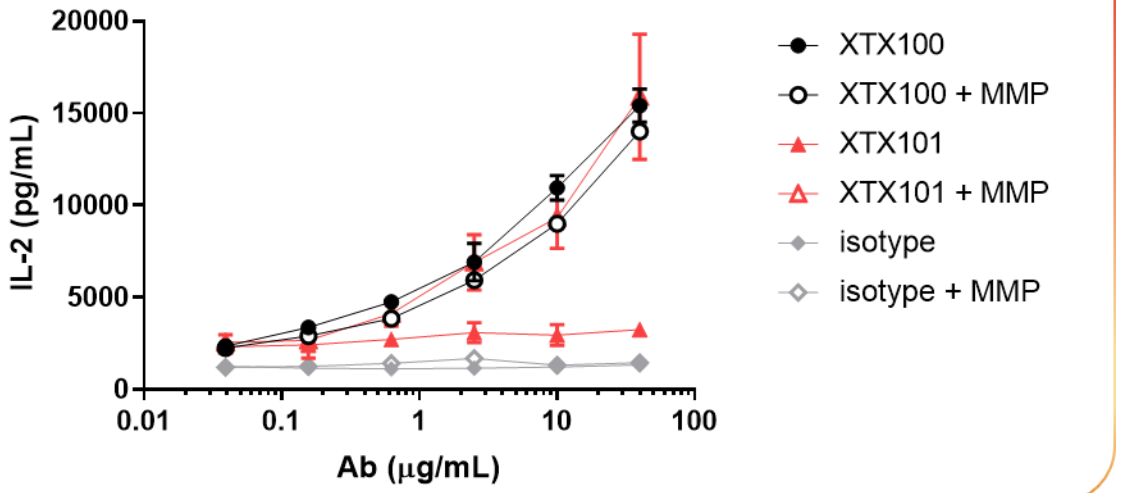


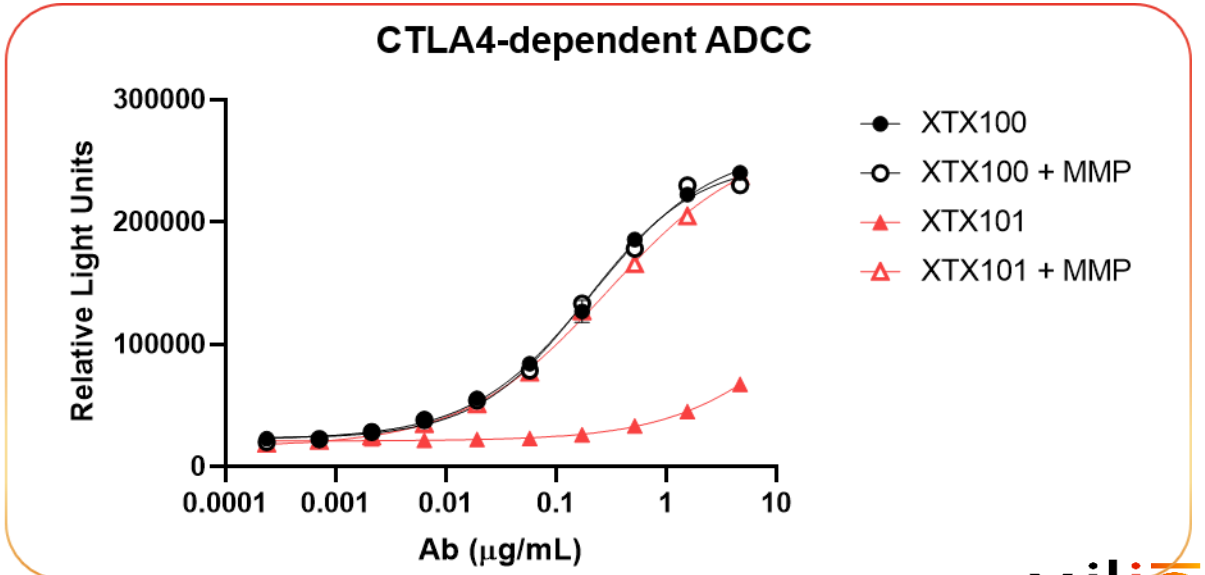
- Protease-dependent activation of XTX101 *in vitro*: competitive ELISA

After proteolytic activation, XTX101 mediates cellular activity in PBMC and ADCC reporter bioassays



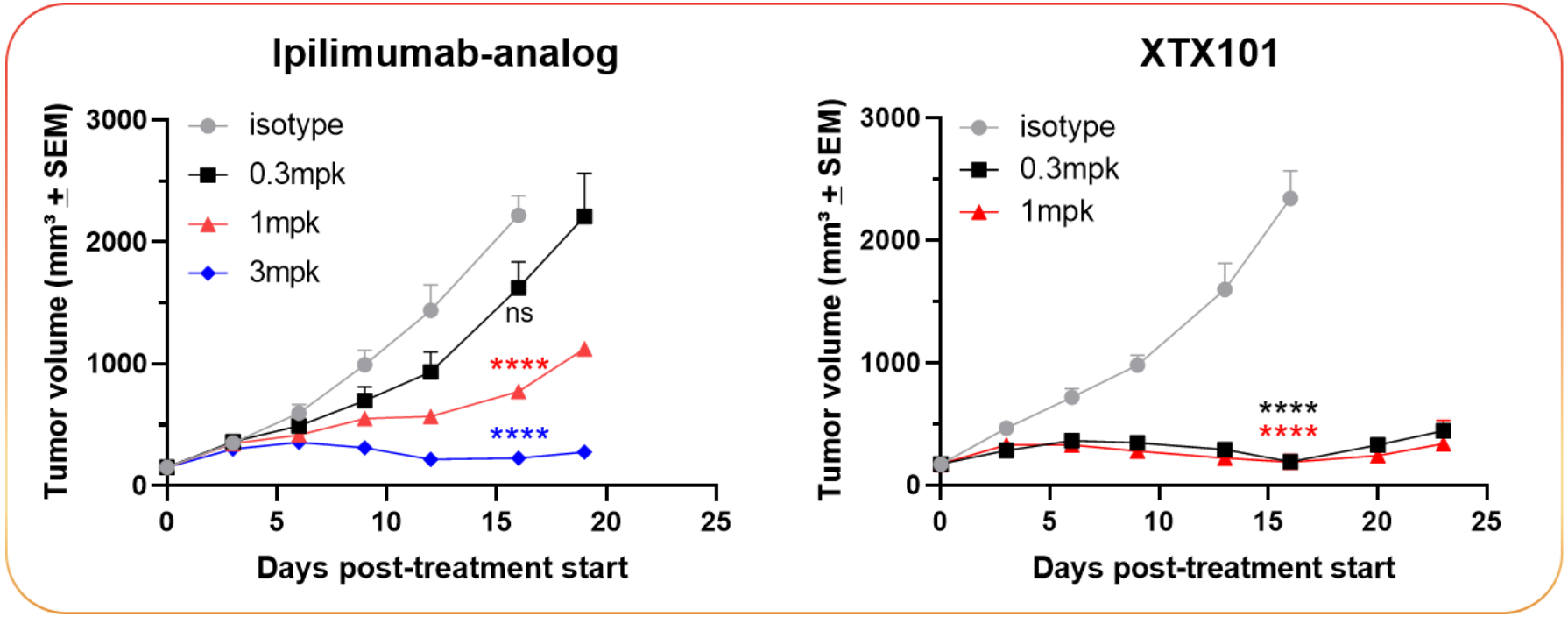
XTX100 **XTX101** **XTX101 + MMP**





- Protease-dependent activation of XTX101 *in vitro*: cell-based functional assays

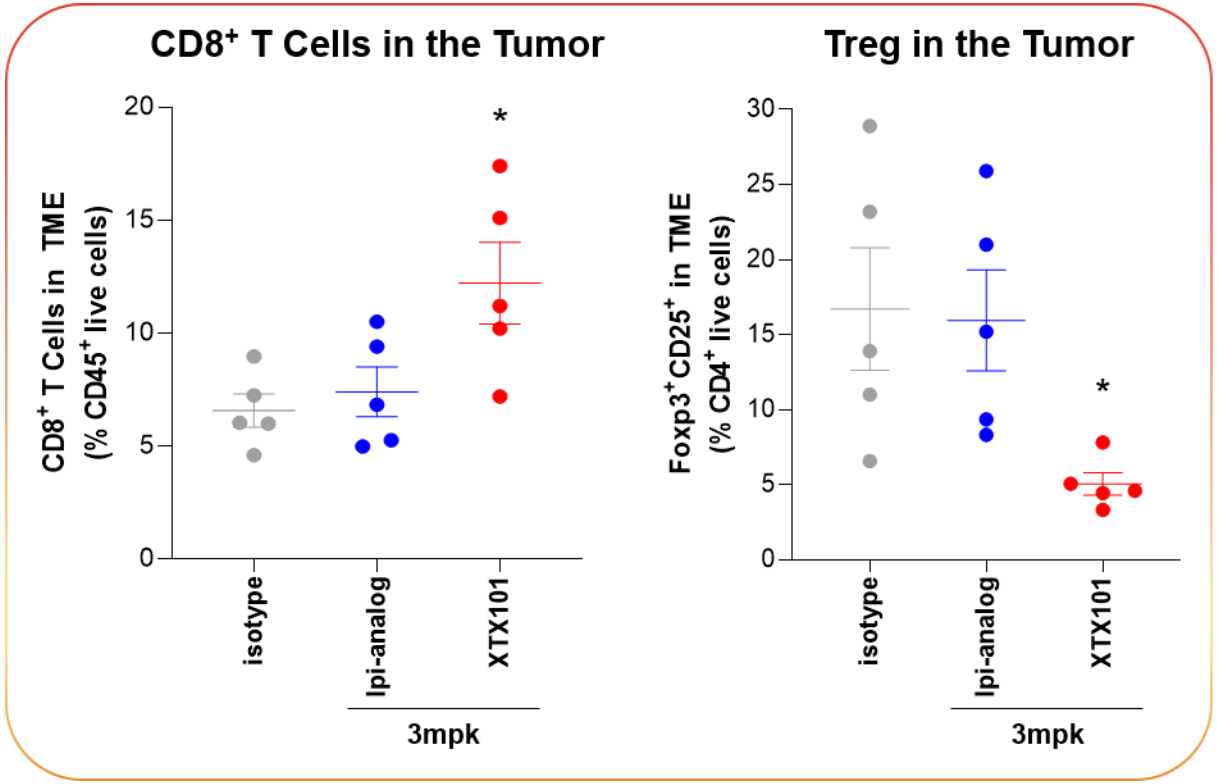
XTX101 is more potent than ipilimumab-analog

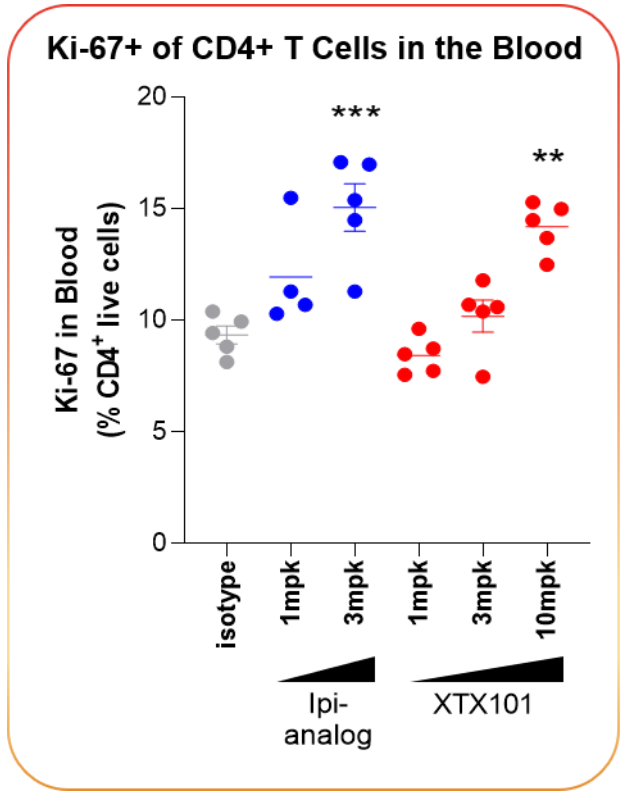


- XTX101 drives potent TGI, superior to ipilimumab-analog
- A dose of 3 mg/kg of ipilimumab-analog is required to achieve similar efficacy and CR rate as XTX101 at 0.3 mg/kg, suggesting XTX101 has 10x higher potency

MB49 cells were inoculated subcutaneously into C57BL/6-huCTLA4 mice. Mice were dosed single-dose i.v., 8 mice per dose group. A two-way ANOVA with Bonferonni's multiple comparisons post-test was performed to determine the statistical significance of treatment vs. isotype on Day 16 (ns not significant,;*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

XTX101 demonstrates potent intratumoral PD, superior to ipilimumab-analog

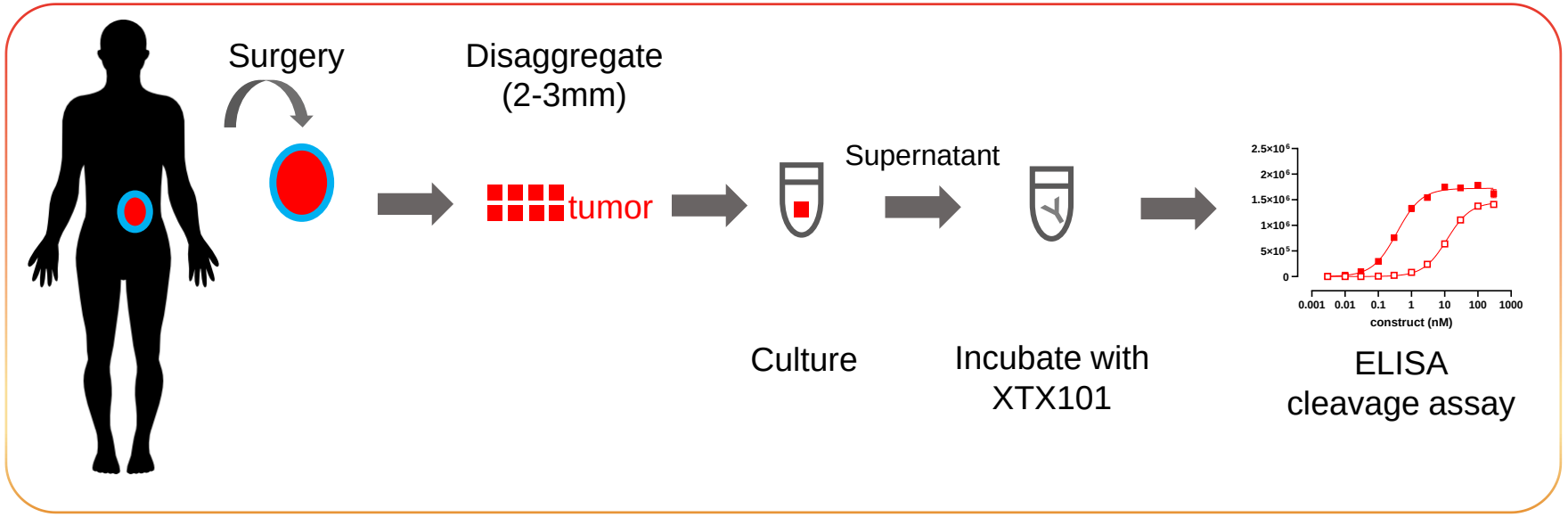




- XTX101 shows Treg depletion in the tumor and no effect on peripheral Treg levels
- A dose of 3 mg/kg of ipilimumab-analog is required to achieve similar peripheral PD as XTX101 at 10 mg/kg, consistent with better tolerability of XTX101 in mice

MB49 cells were inoculated subcutaneously into C57BL/6-huCTLA4 mice. Mice were dosed single-dose i.v. A one-way ANOVA with Bonferroni's multiple comparisons post-test was performed to determine the statistical significance of treatment vs. isotype (*P<0.05; **P<0.01; ***P<0.001).

Broad activation of XTX101 across human tumors in *ex vivo* assay



Cancer type	Melanoma	RCC	Ovary	Bladder	Colon	NSCLC	Breast	Liver	Total
% Tumors that activate XTX101	71%	57%	58%	67%	91%	67%	75%	67%	66%
Sample size	7	30	12	6	11	9	4	6	85

- Broad potential for tumor selective XTX101 activity leads to opportunities in many indications
- Given the broad activation of XTX101 across diverse tumor types, Companion Diagnostic (CDx) biomarker for protease activity or expression likely not required

Conclusion

- The clinical benefit of CTLA-4 blockade is well established, but remains limited due to toxicities arising from systemic immune activation
- XTX101 is a potent, tumor-selective anti-CTLA4 mAb with the potential to significantly expand the TI relative to ipilimumab and therefore potentially reduce toxicity while enhancing efficacy

- Functional characterization of XTX101 confirmed protease-dependent activity
  - XTX101 binds to CTLA-4 with high affinity in a protease-dependent manner
  - XTX101 exhibits protease dependent inhibition of CD80/86 binding to CTLA-4
  - Protease-dependent activation of XTX101 overcomes CTLA-4 inhibition of T cell activation
  - Enhanced effector function of XTX101 confers potent protease & CTLA-4 binding dependent ADCC activity
- Tumor-selective activity of XTX101 was demonstrated by *in vivo* studies
  - XTX101 demonstrates 10x improvement in potency in tumor growth inhibition studies
  - XTX101 exhibited enhanced Treg depletion in tumors
  - XTX101 showed reduced peripheral immune activation by comparison to ipilimumab-analog

- XTX101 is activated broadly across multiple tumor indications based on *ex vivo* studies in fresh human tumor tissue
- These data support evaluation of XTX101 in clinical studies

Title: Tumor-activated Fc-engineered anti-CTLA-4 monoclonal antibody, XTX101, demonstrates tumor-selective PD and efficacy in preclinical models
Abstract ID: 587

See also:
Title: XTX201, a protein-engineered IL-2, exhibits tumor-selective activity in mice without peripheral toxicities in non-human primates
Abstract ID: 568

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