Tumor-Activated Anti-CTLA-4 Monoclonal Antibody, XTX101, Demonstrates Monotherapy and Anti-PD-1 Combination Benefit in Preclinical Models

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BACKGROUND

- The clinical benefit of CTLA-4 blockade to cancer patients has been well established
- However, efficacy of current therapies is limited 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** is designed for improved potency
  - Higher affinity binding to the target CTLA-4
  - Enhanced Fc effector function
- **XTX101** is designed to have reduced peripheral immune activity
  - CDR sequences are masked while in circulation
  - Activated by protease-dependent release of the masks
  - Selectively active in the tumor microenvironment and avoids toxicity associated with systemic immune activation

Ipilimumab data strongly validate potential for improved α-CTLA-4 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 overall survival (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]
- Ipilimumab is more active when combined with nivolumab, including increased rate of irAEs[7]
- The therapeutic potential of ipilimumab monotherapy 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-CTLA-4 treatment to achieve an improved therapeutic index (TI)

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] Lebbé C, J. Clin. Onc (2019)

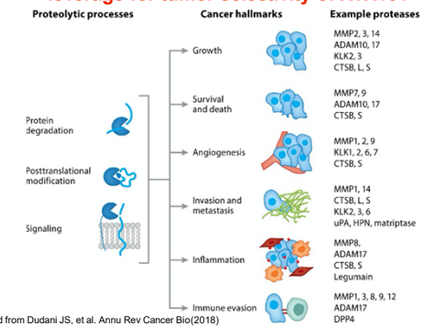
BACKGROUND

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

- α-CTLA-4 mAb
- Fc mutations for enhanced FcγR binding and ADCC
- Improved affinity over ipilimumab

- 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

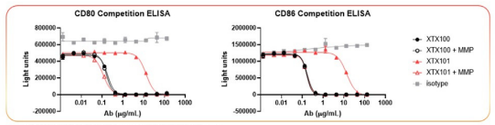
Tumors are proteolytically active environments that we leverage for tumor selectivity of XTX101



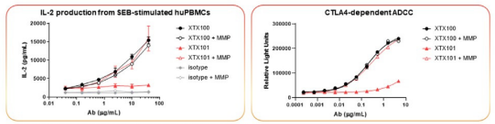
Modified from Dudani JS, et al. Annu Rev Cancer Bio(2018)

RESULTS

After proteolytic activation, XTX101 inhibits binding of CTLA-4 to its cognate ligands CD80 and CD86, and mediates cellular activity in PBMC and ADCC assays



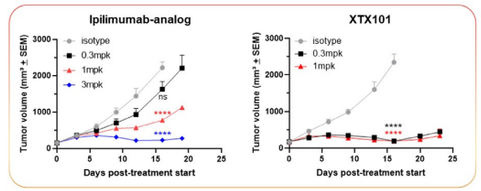
- Protease-dependent activation of XTX101 *in vitro*: competitive ELISA



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

RESULTS

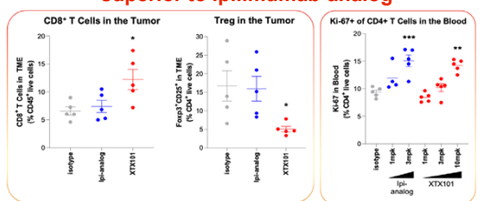
XTX101 is more potent than ipilimumab-analog



- XTX101 drives potent tumor growth inhibition (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-huCTLA-4 mice. Mice were dosed single-dose i.v. 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).

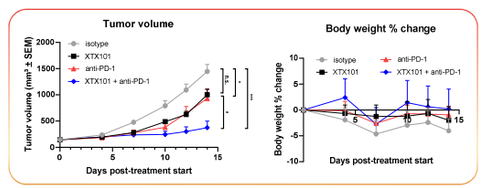
XTX101 demonstrates potent intratumoral pharmacodynamic (PD) response, superior to ipilimumab-analog



- 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-huCTLA-4 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).

Significant combination benefit observed with XTX101 and anti-muPD-1

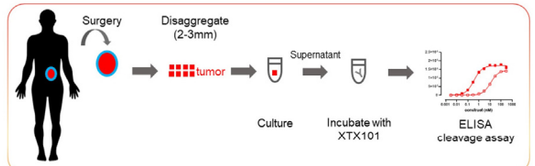


- Significant TGI observed in mice treated with XTX101 and anti-PD-1 combination, and no significant decrease of body weight was observed.

MC38 cells were inoculated subcutaneously into the right flank of C57BL/6-huCTLA-4 mice. Therapy started when the tumors reached ~144 mm³. Mice were dosed i.v with 0.3 mg/kg of XTX101 on day 0; i.p. with 10 mg/kg of anti-PD-1 on day 0, 3, 6. A Two-way ANOVA with Dunnett's multiple comparisons post-test was performed to determine the statistical significance of treatment between groups (n.s.=not significant; *p<0.05; **p<0.01; ***p<0.001).

RESULTS

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-CTLA-4 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 murine tumor growth inhibition studies
  - XTX101 exhibited enhanced Treg depletion in tumors
  - XTX101 showed reduced peripheral immune activation by comparison to ipilimumab-analog
  - Significant benefit observed with XTX101 and anti-PD-1 combination, inducing increased TGI without an increase in toxicity

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