XTX201, a protein-engineered IL-2, exhibits tumor-selective activity in mice without peripheral toxicities in non-human primates

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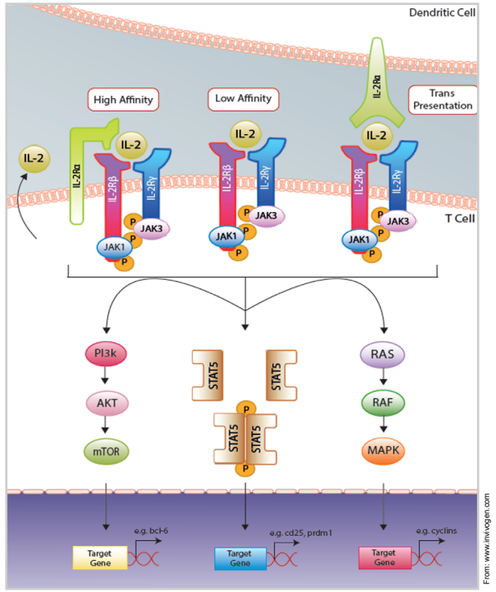
BACKGROUND

IL-2 Receptor Complexes and Signaling Pathway



The IL-2 cytokine binds with high affinity to the trimeric receptor complex consisting of the α (CD25), β (CD122), and γ (CD132) subunits. IL-2 can also bind to the dimeric receptor complex consisting of the β and γ subunits with lower affinity. Binding of IL-2 to either the trimeric or dimeric receptor complex leads to downstream signaling including activation of STAT5 and activation of T cells. T regulatory cells express high levels of the α subunit and are activated by low levels of IL-2. Conventional naïve CD4+ and CD8+ T cells express the dimeric IL-2 receptor. Only high concentrations of IL-2 activate conventional CD4+ and CD8+ T cells[1,2].

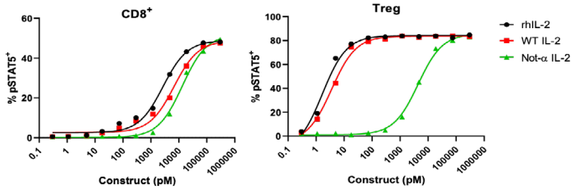
The immunostimulatory effects of high concentrations of IL-2 has been exploited for cancer therapy. High-dose recombinant human interleukin-2 (aldesleukin) elicits durable anti-tumor immunity and gained FDA approval two decades prior to checkpoint blockers. However, use of aldesleukin is limited due to treatment-related life-threatening toxicities[3]. Second generation efforts to alleviate toxicities have largely focused on eliminating binding to IL-2Rα, often with half-life extension.

We have determined that mice and non-human primates (NHPs) treated with a 2nd generation IL-2 surrogate that does not bind IL-2Rα (not-α IL-2) still experience characteristic dose-limiting toxicities, including vascular leak syndrome (VLS), and exhibit dysregulated peripheral immune function due to reduced Treg activation. To overcome these toxicities and improve the therapeutic index of IL-2 as an anti-tumor immunotherapy, we employed protein engineering to generate XTX201. XTX201 is a highly potent masked not-α IL-2 that is designed to be pharmacologically inactive until it is unmasked in tumors by proteases that are selectively active in the TME, stimulating cytolytic responses against tumor cells while sparing systemic immune activation.

[1]Malek, T.R. The biology of interleukin-2. Annu Rev Immunol 26, 453-479 (2008).
[2]Malek, T.R. & Castro, I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity 33, 153-165 (2010).
[3]Jeal, W. & Goa K.L. Aldesleukin (recombinant interleukin-2): a review of its pharmacological properties, clinical efficacy and tolerability in patients with renal cell carcinoma. BioDrugs 7(4): 285-317 (1997).

RESULTS

IL-2 mutations reduce Treg activation and improve CD8/Treg ratio

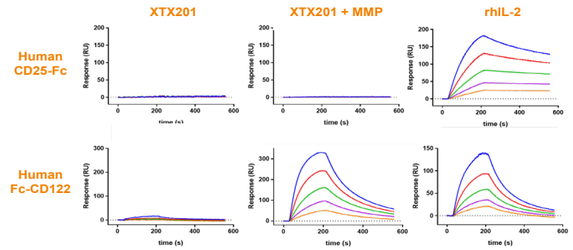


	CD3+ CD4+ FoxP3+		CD3+ CD8+		CD8/Treg
Test Molecule	EC_{50} (pM)	Reduction in Treg Activity	EC_{50} (pM)	Reduction in CD8 Activity	EC_{50} Ratio
rhIL-2	1.67		2710		1,622
WT IL-2	3.59	1.0	6700	1.0	1,866
Not-α IL-2	4390	1222	13200	2.0	3.0

- IL-2 mutations reduced Treg activation ~ 1200-fold and improve CD8/Treg ratio by ~ 600-fold
- This mutant has been selected for XTX201

Primary human peripheral blood mononuclear cells were treated with IL-2 for 20 minutes. Cells were fixed, permeabilized and stained with Foxp3, pSTAT5, CD3, CD4 and CD8 antibodies. Cell populations and STAT activation were determined by flow cytometry.

XTX201 exhibits protease-dependent binding to CD122, and neither masked or active forms of XTX201 bind to IL2Ra



Binding to Human CD25-Fc

	ka (1/(M*s))	kd (1/s)	KD (M)
XTX201	ND	ND	ND
XTX201 + MMP	ND	ND	ND
rhIL-2	8.30e+5	7.87e-4	0.88 nM

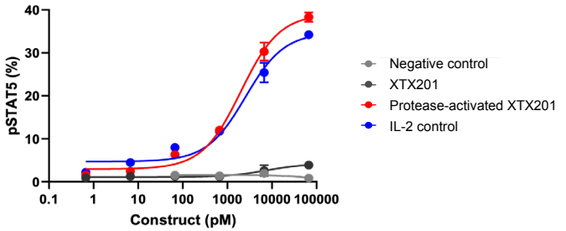
Binding to Human Fc-CD122

	ka (1/(M*s))	kd (1/s)	KD (M)
XTX201	ND	ND	ND
XTX201 + MMP	3.58e+4	5.40e-3	151 nM
rhIL-2	2.01e+4	7.87e-3	392 nM

Surface plasmon resonance (SPR) was used to measure binding kinetics of XTX201, activated XTX201 and recombinant human IL-2 to IL-2 receptors. XTX201, activated XTX201, and IL-2 were immobilized to a sensor chip, with IL-2 receptors flowed over at concentrations of 1 nM to 16 nM for CD25 and 31.3 nM to 500 nM for CD122.

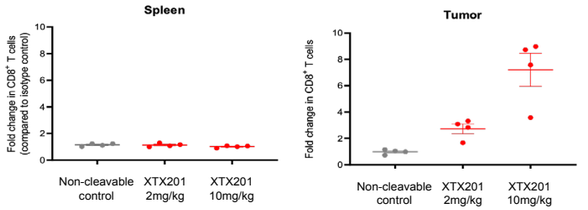
RESULTS

XTX201 stimulates IL-2 signaling in human CD8 T cells only after protease activation



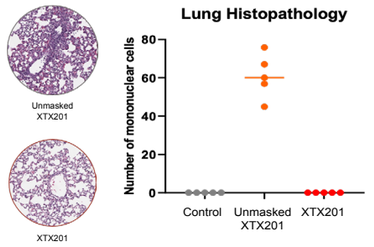
Primary human peripheral blood mononuclear cells were treated with IL-2, XTX201, protease-activated XTX201 or negative control for 20 minutes. Cells were fixed, permeabilized and stained with Foxp3, pSTAT5, CD3, CD4 and CD8 antibodies. Cell populations and STAT activation were determined by flow cytometry.

XTX201 induces tumor selective immune activation in mice



MC38 tumor bearing C57bl/6 mice were administered a single intravenous dose of XTX201 at 2mg/kg or 10mg/kg. Flow cytometry analysis was performed 5 days after mice were dosed. Fold change in CD8+ compared to noncleavable masked IL-2 control molecule was determined.

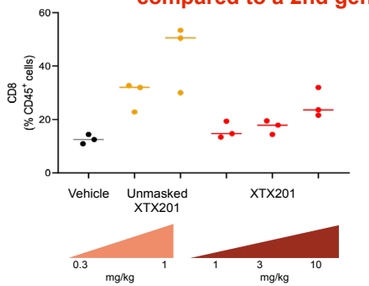
XTX201 eliminates IL-2-induced lethal vascular leak syndrome in mice



MC38 tumor bearing C57Bl/6 mice were administered 4 daily doses of unmasked XTX201 (2nd gen IL-2)1 or XTX201. Mice administered unmasked XTX201 die at day 4. H&E staining of lung sections was performed to detect mononuclear cell infiltrate.

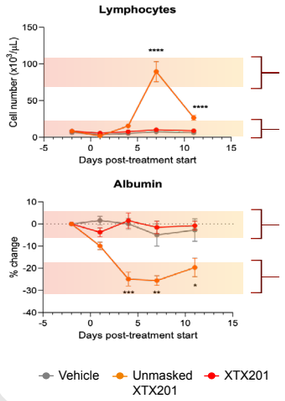
RESULTS

XTX201 effect on CD8+ T cell expansion in peripheral blood of non-human primates is reduced compared to a 2nd gen IL-2



Cynomolgus monkeys were intravenously administered the indicated doses of unmasked XTX201 (2nd gen IL-2) or XTX201. Immunophenotyping in peripheral blood was performed seven days after dosing.

XTX201 is well tolerated in non-human primates



Unmasked molecule induces lymphocyte proliferation

XTX201 has no effect on lymphocyte proliferation

XTX201 has no effect on blood albumin

Unmasked molecule induces vascular leak syndrome

Cynomolgus monkeys were administered a single intravenous dose of vehicle, or 1mg/kg of unmasked XTX201 (2nd gen IL-2 or XTX201. Blood lymphocyte counts and serum albumin levels were measured at multiple timepoints after dosing. A repeated measurement two-way ANOVA with Bonferroni's multiple comparison correction was performed to determine the statistical significance of treatment vs vehicle (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

CONCLUSION

- XTX201 is a masked, tumor selective IL-2 molecule that has the potential to have a broader therapeutic index than aldesleukin and 2nd gen IL-2 therapies currently under development.
- In its native form, XTX201 does not bind to IL-2 receptors.
- Upon activation by proteases that are preferentially active in the tumor microenvironment, XTX201 binds to the IL-2 receptor β and γ subunits and drives immune cell activation.
- In mouse models, XTX201 demonstrates tumor selective immune activation and no vascular leak syndrome.
- In non-human primates, XTX201 effect on peripheral immune cell activation is reduced compared to unmasked XTX201, and XTX201 does not induce peripheral lymphocyte expansion or serum albumin decrease (a marker of vascular leak syndrome).