XTX202, a protein-engineered IL-2, exhibits tumor-selective activity in mice without peripheral toxicities in non-human primates

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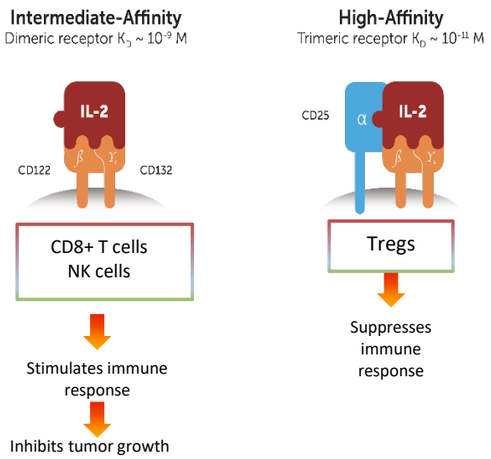
BACKGROUND

IL-2 Receptor Complexes and Downstream Functional Effects



Adapted from Boder, E. T. Tighter ties that bind. Nature 484: 463–464 (2012).

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 and can only be activated at high concentrations of IL-2[1,2].

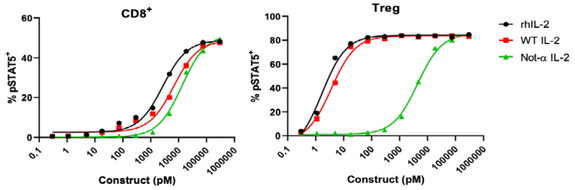
The immunostimulatory effects of high concentrations of IL-2 have been exploited for cancer therapy. High-dose recombinant human interleukin-2 (aldesleukin) elicits durable anti-tumor immunity and gained FDA approval two decades prior to checkpoint inhibitors However, use of aldesleukin is limited due to treatment-related life-threatening toxicities[3]. Efforts to develop second generation IL-2 molecules have largely focused on eliminating binding to IL-2Rα to alleviate toxicities 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 seek to overcome these toxicities and improve the therapeutic index of IL-2 as an anti-tumor immunotherapy, we employed protein engineering to generate XTX202. XTX202 is designed to be 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 tumor microenvironment, 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

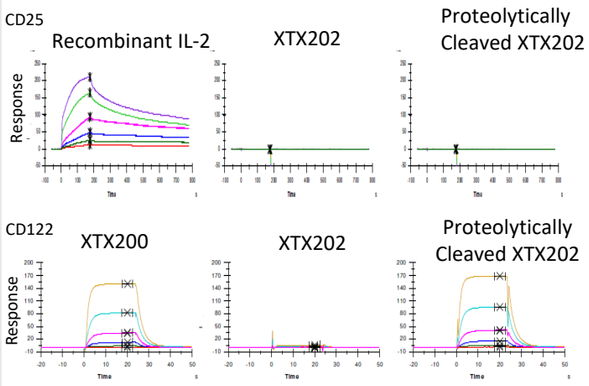


	CD3+ CD4+ FoxP3+		CD3+ CD8+		CD8/Treg EC_{50} Ratio
Test Molecule	EC_{50} (pM)	Reduction in Treg Activity	EC_{50} (pM)	Reduction in CD8 Activity	
rhIL-2	1.67		2710		1,622
WT IL-2	3.59	1.0	6700	1.0	1,866
Not-α IL-2	4390	1222	13200	2.0	3.0

- IL-2 mutations reduced Treg activation ~ 1200-fold and improve CD8/Treg ratio by ~ 600-fold
- This mutant has been selected for XTX202

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.

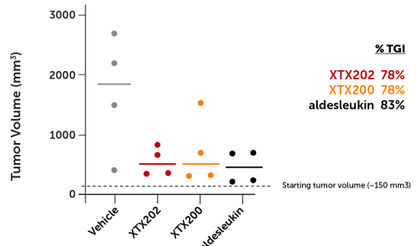
XTX202 is a not-α IL-2 molecule that binds IL-2Rβ upon activation



Surface plasmon resonance (SPR) was used to measure binding kinetics of XTX202, proteolytically cleaved XTX202, XTX200 (unmasked parental form of XTX202, 2nd generation IL-2 surrogate) and recombinant human IL-2 to IL-2 receptors. XTX202, proteolytically cleaved XTX202, XTX200 and IL-2 were immobilized to a sensor chip, with IL-2 receptors flowed over at concentrations of 1.56, 3.125, 6.25.12.5, 25, 50 and100 nM for CD25 and 16.5, 49, 148, 444, 1333 and 4000 nM for CD122. Receptor concentrations decrease from top to bottom in panels.

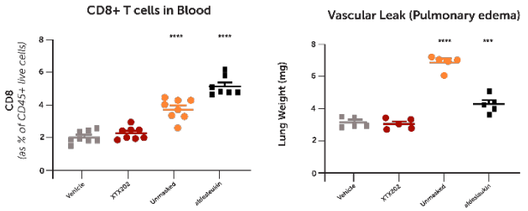
RESULTS

XTX202 demonstrates similar tumor growth inhibition as aldesleukin and XTX200 in mice



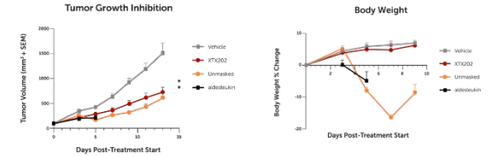
Female C57BL/6 mice (n=4 per treatment group) were inoculated with 0.5x106 MC38 tumor cells subcutaneously in the right flank. Treatment was initiated when tumors reached 150 mm^3. Animals received 10 mg/kg of XTX202 or 0.36 mg/kg of XTX200 on day 0 and day 3, or 3 mg/kg BID on days 0 – 4 for aldesleukin.

XTX202 does not induce peripheral immune cell activation or pulmonary edema in mice



Female C57BL/6 hFcRn mice (n=8 in each treatment group) were inoculated with 1x10^6 MB49 tumor cells subcutaneously in the right flank. On day 0, mice received 2 mg/kg of XTX202 or 0.36 mg/kg XTX200 every 2 days. Aldesleukin was given BID at 3mg/kg for three days. Flow cytometry on peripheral blood was performed on day 5. Lung weights were measured on day 8. A One-way ANOVA Dunnet's multiple comparison post-test was performed to determine the statistical significance of treatment vs vehicle(*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

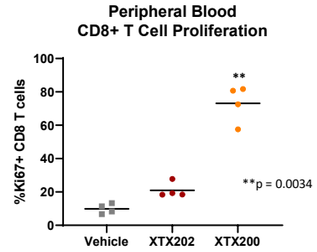
XTX202 inhibits tumor growth in a mouse syngeneic tumor model without body weight loss



Female C57BL/6 hFcRn mice (n=8 in each treatment group) were inoculated with 1x10^6 MB49 tumor cells subcutaneously in the right flank. On day 0, mice received 2 mg/kg of XTX202, 0.36 mg/kg of XTX200 every 2 days. Aldesleukin was given BID at 3mg/kg for three days. Aldesleukin resulted in mortality by day 5. A 2-way ANOVA Dunnet's multiple comparison post-test was performed on day 13 to determine the statistical significance of treatment vs vehicle(*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

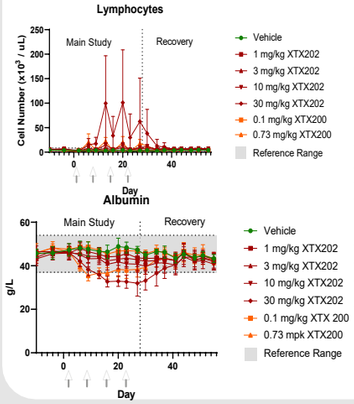
RESULTS

XTX202 does not induce peripheral immune cell activation in NHPs



Cynomolgus monkeys were intravenously administered 1 mpk of XTX200 or XTX202. Immunophenotyping in peripheral blood was performed six days after dosing. A Kruskall-Wallis nonparametric test with Dunn's multiple comparison post-test was performed to determine the statistical significance of treatment vs vehicle (**P<0.001).

XTX202 is well tolerated in non-human primates



Cynomolgus monkeys were intravenously administered repeat doses of vehicle, XTX 202 or XTX200 on Days 1, 8, 15, 22 (indicated by ↑) in a dose range finding toxicology study. Blood lymphocyte counts and serum albumin levels were measured at multiple timepoints after dosing. Repeat dosing of XTX202 up to 10 mg/kg showed no adverse changes in lymphocytes or albumin with equivalent or lesser effects respectively, to 0.73 mg/kg XTX200. 30 mg/kg of XTX202 did result in larger changes in both lymphocytes and albumin than the highest dose of XTX200, but was tolerated. The 0.73 mg/kg dose of XTX200 was not well tolerated, and one animal had a dosing holiday.

CONCLUSIONS

- XTX202 is a masked, tumor selective not-α IL-2 molecule being advanced toward planned clinical trials.
- In its masked form, XTX202 does not bind to IL-2 receptors.
- Upon activation by proteases that are preferentially active in the tumor microenvironment, XTX202 binds to the IL-2 receptor β and γ subunits and drives immune cell activation.
- XTX202 inhibits tumor growth without peripheral immune activation, lung edema or body weight loss in mice.
- In non-human primates, repeat dosing of XTX202 was well tolerated up to 30 mg/kg.
- Based on tumor growth inhibition data in mice and NHP tolerability data, we estimate that XTX202 has a 10-fold improved therapeutic index (TI) compared to 2nd generation IL-2 and a ≥150 fold improved TI compared to aldesleukin.