XTX201, A PROTEIN-ENGINEERED IL-2, EXHIBITS TUMOR-SELECTIVE ACTIVITY IN MICE WITHOUT PERIPHERAL TOXICITIES IN NON-HUMAN PRIMATES


Risk Research Related Activities at Stanford Blood Center

Abstracts

Background 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. 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 still exhibit 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, a highly potent 3rd generation IL-2 that is designed to be selectively active in tumors, stimulating cytolytic responses against tumor cells while sparing systemic immune activation.

Methods XTX201 binding interactions were measured with SPR, and bioactivity was measured using STAT-5 phosphorylation in human PBMCs and reporter cell lines. Anti-tumor efficacy and immune activation was evaluated in tumors compared to peripheral organs in syngeneic tumor mouse models. Safety and pharmacokinetics were evaluated in rodent and NHPs.

Results Non-activated XTX201 showed no detectable binding to IL-2Rα or IL-2Rβ, and limited IL-2R-dependent STAT-5 signaling in vitro. Activation of XTX201 resulted in high-affinity binding to IL-2Rβ and no binding to IL-2Rα, leading to a ~1000-fold reduction in Treg activation as compared to WT IL-2, while retaining CD8+ T and NK cell activation. Mice and NHPs treated with a 2nd generation IL-2 surrogate experienced toxicities that are commonly observed in patients treated with aldesleukin, including pulmonary edema, VLS, fever and lethality. However, XTX201 did not induce toxicities at exposures 100-fold higher than the MTD of the activated version, and achieved similar anti-tumor efficacy in mice. Experiments in primary human solid tumors and human plasma indicated that XTX201 is preferentially activated in the tumor microenvironment.

Conclusions Our data demonstrate that 2nd generation IL-2s that are systemically active and lack binding to IL-2Rα exhibit dose-limiting toxicities unless further engineered for selective activity in tumors. XTX201, a 3rd generation, tumor-selective IL-2, exhibits long half-life and is innocuous outside of tumors. XTX201 is activated within tumors to release an IL-2Rβ/γ biased cytokine that inhibits tumor growth in syngeneic models, and exhibits tumor-specific pharmacodynamic effects without peripheral toxicities. XTX201 has the potential to be a best-in-class IL-2 immunotherapy by expanding the curative anti-tumor activity of aldesleukin while minimizing dose-limiting toxicities.

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IL-15/IL-15Rα HETERODIMERIC COMPLEX AS CANCER IMMUNOTHERAPY IN MURINE BREAST CANCER MODELS

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Background Interleukin 15 (IL-15) has been evaluated as a potential treatment for solid tumors in clinical trials, but the effectiveness of systemic IL-15 administration as a monotherapy has not been realized. IL-15 receptor alpha (IL-15Rα) can stabilize IL-15 and enhance its bioactivity. The goal of this study was to examine the activity of IL-15/IL-15Rα complex (IL-15cx) to CD8+ T cells and evaluate its potential efficacy in murine breast cancer models.

Methods The bioactivity of IL-15cx to CD8 T cells was assessed by ex vivo and in vivo cell proliferation assays. The antitumor efficacy was studied in mouse mammary carcinoma models (Her2/neu transgenic and 4T1-luc mammary cancers) treated with systemic recombinant protein with/without the depletion of myeloid-derived suppressor cells or intratumoral gene electroporation (GET). Systemic and regional changes of immune cells were examined by flow cytometry, and tumor specific IFN-γ release from immune cells was measured by ELISA assays.

Results IL-15cx shows superior in vivo bioactivity to expand CD8 T cells in comparison to an equimolar single chain IL-15. T-bet is partially involved in CD8 T cell expansion ex vivo and in vivo due to IL-15 or IL-15cx. Intraperitoneal administration of IL-15cx results in a moderate inhibition of breast cancer growth that is associated with an increase in the frequency of cytotoxic CD8 T cells and the improvement of their function. The depletion of myeloid-derived suppressor cells (MDSCs) has no impact on mouse breast cancer growth. IL-15cx treatment diminishes MDSCs in murine tumors. However, it also antagonizes the effects of depleting antibody. Intratumoral GET with plasmid IL-15/IL-15Rα leads to a long-term survival benefit in 4T1 mammary carcinoma model. An early increase of local cytotoxic cells correlates with GET treatment and an increase of long-term memory T cells results from animals with complete tumor regression.

Conclusions Systemic and local administration of IL-15cx shows two distinct therapeutic responses, a moderate tumor growth inhibition or heterogeneous tumor regressions with survival improvement. Further studies are warranted to improve the efficacy of IL-15cx as an immunotherapy for breast cancer.

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Trial Registration N/A

Ethics Approval Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Old Dominion University (S. Guo) and by the IACUC at Virginia Commonwealth University (R.B. Smeltz).

ANV419 IS A NOVEL CD122-SELECTIVE IL-2/ANTI-IL-2 ANTIBODY FUSION PROTEIN WITH POTENT CD8 T CELL AND NK CELL STIMULATORY FUNCTION IN VITRO AND IN VIVO

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Background ANV419 is a uniquely engineered IL-2 fusion to an antibody selectively blocking the IL-2 receptor alpha (CD25) binding site. It signals selectively through the CD122/CD132 dimeric IL-2 receptor and stimulates the proliferation of CD8 T cells and NK cells while avoiding the proliferation of immunosuppressive regulatory T cells (Treg). Therefore, ANV419 has the potential to substantially separate targeted T-cell and NK cell proliferation and anti-tumor responses from the dose limiting toxicities of recombinant IL-2 (aldesleukin). ANV419 has antibody like stability and behavior and is currently in late preclinical development for tumor immunotherapy.

Methods The preclinical data suggest that ANV419 possesses a unique structure and is potent in expanding CD8 T-cells and NK cells with a marked safety window in non-human primates. The ability of ANV419 to inhibit tumor growth has been studied in mouse syngeneic models.

Results Structural analysis demonstrates that the CD25 binding site of IL-2 is completely blocked in ANV419 while the CD122/CD132 sites are available for binding. As a result, ANV419 lacks CD25 binding activity but retains IL-2 receptor beta (CD122) affinity comparable to native IL-2. In human peripheral blood monocyte cultures, ANV419 induces STAT5 phosphorylation with high selectivity for CD8 and NK cells but not Treg. Concordantly, it stimulates the proliferation of purified human CD8 T cells and NK cells but not CTL-L2 cells. A single injection of ANV419 in mice results in strong induction of the proliferation marker Ki67 specifically in CD8 T cells and NK cells but not Treg and a selective increase of the respective cell numbers in the spleen and peripheral blood of animals. Single agent anti-tumor activity was observed in checkpoint sensitive (H22) and resistant (Renca, B16F10) syngeneic mouse tumor models. Combination of ANV419 with trastuzumab in the gastric cancer N87 xenograft model in BALB/c nude mice led to significant tumor reduction relative to trastuzumab monotherapy. In non-human primates, ANV419 is well tolerated and induces expression of Ki67 and sustained expansion in CD8 T cells and NK cells with no signs of vascular leak syndrome observed with high dose aldesleukin in patients.

Conclusions The preclinical data suggest that ANV419 possesses a unique structure and is potent in expanding CD8 T-cells and NK cells with a marked safety window in non-human primates. This data warrants further translational development of ANV419 as an immune therapeutic in oncology.

REFERENCES

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