# WTX-124 is a Novel IL-2 Prodrug that is Conditionally Activated in Tumors and Drives Anti-Tumor Immunity by Activating Tumor Infiltrating CD8+ T Cells

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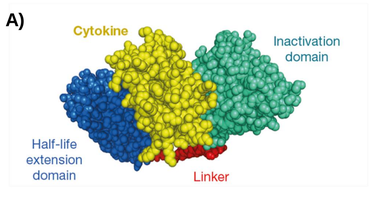
## Introduction

- Preclinical and clinical studies have demonstrated the promise of cytokine therapy to increase anti-tumor immunity, however, systemic toxicity and poor pharmacokinetic (PK) profiles have limited their clinical application
- Interleukin-2 (IL-2) is approved for use in metastatic melanoma and renal cell carcinoma; unfortunately, high-dose IL-2 is linked to serious toxicities which limits its utility
- Our approach takes advantage of dysregulated protease expression in the tumor microenvironment to activate an IL-2 pro-drug (IL-2 INDUKINE™ molecule, Figure 1a) only at the desired site of activity
- Peripheral inactivation is achieved by linking the cytokine to an inactivation domain using a tumor protease-sensitive linker (Figure 1)
- The INDUKINE™ molecule is also engineered with a half-life extension element to improve tumor exposure
- Once the IL-2 INDUKINE™ molecule reaches the tumor, tumor-associated proteases cleave the linker and release a fully-active, wild-type IL-2 cytokine
- The data presented summarize the biochemical, cellular, and in vivo activity of our lead IL-2 INDUKINE molecule (WTX-124) and reviews the mechanism of action of these molecules

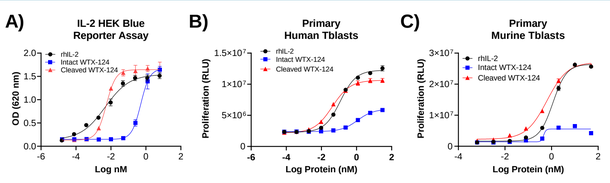
- Please see our recent publication in Cancer Immunology Research titled “Discovery of a Conditionally Activated Interleukin-2 that Promotes Anti-tumor Immunity and Induces Tumor Regression” for more information

## Figure 1: Key Features of WTX-124



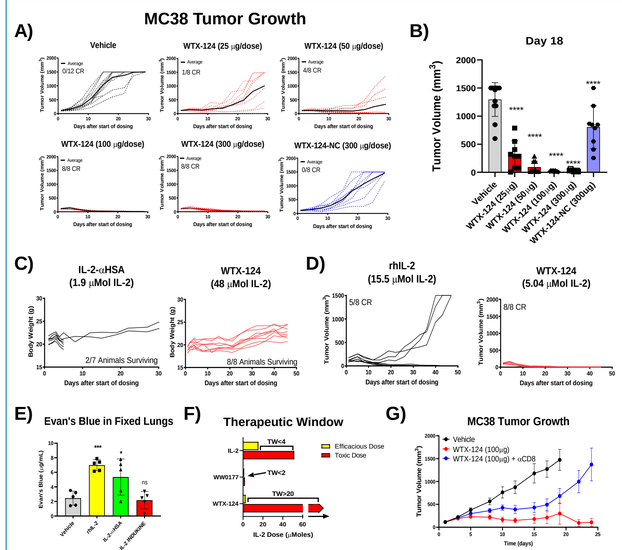

***A)*** *Diagram of the components of an IL-2 INDUKINE™ molecule. The yellow section represents native IL-2, the blue section represents the half-life extending HSA-specific single-domain antibody, the teal section represents the activity blocking antibody, and the red sections represent the protease-cleavable linkers.* ***B)*** *Non-reduced SDS-PAGE comparing intact and protease-cleaved WTX-124 (IL-2, anti-HSA half-life extension domain, and the Fab inactivation domain).*

## Figure 2: Cleavage of WTX-124 Releases the Inactivation Domain and Restores Full IL-2 Activity



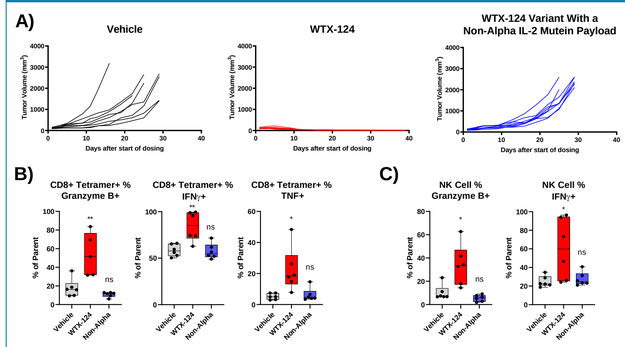

***A)*** *In vitro activity of WTX-124 in the HEK-Blue IL-2 reporter assay comparing intact (blue), and protease-activated (cleaved) WTX-124 (red) to rhIL-2 (black). In vitro activity of intact (blue) and cleaved (red) WTX-124 in* ***B)*** *primary human or* ***C)*** *murine Tblasts compared with rhIL-2 (black).*

## Figure 3: WTX-124 is Well Tolerated and Induces Tumor Regression in a Cleavage-Dependent Manner



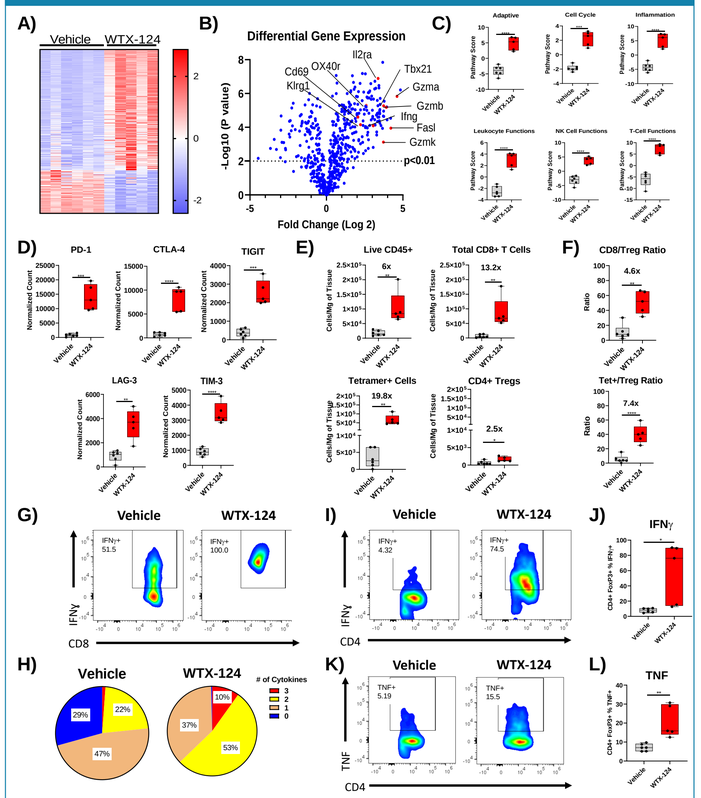

***A)*** *MC38 tumor bearing mice were treated twice a week for two weeks with various doses of either WTX-124, WTX-124-NC (non-cleavable control), or vehicle, and tumor volume was measured over time. Spider plots for individual mice are reported (dashed lines), and the average tumor volume for the group is in bold.* ***B)*** *Tumor volume on Day 18.* ***C)*** *Mice were treated with either WTX-124 or IL-2-αHSA and body weight was measured over time. Total IL-2 delivered throughout the experiment is specified in the figure.* ***D)*** *Mice were treated with either WTX-124 (5.04 μM total) or rhIL-2 (15.5 μM total), and tumor volume was measured over time.* ***E)*** *Mice were dosed with equimolar amounts of recombinant human IL-2 (yellow), IL-2-αHSA (green), or an IL-2 INDUKINE™ molecule (red), and Evan's Blue extravasation into the lungs was measured to assess treatment induced Vascular Leak Syndrome (VLS).* ***F)*** *Representation of the therapeutic window of rhIL-2, IL-2-αHSA, or WTX-124 in MC38 tumor-bearing mice based on the minimum efficacious dose and the maximum tolerated dose of each molecule.* ***G)*** *Anti-tumor activity of WTX-124 when CD8+ T cells are depleted by antibody treatment twice a week.*

## Figure 4: Native IL-2 INDUKINE™ generates superior anti-tumor immunity compared to a non-alpha IL-2 INDUKINE™



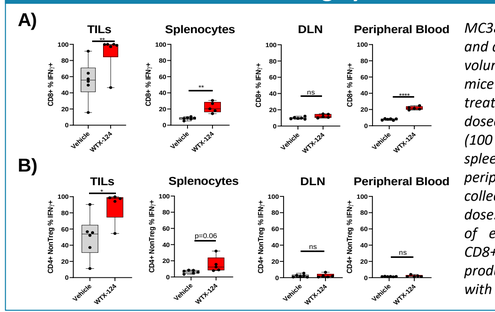

***A)*** *MC38 tumor bearing mice were treated with either vehicle, WTX-124 (containing a native IL-2 payload, 100μg/dose), or a WTX-124 variant containing a non-alpha IL-2 mutein as the payload (100μg/dose), and tumor volume was measured over time. WTX-124 and the non-alpha INDUKINE™ variant were given at identical doses. Spider plots for individual mice are reported.* ***B)*** *The frequency of tumor infiltrating tetramer+ CD8+ T cells producing Granzyme B, IFNγ, or TNF.* ***C)*** *The frequency of tumor infiltrating NK cells producing Granzyme B or IFNγ.*

## Figure 5: WTX-124 Treatment Increases Immune Cell Activation and Infiltration Within MC38 Tumors



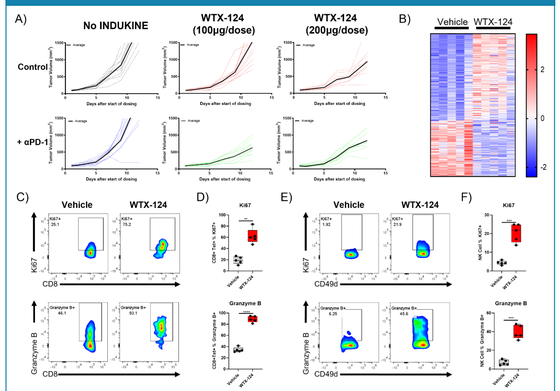

*MC38 tumor bearing mice were dosed twice a week with WTX-124 or vehicle, and tumors were collected 24 hours after the second dose.* ***A,B)*** *RNA from each tumor was isolated and subjected to immune profiling with the NanoString PanCancer Mouse Immune Profiling Panel.* ***A)*** *Heatmap of transcripts with statistically significant differences in expression between the two treatments. Each lane represents an individual animal.* ***B)*** *Volcano plot of transcripts differentially expressed between WTX-124 and vehicle-treated mice.* ***C)*** *Specific pathway scores for WTX-124 or vehicle-treated mice.* ***D)*** *Normalized gene counts from selected immune checkpoint genes.* ***E)*** *Flow cytometry analysis of TIL density of various immune populations.* ***F)*** *The ratio of total CD8+ T cells or tetramer-positive CD8+ T cells to Tregs within the TILs.* ***G)*** *The frequency of tetramer positive CD8+ T cells producing IFNγ.* ***H)*** *The frequency of polyfunctional tetramer positive CD8+ T cells by examining co-expression of IFNγ, TNF, and granzyme B. The frequency of tumor infiltrating FoxP3+ Tregs producing* ***I,J)*** *IFNγ or* ***K,L)*** *TNF after PMA/Ionomycin restimulation.*

## Figure 6: WTX-124 Selectively Activates Tumor Infiltrating T Cells Without Causing Systemic Activation



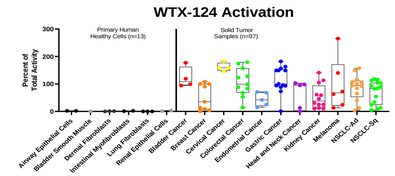

*MC38 tumor cells were implanted and allowed to grow to an average volume of 100–150 mm³ before mice were randomized into treatment groups. Mice were dosed twice a week with WTX-124 (100 μg) or vehicle. Tumors, spleens, DLNs, non-DLNs, and peripheral blood samples were collected 24 hours after the second dose. Graphs show the frequency of either* ***A)*** *tetramer negative CD8+ T cells or* ***B)*** *CD4+ non-Tregs producing IFNγ after re-stimulation with PMA/Ionomycin.*

## Figure 7: WTX-124 Treatment Increases Immune Cell Activation Within B16-F10 Tumors




*B16-F10 tumor bearing mice were dosed twice a week with either PBS or with various doses of WTX-124. Some mice also received PD-1 blockade in addition to WTX-124 treatment.* ***A)*** *Spider plots for individual mice are reported (dashed lines), and the average tumor volume for the group is in bold. Tumors from mice treated with either the vehicle or WTX-124 (200 μg/dose) were harvested 24 hours after the second dose.* ***B)*** *RNA from each tumor was isolated and subjected to immune profiling with the NanoString nCounter® PanCancer Mouse Immune Profiling panel. Heatmap of transcripts with statistically significant differences in expression between the two treatments.* ***C-F)*** *TILs were re-stimulated with PMA/Ionomycin and examined for effector cytokine production and proliferation.* ***C-D)*** *The frequency of tumor infiltrating tetramer positive CD8+ T cells producing granzyme B or expressing Ki67.* ***E-F)*** *The frequency of tumor infiltrating NK cells producing granzyme B or expressing Ki67.*

## Figure 8: WTX-124 is Selectively and Efficiently Processed by Human Tumor Samples




*WTX-124 was exposed to primary human tumor samples (n = 97) or primary human healthy cells (n = 13) for 48 hours before activation of the INDUKINE™ protein was measured. WTX-124 cleavage was measured by assessing the activity of the INDUKINE™ protein with a Promega IL-2 assay. Activity was normalized to that of pre-cut WTX-124 (100% activity) or an uncleavable control (0% activity).*

## Conclusions

- WTX-124 is a tumor selective inducible IL-2 prodrug that generates significant anti-tumor activity in a CD8+ T Cell dependent manner
- WTX-124 has a better therapeutic window than rhIL-2 or half-life extended rhIL-2
- A WTX-124 variant INDUKINE™ molecule containing a non-alpha IL-2 mutein payload is not active at the same dose as WTX-124
- WTX-124 treatment significantly shifts the transcriptional profile of the tumor microenvironment towards activation of various immune cell populations in both the MC38 and B16F10 model
- WTX-124 preferentially activates tumor infiltrating CD8+ and CD4+ T cells, with limited evidence of systemic T cell activation
- Combinatorial activity was observed with WTX-124 and αPD-1 treatment in a less immunogenic model
- WTX-124 is selectively and efficiently processed by primary human tumor samples