WTX-124 is a novel IL-2 pro-drug that is conditionally activated in tumors and drives anti-tumor immunity in murine syngeneic cancer models

Christopher J. Nirschl, Heather R. Brodkin, Daniel J. Hicklin, Nesreen Ismail, Kristin Morris, Cynthia Seidel-Dugan, Philipp Steiner, Zoe Steuert, Jenna M. Sullivan, William M. Winston and Andres Salmeron

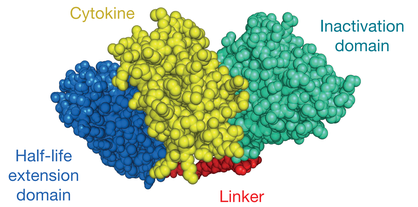
Werewolf Therapeutics, Cambridge, Massachusetts, USA

#718

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 that limit its utility
- Our approach takes advantage of the dysregulated protease tumor microenvironment to activate an IL-2 pro-drug (IL-2 INDUKINE™ molecule) 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 the active cytokine
- The data presented summarize the biochemical, cellular, and *in vivo* activity of our lead IL-2 INDUKINE™ molecule (WTX-124) and review the mechanism of action of these molecules

Figure 1. INDUKINE™ molecule structural design

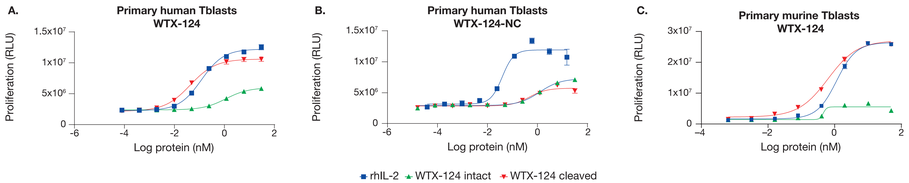


RESULTS

- *In vitro* WTX-124 activity is inducible by protease activation (**Figure 2**)
- *In vivo* treatment with WTX-124 results in complete regression of tumors driven by the expansion and activation of CD8+ T cells and natural killer (NK) cells, which increase the production of effector cytokines in the tumor (**Figures 3 and 4**)
- *In vivo* activity is dependent on the proteolytic processing of the INDUKINE™ molecule, as a non-cleavable control INDUKINE™ molecule lacks efficacy (**Figure 3A**)
- Tumor-infiltrating lymphocyte stimulation driven by localized activation of WTX-124 is sufficient to induce tumor efficacy (**Figure 3**)
- WTX-124 treatment generates memory formation against the tumor (increased frequency of tumor-specific effector memory cells), as animals that rejected primary tumors were protected against follow-up re-challenge with the same tumor cell line (**Figure 5**)
- PK analysis in mouse models demonstrates a favorable accumulation of free IL-2 in tumors compared with plasma. Non-human primate (NHP) PK analysis demonstrates a favorable exposure profile and tolerability (**Figure 6**)

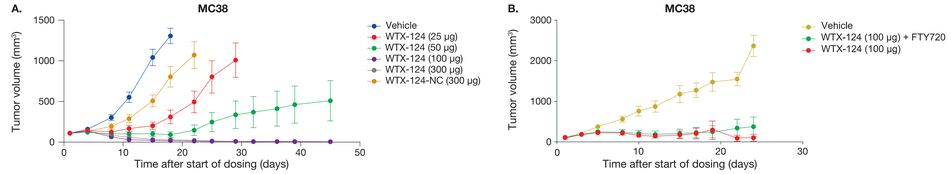
RESULTS

Figure 2. Design and development of the selectively active IL-2 INDUKINE™ molecule WTX-124



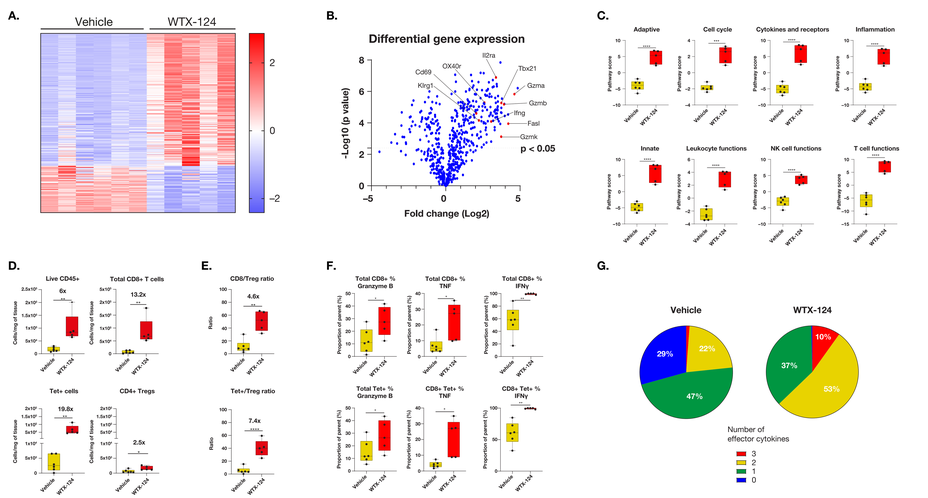
*Activity in primary human Tblasts of (**A**) WTX-124 or a (**B**) non-cleavable IL-2 INDUKINE™ molecule. **C**) Activity of WTX-124 in primary murine Tblasts of WTX-124. IL-2, interleukin-2; RLU, relative light units.*

Figure 3. WTX-124 inhibits MC38 tumor growth by activating tumor-infiltrating lymphocytes



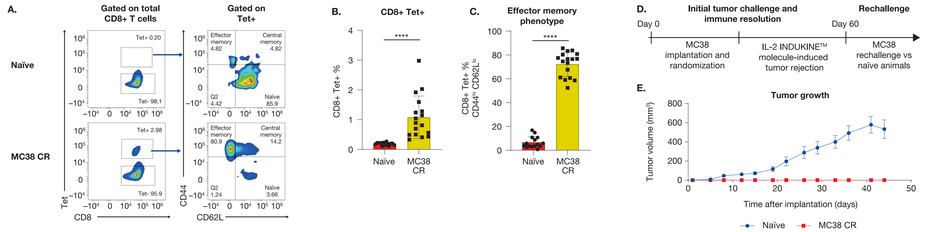
***A**) MC38 tumor model efficacy data. Mice were dosed via IP injection with different dose levels of WTX-124. WTX-124-NC group (uncleavable control IL-2 INDUKINE™ molecule) was only given at highest dose level. Tumor volume was measured over time. **B**) Efficacy of WTX-124 when peripheral immune cell egress was restricted by daily FTY-720 treatment.*
IL-2, interleukin-2; IP, intraperitoneal.

Figure 4. Treatment with WTX-124 increases immune cell infiltration and activation in MC38 tumors



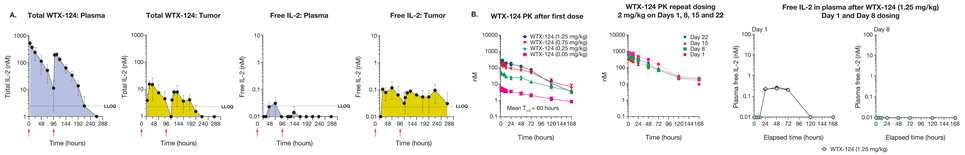
*MC38 tumors were implanted and allowed to grow before mice were randomized into treatment groups. Mice were dosed with either WTX-124 or vehicle control. **A–B**) Total RNA from each tumor was isolated and subjected to immune profiling with the NanoString™ PanCancer Mouse Immune Profiling Panel. **A**) Heatmap of transcripts with statistically significant differences in expression between the two treatment groups. Each lane represents an individual animal. **B**) Volcano plot of differentially expressed genes. P values are derived from the Nsolver software. **C**) Pathway scoring using Nsolver software with the advanced analysis module. **D–E**) Flow cytometry analysis of tumor-infiltrating lymphocyte density, represented either as the (**D**) cells per mg of tissue or (**E**) overall ratio. **F**) The frequency of tumor-infiltrating immune cells producing effector cytokines. **G**) Tetramer+ CD8+ T cells were examined for polyfunctionality (IFNγ, TNF, and Granzyme B) after restimulation. Unless otherwise stated, data are presented as the mean ± standard deviation, and p values are derived from T tests (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).*
IFNγ, interferon gamma; NK, natural killer; Tet, tetramer; TNF, tumor necrosis factor; Treg, regulatory T cell.

Figure 5. Treatment with IL-2 INDUKINE™ molecule generates an anti-tumor memory response



*MC38 tumors were implanted and randomized before starting treatment. Animals that rejected tumors after treatment with an IL-2 INDUKINE™ molecule were then used for additional analysis. **A**) 180 days following the initial implantation, spleens were harvested from CR or age-matched naïve animals and examined by flow cytometry. The presence of (**B**) Tet+ CD8+ T cells (gp70 antigen) and (**C**) the expression of the memory cell markers CD44 and CD62L on Tet+ cells were determined. **D**) Naïve animals or animals that had previously rejected MC38 tumors (achieved complete response) after treatment with the IL-2 INDUKINE™ molecule were rechallenged with MC38 tumor cells 60 days following the initial implantation. No treatment was administered to these animals during the rechallenge. **E**) Tumor volume was measured over time. Data are presented as mean ± standard deviation, with p values derived from t tests (**** p < 0.0001).*
CR, complete responders; IL-2, interleukin-2; Tet, tetramer.

Figure 6. WTX-124 has favorable PK characteristics in both mice and NHPs



***A**) Mouse PK analysis. Plasma and tumor samples from MC38 tumor-bearing animals were analyzed at various time points for either the presence of the total WTX-124 molecule or free human IL-2. Animals received two doses, and the timing of the doses is indicated by the red arrows on the figure. Data demonstrated exposure of WTX-124 and a favorable activation rate to free IL-2 within the tumor compared with activation in the periphery. **B**) NHP PK analysis. Left panels show dose-dependent exposure of WTX-124 and consistent exposure achieved after repeated dosing. Right panels show that minimal or undetectable amounts of free IL-2 were found in the plasma of these animals, demonstrating the stability of WTX-124.*
IL-2, interleukin-2; LLOQ, lower limit of quantification; NHP, non-human primate; PK, pharmacokinetics; $T_{1/2}$ half-life.

CONCLUSIONS

- Our work shows that WTX-124, a pro-drug containing wild-type IL-2, is selectively processed and activated in tumors and is efficacious in murine models, even in the presence of regulatory T cells
- WTX-124 activity is dependent on the processing of the pro-drug as a non-cleavable version of WTX-124 (WTX-124-NC) is not efficacious in our models
- Mechanistically, WTX-124 induces intratumoral activation of NK cells and CD8+ T cells and generates long-term memory in treated animals
- WTX-124 possesses good PK characteristics in mouse and NHP models and is stable in the periphery, with minimal release of free IL-2

ACKNOWLEDGMENTS

The authors were responsible for all content and editorial decisions; editorial support was provided by MediTech Media, funded by Werewolf Therapeutics.

Poster presented at SITC Annual Meeting, Nov. 10–14, 2021; Washington, D.C.