WTX-124 is an IL-2 Pro-Drug Conditionally Activated in Tumors and Able to Induce Complete Regressions in Mouse Tumor Models

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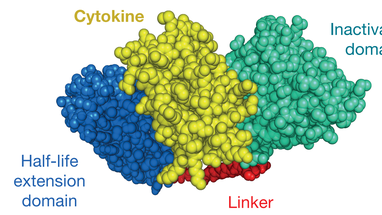
INTRODUCTION

- 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 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 reviews the mechanism of action of these molecules

Figure 1. INDUKINE™ molecule structural design

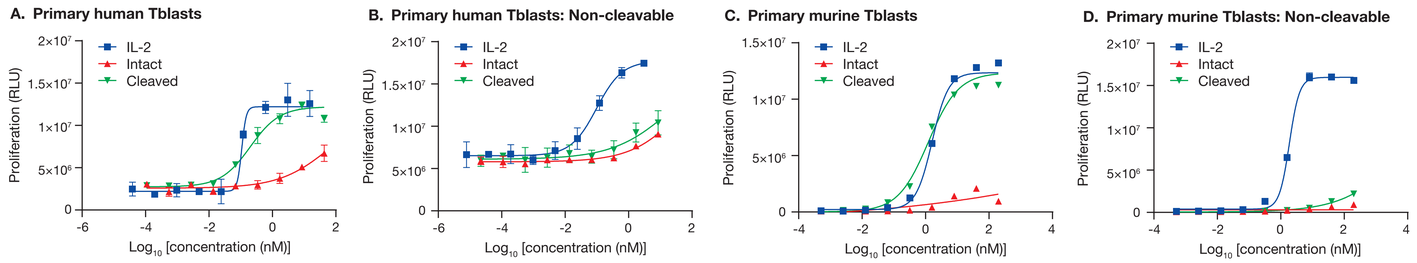


RESULTS

- *In vitro* WTX-124 activity in primary cells 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 resulting in an increased production of effector cytokines in the tumor (**Figure 3; Figure 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**)
- WTX-124 treatment generates memory formation against the tumor (increase in tumor-specific effector memory cells) (**Figure 5**)
  - The animals that rejected the tumors were protected against follow-up rechallenge with the same tumor cell line (**Figure 5E**)
- PK analysis in mouse models demonstrates a favorable accumulation of free IL-2 in tumors compared with plasma (**Figure 6A**)
- Non-human primate (NHP) PK analysis demonstrates a favorable exposure profile and tolerability (**Figure 6B**)

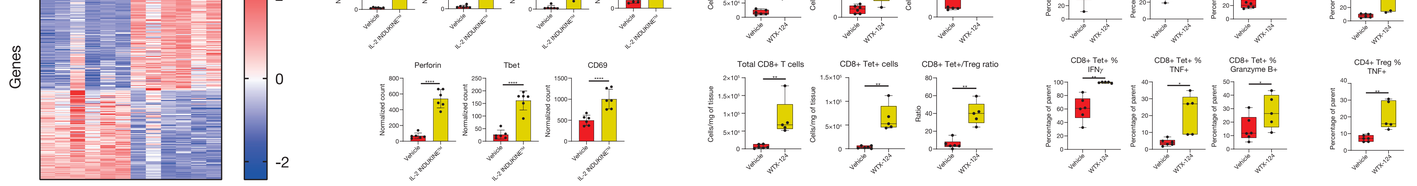
RESULTS

Figure 2. Design and development of a selectively active IL-2 INDUKINE™ molecule



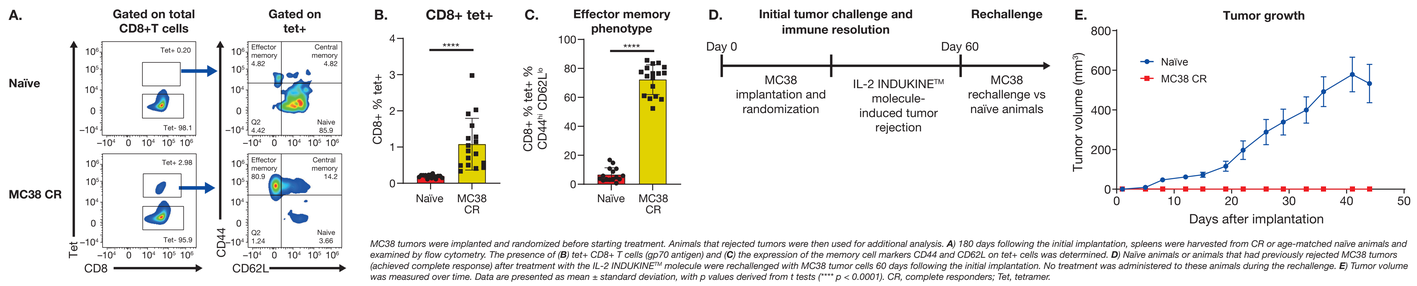
*Activity in primary human Tblasts of (**A**) IL-2 INDUKINE™ molecule and (**B**) non-cleavable IL-2 INDUKINE™ molecule. Activity in primary murine Tblasts of (**C**) IL-2 INDUKINE™ molecule and (**D**) non-cleavable IL-2 INDUKINE™ molecule. RLU, relative light units.*

Figure 4. Treatment with IL-2 INDUKINE™ molecule increases immune cell infiltration and activation in MC38 tumors



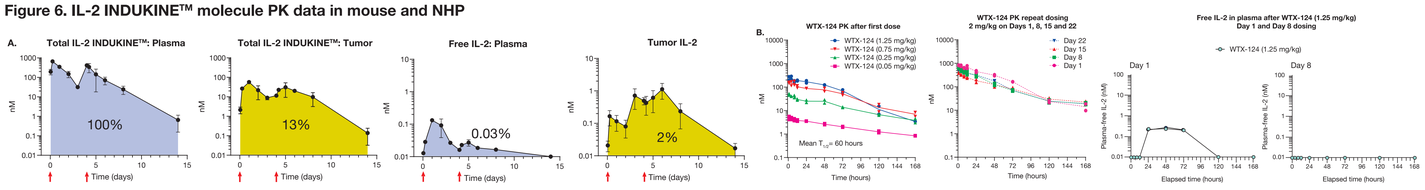
*MC38 tumors were implanted and allowed to grow before mice were randomized into treatment groups. Mice were dosed with either an IL-2 INDUKINE™ molecule or vehicle control. (**A–B**) Total RNA from each tumor was isolated and subjected to immune profiling with the NanoString PanCancer Mouse Immune Profiling. Panel (**A**) shows a heatmap of transcripts with statistically significant differences in expression between the two treatments groups. Each lane represents an individual animal. **B)** Normalized gene counts from specific genes comparing IL-2 INDUKINE™ molecule treated animals vs the control group. P values are derived from the Nsolver software. **C)** Flow cytometry analysis of tumor infiltrating lymphocyte density, represented as the cells per mg of tissue. **D)** The frequency of tumor infiltrating immune cells producing effector cytokines. **E)** Effector cytokine production by FoxP3+ Tregs following IL-2 INDUKINE™ molecule treatment (induction of a Treg proinflammatory phenotype).*
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 induces an anti-tumor memory response



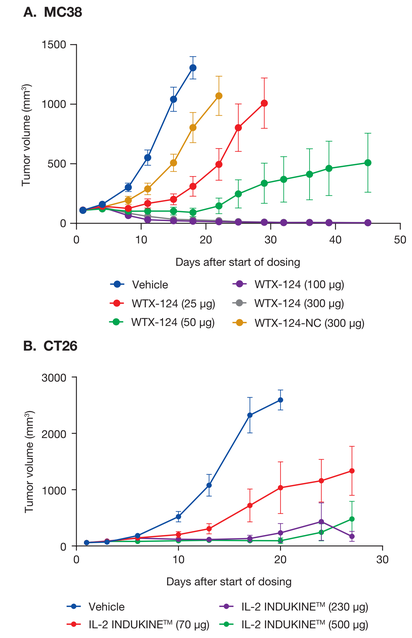
*MC38 tumors were implanted and randomized before starting treatment. Animals that rejected tumors were then used for additional analysis. **A)** 180 days following the initial implantation, spleens were harvested from CR or age-matched naïve animals and examined by flow cytometry. The presence of (**B**) tet+ CD8+ T cells (gp70 antigen) and (**C**) the expression of the memory cell markers CD44 and CD62L on tet+ cells was 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; Tet, tetramer.*

Figure 6. IL-2 INDUKINE™ molecule PK data in mouse and NHP



***A)** Mouse PK analysis. Plasma and tumor samples from tumor bearing animals were analyzed at various time points for either the presence of the total IL-2 INDUKINE™ 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 tumor exposure for the IL-2 INDUKINE™ molecule and a favorable conversion rate to free IL-2 within the tumor compared with the conversion in plasma. **B)** NHP PK analysis. Left panels show dose-dependent exposure of the IL-2 INDUKINE™ molecule and consistent exposure achieved after repeated dosing. Right panels shows that minimal or undetectable amounts of free IL-2 were found in the plasma of these animals, demonstrating the stability of the IL-2 INDUKINE™ molecule. NHP, non-human primate; PK, pharmacokinetics; $T_{1/2}$, half-life.*

Figure 3. IL-2 INDUKINE™ molecule is efficacious in tumor models in a cleavage-dependent manner



***A)** MC38 tumor model efficacy data. Mice were dosed via IP injection with different dose levels of WTX-124 INDUKINE™ molecule. 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 IL-2 INDUKINE™ molecule in the CT26 tumor model. NC, non-cleavable; IP, intraperitoneal.*

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 as well as a proinflammatory phenotype in Tregs, 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
  - WTX-124 was also well tolerated in NHP

Poster presented at AACR Annual Meeting 2021; Virtual Meeting