Pharmacokinetic Insight into the IL-2 INDUKINE Prodrug WTX-124: Real-Time Assessment of Tumor Specific Activation and Immune Modulation

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Werewolf THERAPEUTICS
Building a Novel Class of Conditionally Activated Immunotherapies for Patients with Cancer

Abstract

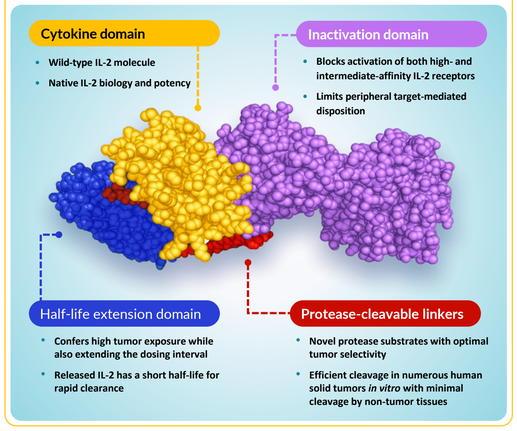
Background: Cytokine prodrugs represent a promising strategy in the field of cancer immunotherapy, aiming to bolster antitumor immunity while minimizing systemic toxicity. WTX-124 is a clinical stage IL-2 prodrug (INDUKINE molecule) comprised of wild-type IL-2 linked to a half life extension domain and an inactivation domain by protease cleavable linkers. In the periphery, WTX-124 remains inactive, while exposure to dysregulated proteases in the tumor microenvironment (TME) results in cleavage of the linkers and the release of active, wild-type IL-2 within the TME. Ultimately, WTX-124 was designed to improve the safety of high-dose IL-2 without compromising its clinical efficacy. Although the potent anti-tumor effect of WTX-124 has been confirmed in patients and preclinical models, traditional preclinical assessments have relied heavily on static tumor homogenate analysis, hindering the ability to understand the spatiotemporal dynamics of intact (inactive) and cleaved (active/free or released IL-2) drug concentrations, and their effects at the tumor site.

Methods: To address this limitation, we employed a large pore microdialysis technique in a syngeneic murine tumor model, enabling real-time monitoring of IL-2 INDUKINE pharmacology directly at the site of action. Tumor-bearing mice received either vehicle, a single dose of WTX-124, or its non-cleavable analog (WTX-124-NC) intravenously (IV). Tumor interstitial fluid (TIF) and plasma were collected at various time points, and tumor tissues were harvested at the end of treatment for immunoassay and multiplex immunofluorescence (IF) analysis.

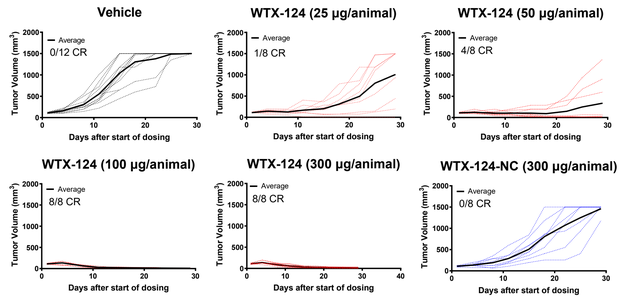
Results: A single IV dose of WTX-124 or WTX-124-NC led to consistently low levels of active IL-2 in plasma, suggesting that prodrug conversion to active cytokine does not occur systemically. Both the intact prodrug and non-cleavable variant exhibited continuous uptake and accumulation in the tumor; however, only treatment with the cleavable INDUKINE molecule resulted in a significant, time-dependent increase in tumor active IL-2 exposure. In stark contrast, the WTX-124-NC did not yield detectable active IL-2 levels, highlighting the importance of linker cleavage for effective payload release. Additionally, cytokine profiling of the TIF showed a notable increase in IFNγ production that correlated with free IL-2 exposure, indicating robust immune activation associated with WTX-124. Ex vivo multiplex IF analysis of tumor tissues revealed an elevation in the density of infiltrating immune cells, including natural killer cells and CD8+ T cells, as well as enhanced expression of key effector molecules such as IFNγ, Granzyme B, and Perforin in response to active IL-2 derived from WTX-124. Importantly, this vigorous immune response was not observed in tumors treated with WTX-124-NC, suggesting that effective immune modulation is driven by localized IL-2 release.

Conclusions: These preclinical data clearly demonstrate tumor-selective activation of WTX-124, further substantiating the INDUKINE prodrug design capable of harnessing enzymatic cleavage mechanisms for robust immune modulation.

INDUKINE Design of WTX-124 an IL-2 Prodrug

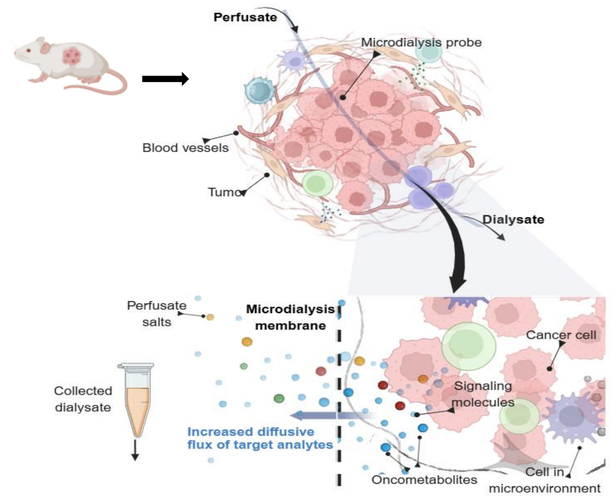


Anti-Tumor Activity of WTX-124 Treatment is Cleavage Dependent



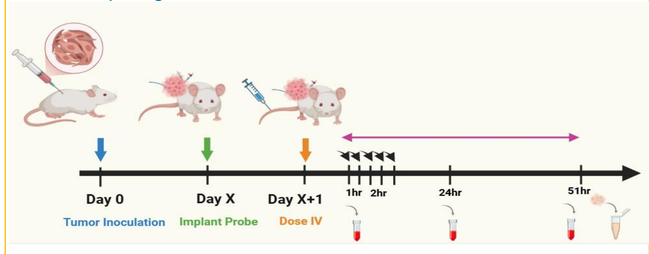
MC38 tumor cells were implanted and allowed to grow to an average volume of 100–150 mm³ before mice were randomized into treatment groups. Mice were dosed twice a week with INDUKINE proteins. Mice were treated with various doses of WTX-124, WTX-124-NC (noncleavable control), or vehicle, and tumor volume was measured over time. Spider plots for individual mice are reported (dashed lines), and the average tumor volume for the group is in bold.†

Microdialysis Approach to Understanding Real-Time INDUKINE Cleavage



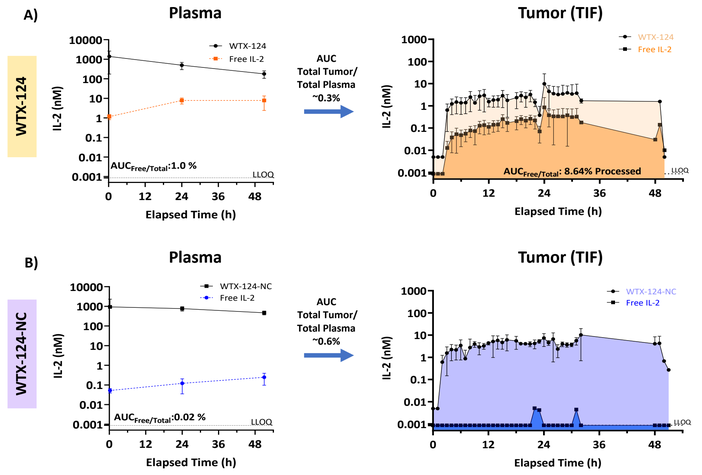
Murine tumors are implanted and allowed to grow to a reasonable size for probe implantation. The probe is made of a selectively permeable membrane and is surgically implanted through the core of the tumor in a live animal. Dialysate is then collected at various timepoints to measure drug exposure, cleavage status, or pharmacodynamic readouts in real time in a living animal.

Overall Study Design



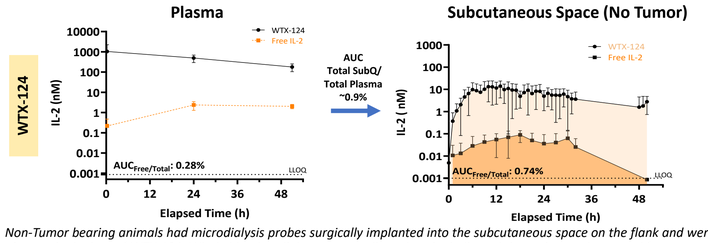
MC38 tumor cells were implanted and allowed to grow to 300-400 mm³ before mice were randomized into treatment groups and had probes surgically implanted. Mice were dosed once with either WTX-124, an IL-2 prodrug that delivers wild-type IL-2 and which is selectively activated in the TME, or a variant of WTX-124 where all the linkers have been replaced with non-cleavable sequences, resulting in a prodrug that cannot be activated (WTX-124-NC). Dialysate was collected at various timepoints, and tumors were collected for multiplex immunofluorescence at the end of the experiment.

Tumor Specific Cleavage of WTX-124 Can Be Measured In Real Time



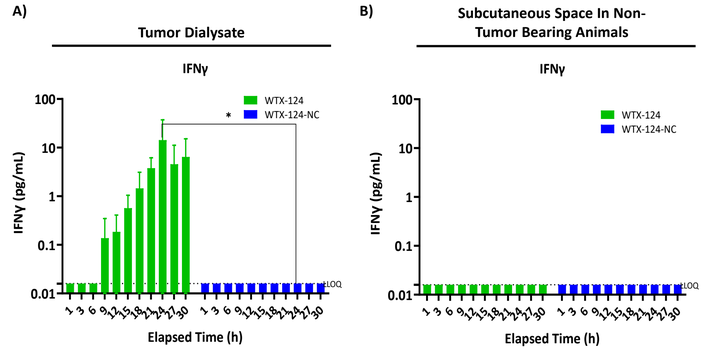
*Tumor bearing animals had microdialysis probes surgically implanted and were given a single dose of either **A)** WTX-124 or **B)** a non-cleavable variant of WTX-124. Whole blood or dialysate was collected at the indicated timepoints, and assessed for presence of the fully intact prodrug, or free IL-2, signifying cleavage of the molecule. Error bars represent the standard deviation from n=2-4 animals per group, sampled repeatedly over the course of the experiment. LLOQ of this assay was calculated to be 0.086 pM.*

Minimal Cleavage of WTX-124 Detected in Non-Tumor Bearing Animals



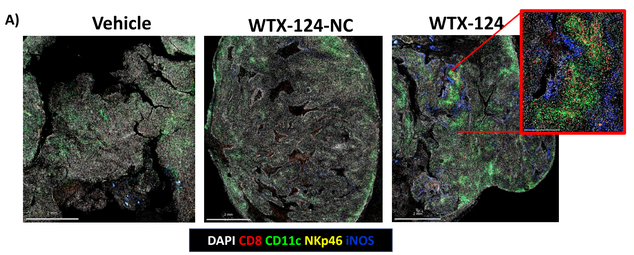
Non-Tumor bearing animals had microdialysis probes surgically implanted into the subcutaneous space on the flank and were given a single dose of WTX-124. Whole blood or dialysate was collected at the indicated timepoints and assessed for presence of the fully intact prodrug, or free IL-2, signifying cleavage of the molecule. Error bars represent the standard deviation from n=2-4 animals per group, sampled repeatedly over the course of the experiment.

Effector Molecule Detection is Dependent on Cleavage in the TME



***A)** Tumor bearing animals had a microdialysis probe surgically implanted into the tumor or **B)** non-tumor bearing mice had a microdialysis probe surgically implanted into the subcutaneous space on the flank. Mice were given a single dose of either WTX-124 (green bars) or a non-cleavable variant of WTX-124 (blue bars). Dialysate was collected at the indicated timepoints and assessed for presence of pharmacodynamic molecules (IFNγ) downstream of immune cell activation. Error bars represent the standard deviation from n=2-4 animals per group, sampled repeatedly over the course of the experiment. LLOQ of this assay was calculated to be 0.016 pg/mL.*

Tumor Dependent Cleavage Leads to Accumulation and Activation of Various Immune Cell Populations in the TME



*MC38 tumors from mice treated with either PBS (vehicle), WTX-124-NC, or WTX-124 were harvested, fixed, and made into FFPE blocks 48 hours after a single dose. Multiplex immunofluorescence was performed. **A)** Low magnification images depict DAPI (grey), CD8 (red), CD11c (green), NKp46 (yellow), and iNOS (blue). **B)** High magnification images depicting DAPI (grey), CD45(pink), and NKp46 (yellow). **C)** High magnification images depict DAPI (grey), CD8 (red), granzyme B (green), perforin (pink), and iNOS (blue). **D)** High magnification images depict DAPI (grey), CD8 (red), and LAG-3 (green).*

CONCLUSIONS

- Efficacy of WTX-124 is dependent on the tumor dependent cleavage of the molecule *in vivo*
- Microdialysis can be utilized to measure *in vivo* cleavage in real time in murine tumor models
  - Analysis reveals real-time cleavage of the WTX-124 prodrug, leading to accumulation of free IL-2 within the TME
  - Cleavage is tumor selective, as a substantially higher fraction of the pro-drug is converted into free cytokine in the TME compared to plasma and subcutaneous space without a tumor present
- Microdialysis analysis also confirms real-time pharmacodynamic effects of cleavage, including increased IFNγ in the TME
- Cleavage was required for downstream pharmacodynamic effects, as no downstream effects were observed when a noncleavable prodrug was used (WTX-124-NC)
  - No increases were observed in the subcutaneous space of non-tumor bearing animals, demonstrating that processing was dependent on the tumor and not the anatomical location
- Increased accumulation of activated immune cells in the tumor is only detected following treatment with the cleavable INDUKINE molecule, WTX-124
- Together, these data demonstrate that the INDUKINE design is working as intended, with tumor selective activation leading to a demonstrable antitumor response.

†Nirschl CJ, Brodkin HR, Hicklin DJ, Ismail N, Morris K, Seidel-Dugan C, et al. Discovery of a Conditionally Activated IL-2 that Promotes Antitumor Immunity and Induces Tumor Regression. Cancer Immunol Res [Internet]. American Association for Cancer Research (AACR); 2022 [cited 2022 Apr 11];10:581–96.

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