Werewolf THERAPEUTICS

Shifting the Balance In Cytokine Therapeutics

SITC 2023

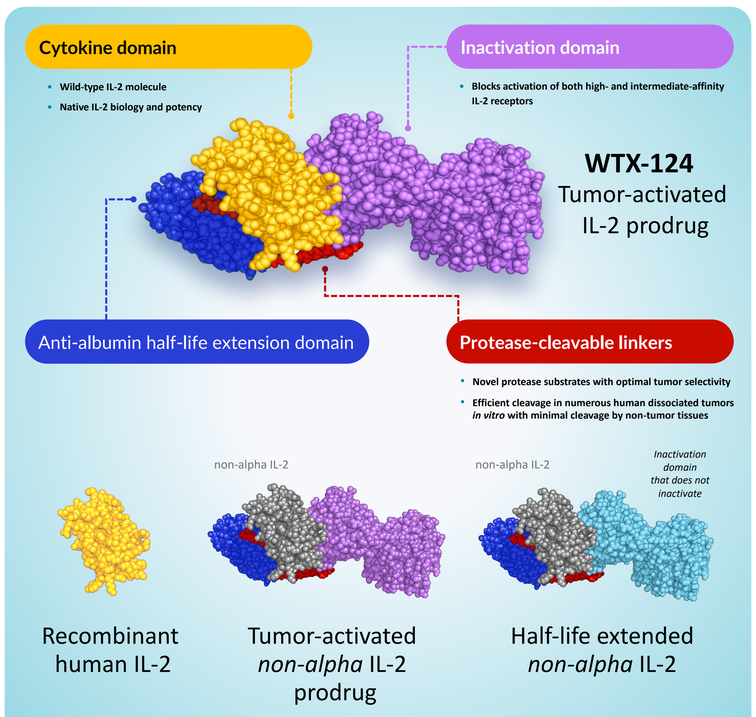
PK/RO modeling of WTX-124, a tumor-activated IL-2 prodrug, highlights the potential for a substantially improved therapeutic index compared to other IL-2 molecules

Kulandayan K. Subramanian, PhD; Sameer S. Chopra, MD, PhD; Kristin Morris, PhD; Christopher Nirschl, PhD; Celesztina Domonkos, PhD; Kyriakos Economides, PhD; Andres Salmeron, PhD; Cynthia Seidel-Dugan, PhD; Randi Isaacs, MD; Daniel Hicklin, PhD

Werewolf Therapeutics Inc., Watertown, MA

BACKGROUND

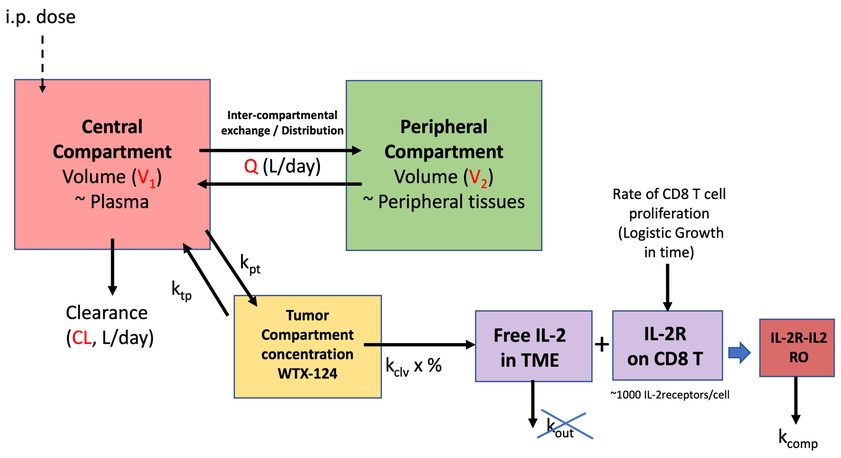
Model based comparison of WTX-124 and other IL-2 molecules



Although high dose recombinant human IL-2 (rhIL-2) is an effective immunotherapy for certain patients with metastatic melanoma and renal cell carcinoma, the clinical benefits are counterbalanced by life-threatening toxicities. To address this challenge, many novel IL-2 molecules that do not bind to the alpha subunit of the high-affinity IL-2 receptor ('non-alpha' IL-2s) have entered the clinic. However, minimal monotherapy antitumor activity has been observed at doses tolerated by patients. Conditional activation is a novel engineering strategy for toxic drug molecules that could provide an alternative approach to improve the tolerability of rhIL-2 without compromising its activity. Here we use plasma and tumor homogenate data from mice to perform PK/RO modeling to predict IL-2 receptor occupancy on peripheral lymphocytes and tumor infiltrating lymphocytes (TILs) after dosing with rhIL-2, half-life extended non-alpha IL-2, WTX-124 (an activatable, tumor selective IL-2 prodrug or INDUKINE™ molecule), or a non-alpha IL-2 tumor-activated prodrug.

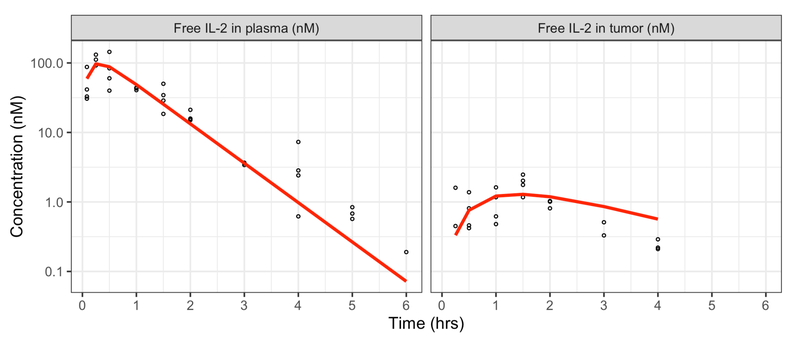
METHODS AND DATA

Model Schema: Cleavage of WTX-124 in the tumor microenvironment



- *Model fit to WTX-124 and Free IL-2 PK data in plasma and MC38 tumor model*
- *Model includes cleavage of WTX-124 in tumor to release free IL-2*
- *k_{out}, clearance rate of free IL-2 in tumor could not be reliably estimated*
- *Receptor binding in plasma and tumor predicted using the K_D for high affinity and intermediate affinity IL-2R binding*
- *IL-2-IL-2R complex internalization included in model*

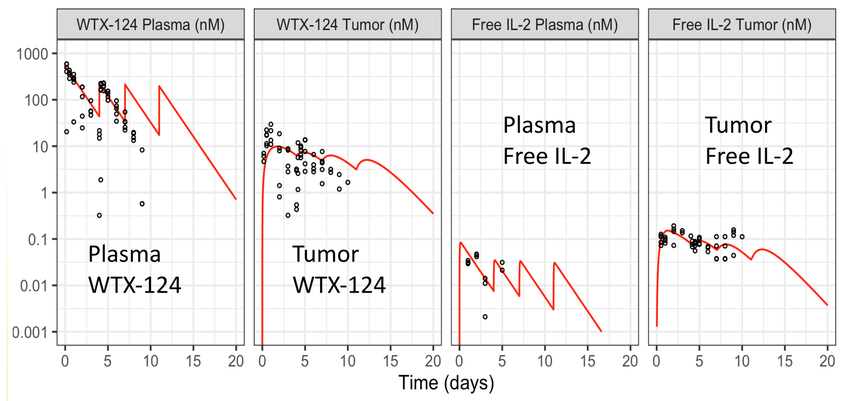
Model fit to rhIL-2 plasma and tumor data from MC38 mice



PK data in plasma and tumor from MC38 mice after a single 50 µg intraperitoneal dose of rhIL-2

METHODS AND DATA

Model fit to WTX-124 plasma and tumor data [1] from MC38 mice



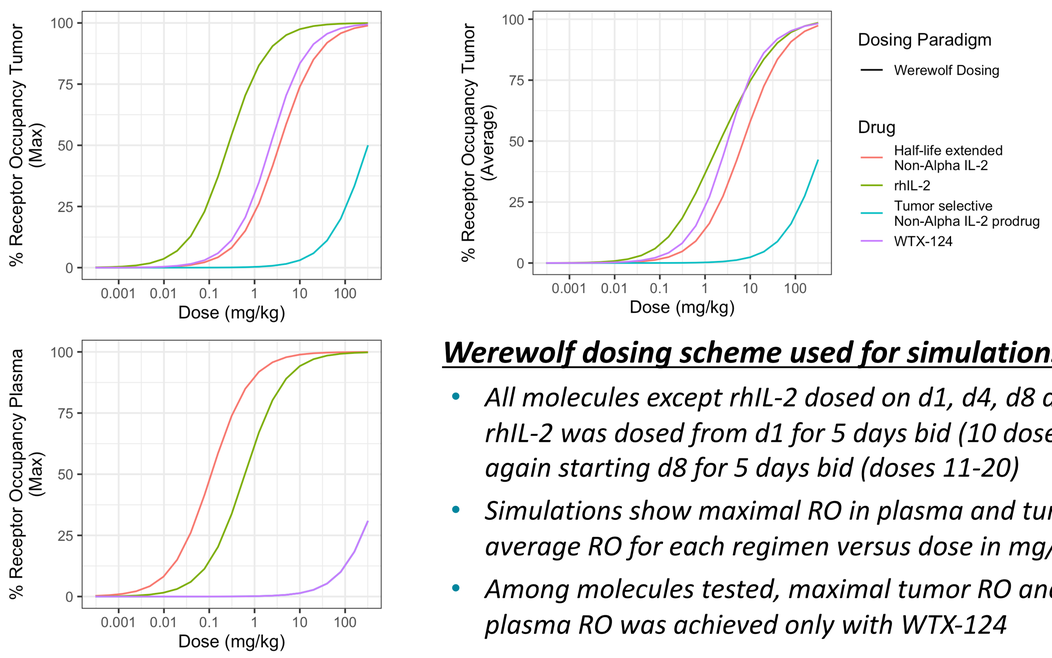
- *PK data from mice dosed with 2.5 mg/kg WTX-124 intraperitoneally (IP) on d1 and d5*
- *Plots show model captures WTX-124 and free IL-2 PK in plasma and tumor*

KEY MODELING ASSUMPTIONS

- *WTX-124 plasma and tumor exposure data were used to represent half-life extended (HLE) non-alpha active IL-2 PK*
- *The same plasma and tumor WTX-124 and free IL-2 PK data were used to represent tumor activated non-alpha IL-2 prodrug and free non-alpha IL-2 exposures*
- *Model for RO and TIL proliferation kinetics based on published work [3]*
- *K_D used for high affinity and intermediate affinity mouse IL-2R (based on [4]):*
  - *High affinity K_D IL-2R $\alpha\beta\gamma$ = 0.13 nM ~14 fold weaker in mouse relative to human x 0.096 nM/10 (using Werewolf laboratory potency measurements)*
  - *Intermediate K_D IL-2R $\beta\gamma$ = 239 nM/10 = 23.9 nM (no cross-species potency difference included)*
- *Peripheral lymphocytes such as NK cells express intermediate affinity receptors – high activation hypothesized to lead to safety risks*
- *TILs such as "activated" CD8s express high affinity receptors – activation of these cells result in tumor killing [2]*

MODEL PREDICTIONS

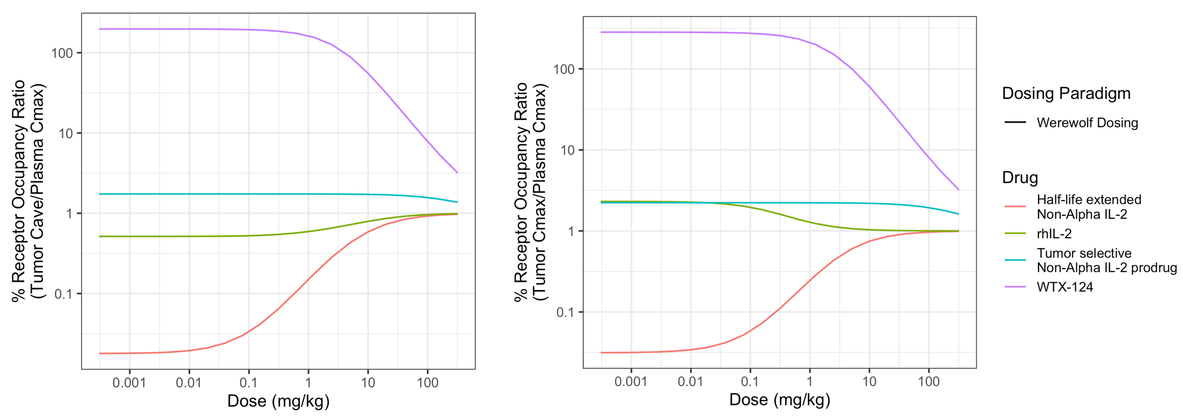
Receptor occupancy (RO) metrics on peripheral lymphocytes and TILs suggests that WTX-124 is best in class with respect to therapeutic index



Werewolf dosing scheme used for simulations:

- *All molecules except rhIL-2 dosed on d1, d4, d8 and d11. rhIL-2 was dosed from d1 for 5 days bid (10 doses) and then again starting d8 for 5 days bid (doses 11-20)*
- *Simulations show maximal RO in plasma and tumor, and average RO for each regimen versus dose in mg/kg*
- *Among molecules tested, maximal tumor RO and minimal plasma RO was achieved only with WTX-124*

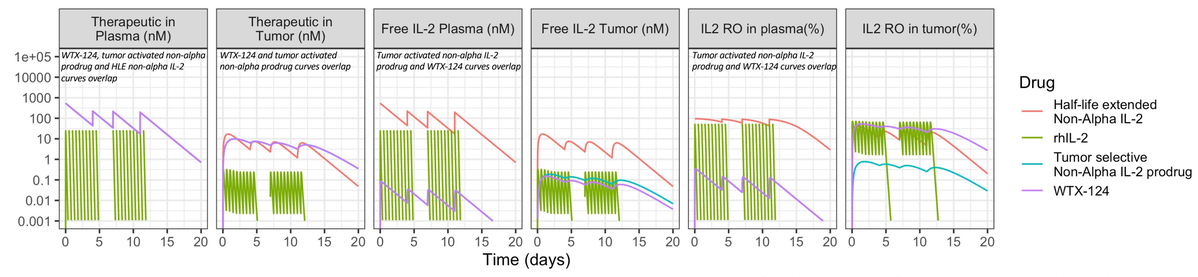
Tumor versus plasma receptor occupancy (RO): Substantially improved therapeutic index for WTX-124



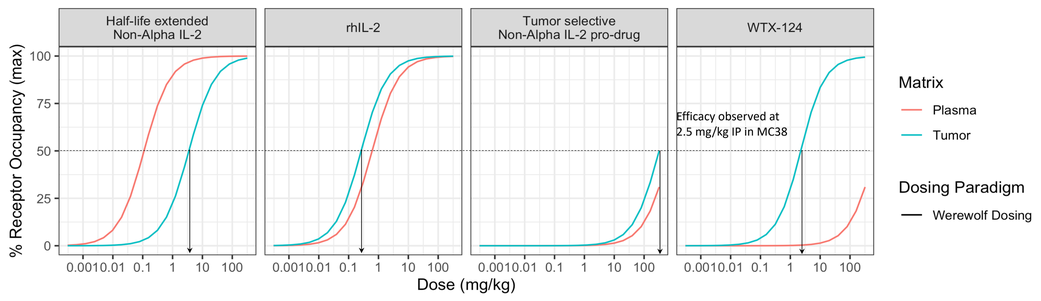
- *Ratio of average tumor RO to maximal plasma RO, and maximal tumor RO to maximal plasma RO are shown versus dose in mg/kg*
- *Maximal plasma RO may be linked to safety, whereas tumoral RO to antitumor effects*
- *As dose is increased to 30 mg/kg, only WTX-124 has a high tumor to plasma RO ratio*

MODEL PREDICTIONS

Receptor occupancy (RO) predictions suggest WTX-124 has best therapeutic index among IL-2 molecules investigated: High RO on TILs and minimal RO on peripheral lymphocytes



The above simulation uses the Werewolf dosing scheme at equimolar doses of 2.5 mg/kg IP of WTX-124 (dose at which WTX-124 efficacy was observed in mice [1])



- *As they are unmasked, rhIL-2 and non-alpha IL-2 have higher systemic RO stemming from intermediate-affinity receptor binding*
- *In comparison to tumor-activated molecules, WTX-124 and non-alpha IL-2 prodrug have lesser systemic RO*
- *Tumor-activated WTX-124 (using wild-type IL-2) has significantly higher tumor RO than tumor activated non-alpha IL-2 due to high-affinity receptor binding on TILs from wild-type IL-2, versus intermediate-affinity receptor binding with the tumor activated non-alpha IL-2*
- *Both rhIL-2 and non-alpha IL-2 can achieve high RO on TILs, however only when systemic RO is high, consistent with toxicity observed in the clinic*

CONCLUSIONS and REFERENCES

- The model predicts that tumor-activated IL-2 molecules (WTX-124 and tumor-activated non-alpha IL-2) produce markedly lower RO on peripheral lymphocytes than rhIL-2 and non-alpha IL-2
- The tumor-activated wild-type IL-2 molecule (WTX-124) produces high RO on TILs, consistent with the effective antitumor response observed in mouse models [1]
- Comparable RO on TILs can be produced with a tumor-activated non-alpha IL-2, but only at substantially higher doses (~100x more), consistent with observations of ineffective antitumor response with high doses of tumor-activated non-alpha IL-2 [2]
- Both rhIL-2 and non-alpha IL-2 can also achieve high RO on TILs but only where peripheral RO is also high, consistent with the observed toxicity in patients

References:
[1] Nirschl C et al., *Cancer Immunol Res*, 2022, 10 (5): 581–596.
[2] Nirschl C et al., *SITC Annual Meeting* 2023, Poster #1058.
[3] Momin N et al., *Nat. Commun.*, 2022, 13: 109
[4] Charych D et al., *PLoS One*. 2017, 12(7): e0179431.

Werewolf Therapeutics
200 Talcott Avenue
Watertown, MA 02472

media@werewolftx.com
info@werewolftx.com
https://werewolftx.com/

POSTER PAGE: