WTX-613, a conditionally activated IFNα INDUKINE™ molecule, induces anti-tumor immune responses resulting in strong tumor growth control in syngeneic mouse tumor models

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

Werewolf Therapeutics, Cambridge, Massachusetts, USA

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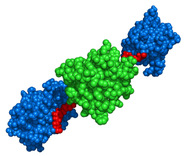
INTRODUCTION

- WTX-613 is designed to be a first-in-class, systemically delivered, conditionally activated interferon alpha (IFNα) INDUKINE™ molecule for the treatment of relapsed/refractory advanced or metastatic solid tumors and lymphomas (**Figure 1**)
- Werewolf is developing WTX-613 to minimize the severe toxicities that have been observed with recombinant human IFNα therapy and maximize clinical benefit when administered as monotherapy or in combination with immune checkpoint inhibitors
- IFNα is a member of the type-I IFN family and activates immune responses either directly, by engaging IFNα receptors (IFNARs) ubiquitously expressed on immune cells, or indirectly, by inducing chemokines that attract myeloid and lymphoid cells to the tumor site
- High-dose IFNα therapy is approved for melanoma, lymphoma and leukemia, but its use is limited by systemic toxicity and modest efficacy
- WTX-613 has the potential to be the only systemically delivered, conditionally activated IFNα therapy with normal IFNAR blockade and with full IFNα potency and function

Figure 1. Key features of WTX-613



Key features of WTX-613 include peripheral blockade of IFNα (green) from IFNα2b receptor interaction to limit systemic toxicity, and the half-life extension (blue) for optimal exposure in tumors and conditional activation in the tumor microenvironment.
IFNα1, interferon alpha; IFNα2b, interferon alpha 2b.

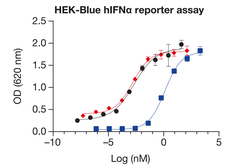
RESULTS

PROOF OF CONCEPT

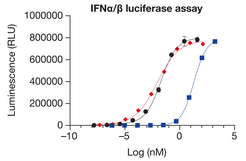
- Three cell-based assays demonstrated the inducibility of the cleaved INDUKINE™ molecule, WTX-613
- Activity was compared with rhIFNα2b to ensure cross-reactivity with the human IFNAR
- The WTX-613 molecule that had *in vitro* activation by cleavage of protease-sensitive linkers was more active than both intact WTX-613 and rhIFNα2b (**Figure 2**)
  - WTX-613 cleaved had 840-fold increased inducibility compared with WTX-613 intact

RESULTS

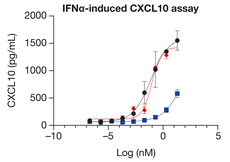
Figure 2. WTX-613 demonstrates inducibility in primary human cell assays



	EC_{50} (pM)
rhIFNα2b	2.17
WTX-613 intact	1342
WTX-613 cleaved	1.6



	EC_{50} (nM)
rhIFNα2b	0.02036
WTX-613 intact	19.82
WTX-613 cleaved	0.008901



	EC_{50} (nM)
rhIFNα2b	0.07979
WTX-613 intact	185.4
WTX-613 cleaved	0.09324

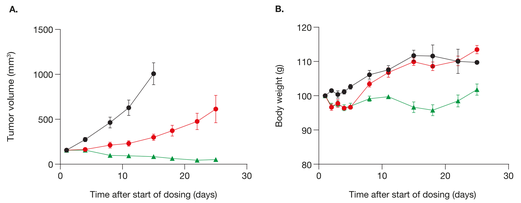
rhIFNα2b ■ WTX-613 intact ◆ WTX-613 cleaved

In vitro activity of WTX-613 in primary human cell assays comparing intact (blue) and protease-activated (cleaved) WTX-613 (red) with rhIFNα2b (black).
CXCL10, C-X-C motif chemokine ligand 10; EC_{50}, half maximal effective concentration; HEK, human embryonic kidney; OD, optical density; rhIFNα2b, recombinant human interferon alpha 2b; RLU, relative light units.

ANTI-TUMOR EFFICACY OF WTX-613 SURROGATE SHOWN IN THE MC38 (MOUSE COLON) TUMOR MODEL

- WTX-613 surrogate or IFNα1 was given to MC38 mice at equivalent molar doses over the treatment period (**Figure 3**)
  - Both treatments were tested at the maximally tolerated dose

Figure 3. WTX-613 surrogate vs IFNα1 in MC38 mouse tumor model



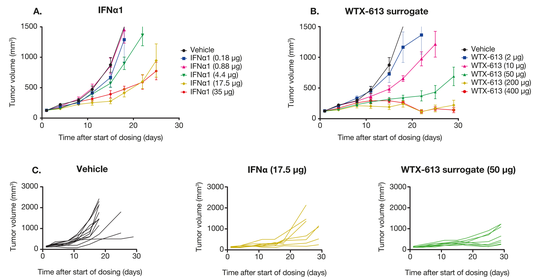
*MC38 mice were treated with WTX-613 surrogate (twice weekly for 2 weeks, via IP), vehicle (IP), or IFNα1 at equivalent molar doses over the treatment period (twice daily [5/2/5/2 regimen] for a total of 20 doses, IP). **A**) Tumor growth with the three treatments. **B**) Body weight of WTX-613, IFNα1- or vehicle-treated mice.*
IFNα1, interferon alpha 1; IP, intraperitoneal.

RESULTS

DOSE RESPONSE WITH WTX-613 SURROGATE VS IFNα1 IN MC38 TUMOR MODEL

- A dose response with WTX-613 surrogate in the syngeneic MC38 tumor model showed (**Figure 4**)
  - WTX-613 surrogate (mouse IFNα1 INDUKINE™) has an improved therapeutic index (TI)
  - All dose levels tested for WTX-613 were efficacious and well tolerated in mice

Figure 4. WTX-613 surrogate vs IFNα1 dose response in MC38 tumor model



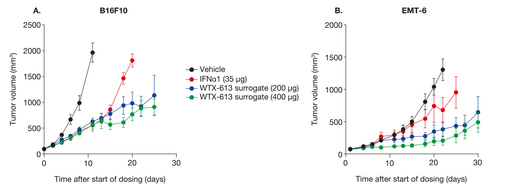
	MTD	Lowest effective dose	TI
IFNα1	80 µg	17.5 µg	4.6
WTX-613	830 µg	50 µg	16.6

*Graphs show average tumor growth with (**A**) IFNα1 vs vehicle, (**B**) WTX-613 surrogate vs vehicle; and the individual mice treated with (**C**) vehicle, IFNα1 (17.5 µg) and WTX-613 surrogate (50 µg). Dose response with IFNα1 and WTX-613 surrogate in the MC38 tumor model. Labels in the legend represent the dose per mouse per dosing day. WTX-613 surrogate and vehicle were dosed twice a week for a total of four doses. IFNα1 was dosed twice a day in a 5/2/5/2 regimen for a total of 20 doses. TI was determined by dividing the MTD by the lowest efficacious dose.*
IFNα1, interferon alpha 1; MTD, minimum therapeutic dose; TI, therapeutic index.

ANTI-TUMOR EFFICACY OF WTX-613 IN MOUSE TUMOR MODELS

- In the difficult-to-treat B16 mouse melanoma model (B16F10), WTX-613 is efficacious (**Figure 5A**)
- Mouse WTX-613 is efficacious and well tolerated in the less immunogenic breast carcinoma (EMT-6) model (**Figure 5B**)

Figure 5. Anti-tumor dose-response curves of WTX-613 surrogate in mouse tumor models



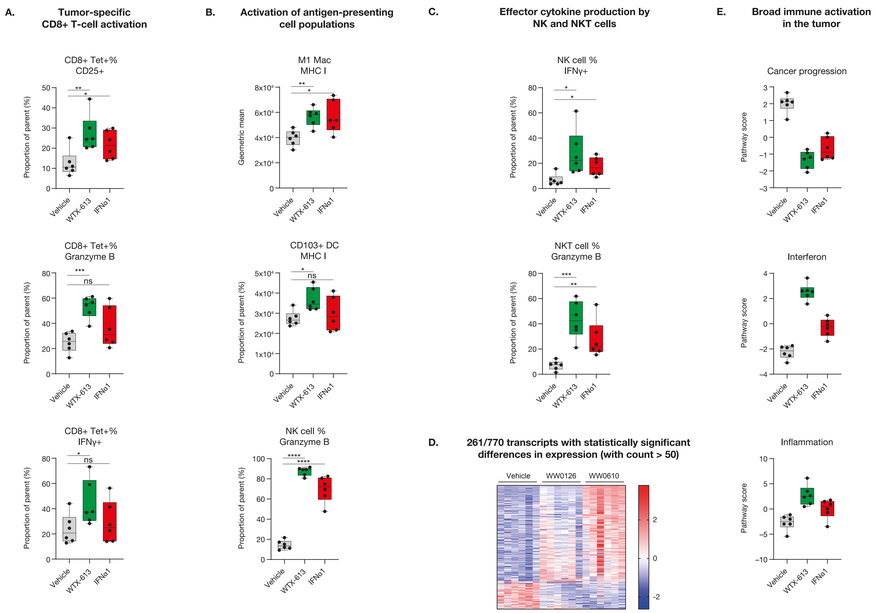
*Dose-response curves showing tumor growth in mice treated with WTX-613 surrogate treatment vs IFNα1 or vehicle (IP) in the (**A**) B16F10 model and (**B**) EMT-6 model.*
B16F10, melanoma model; EMT6, breast carcinoma model; IP, intraperitoneal; IFNα1, interferon alpha 1.

RESULTS

MOUSE WTX-613 SURROGATE ACTIVATES TYPE I INTERFERON IMMUNE RESPONSES IN MC38 MOUSE TUMORS

- The WTX-613 surrogate induces CD8+ T cells and natural killer cells, and dendritic cell activation (**Figure 6A–C**)
- NanoString™ analysis of MC38 tumors treated with WTX-613 surrogate reveals 261/770 transcripts with statistically significant differences in expression (with count > 50) (**Figure 6D**)
- Pathway scores for broad immune activation are elevated after WTX-613 surrogate treatment (**Figure 6E**)

Figure 6. Immune-profiling and NanoString™ analysis of MC38 tumor extracts



*MC38 tumors were implanted and allowed to grow before mice were randomized into treatment groups on Day 0. Mice receiving WTX-613 (400 µg) were dosed on Day 1 and Day 4. Mice receiving IFNα1 (144 µg) were dosed twice daily to give a similar exposure profile. Tumors were harvested on Day 5. **A**) Activation of tumor-specific CD8+ T cells. **B**) Activation of antigen-presenting cell populations. **C**) Effector cytokine production by NK and NKT cells, measured after stimulation. **D**) Total RNA was extracted and NanoString™ analysis was performed to generate a heatmap of differentially expressed transcripts. **E**) Pathway scores for broad immune activation in the tumor were generated by Nsolver software. Unless otherwise stated, data are presented as the mean ± standard deviation, and p values are derived from T tests (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant).*
DC, dendritic cell; IFNα1, interferon alpha 1; IFNγ, interferon gamma; MHC I, major histocompatibility complex class I; NK, natural killer; NKT cell, natural killer T cell; Tet, tetramer.

CONCLUSIONS

- Proof-of-concept assays with WTX-613 surrogate (an IFNα INDUKINE™ lead molecule) demonstrate anti-tumor activity in syngeneic mouse models and better tolerability compared to recombinant IFNα (rIFNα)
- WTX-613 surrogate inhibits tumor growth in MC38, B16F10 and EMT6 mouse tumor models
- Changes in immune profiles in mouse tumors after IFNα INDUKINE™ therapy support a mechanism of action similar to rIFNα
- Further preclinical development is ongoing

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