CONDITIONALLY ACTIVATED IL-12 OR IFNα INDUKINE™ MOLECULES INHIBIT SYNGENEIC LYMPHOMA TUMOR GROWTH IN MICE, INDUCE ANTI-TUMOR IMMUNE RESPONSES AND ARE TOLERATED IN NON-HUMAN PRIMATES

#2258

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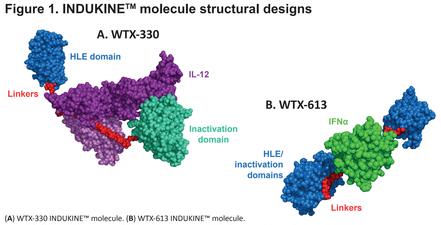
INTRODUCTION

- Cytokine therapy is a promising approach to treat cancer. However, poor pharmacokinetic (PK) properties and dose-limiting toxicities have prevented or limited previous clinical use of cytokines such as interleukin-12 (IL-12) and interferon alpha (IFNα)
- INDUKINE™ molecules, engineered using Werewolf Therapeutics' Predator™ discovery platform, are novel, systemically delivered cytokine pro-drugs designed to prevent systemic toxicity by delivering cytokines to the tumor microenvironment (TME) where they can become active
  - The WTX-330 INDUKINE™ molecule contains a wild-type IL-12 pro-drug, a half-life extension (HLE) domain to support infrequent dosing, and a high-affinity anti-IL-12 neutralizing antibody domain to maintain the molecule in its inactive state in the periphery. Both the HLE and blocking domains are tethered to IL-12 via two identical tumor protease-sensitive linkers. Linker cleavage in the TME removes the HLE domain and the blocker, resulting in active IL-12 within the TME (**Figure 1A**)
  - The WTX-613 INDUKINE™ molecule is an inactive IFNα2b pro-drug with two identical HLE domains tethered to IFNα2b via two identical tumor protease-sensitive linkers. The HLE domains sterically block binding of WTX-613 to IFNα receptor until cleavage of the linkers in the TME releases active IFNα (**Figure 1B**)
- Here we report preclinical findings that demonstrate the use of INDUKINE™ molecules using murine tumor models (for both solid tumors and lymphoma) and PK data from mice and non-human primates (NHPs)

Figure 1. INDUKINE™ molecule structural designs



(**A**) WTX-330 INDUKINE™ molecule. (**B**) WTX-613 INDUKINE™ molecule.
HLE, half-life extension; IFNα, interferon alpha; IFNaR, IFNα receptor; IL-12, interleukin-12.

RESULTS

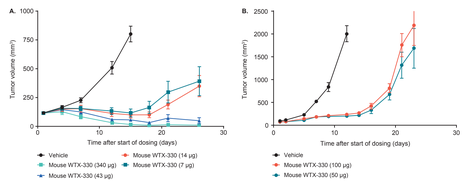
- Since human IL-12 and IFNα2b are not cross-reactive in mice, mouse surrogates for WTX-330 and WTX-613 INDUKINE™ molecules were created, utilizing a mouse/human chimeric IL-12 or a mouse IFNα1 as the respective payloads
- The IL-12 INDUKINE™ molecule WTX-330 inhibited mouse MC38 and B16F10 tumor growth leading to complete regression of all MC38 tumors (**Figure 2**). Pleiotropic immune responses were observed in B16F10 tumors supporting a mechanism of action (MOA) of WTX-330 driven by IL-12 biology (**Figure 3**)
- WTX-330 displayed long half-life in plasma in both mouse and NHP studies. PK profiles were dose-dependent, and animals tolerated the IL-12 INDUKINE™ constructs at higher exposures than is required for anti-tumor efficacy in mouse tumor models (**Figure 4**)
- Anti-tumor efficacy in two subcutaneous mouse lymphoma models with mouse WTX-330 supports clinical exploration in lymphoma that are unresponsive to immuno-oncology (I/O) drugs such as anti-programmed cell death 1 (αPD-1) (**Figure 5**)
- An INDUKINE™ construct using IFNα (mouse WTX-613) demonstrated similar *in vivo* results to the IL-12 INDUKINE™ molecule, with potent anti-tumor efficacy in mouse syngeneic solid tumor (MC38, B16F10) and lymphoma (A20, EG7.OVA) models (**Figures 6–9**)
- Mouse WTX-613 and WTX-613 were well tolerated in mouse and NHP studies, respectively, demonstrating long half-life and dose-dependent PK properties (**Figure 8**)

RESULTS – WTX-330

Anti-tumor efficacy in syngeneic mice

- Mouse WTX-330 induced complete regressions in 100% of the MC38 tumor-bearing mice (**Figure 2A**)
  - All dose levels were well tolerated in mice
- B16F10 is a less immunogenic, difficult-to-treat mouse melanoma model (**Figure 2B**)
  - Mouse WTX-330 was efficacious in B16F10, and tumor regrowth was only seen several days after treatment ended
  - Both dose levels were well tolerated in mice

Figure 2. Mouse WTX-330 dose response in MC38 and B16F10 tumor models

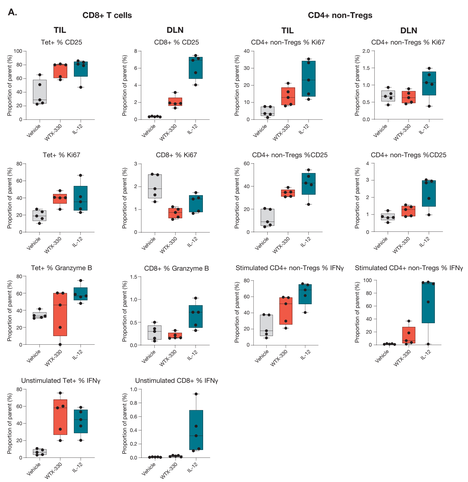


(**A**) Anti-tumor efficacy using MC38 model in female C57Bl/6 mice. Mice were dosed via intraperitoneal injection starting on Day 1.
(**B**) Anti-tumor efficacy using B16F10 model in female C57Bl/6 mice. Mice were dosed via intraperitoneal injection starting on Day 1.

Pleiotropic immune responses

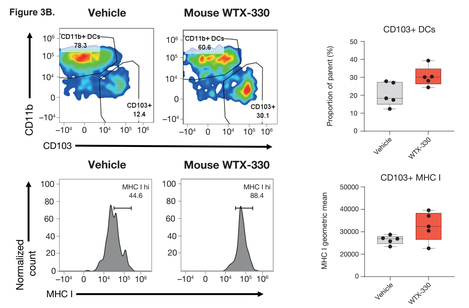
- Mouse WTX-330 activated pleiotropic immune responses in B16F10 mouse tumors (**Figure 3**)
- Mouse WTX-330 treatment amplified tumor infiltration and induced immune cell activation (**Figures 3A–B**)

Figure 3. Immune profiling analysis of B16F10 mouse tumors and draining lymph nodes



(**A**) Mouse WTX-330 led to preferential T cell activation in the tumor, as compared with DLN.
CD, cluster of differentiation; DLN, draining lymph nodes; IFNγ, interferon gamma; IL-12, interleukin-12; Tet, tetramer; TIL, tumor-infiltrating lymphocyte; Treg, regulatory T cell.

Figure 3B.

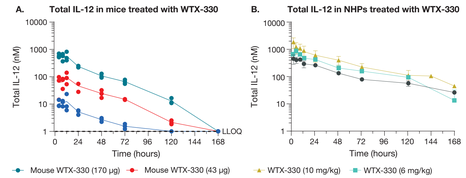


(**B**) Mouse WTX-330 increased cross-presenting DC frequencies and function in tumors.
CD, cluster of differentiation; DCs, dendritic cells; MHC I, major histocompatibility complex class I.

PK and tolerability

- There was an overall increase in exposure (maximum concentration [C_{max}] and area under curve [AUC]) with increasing dose of mouse WTX-330 in naive mice (**Figure 4A**) and NHPs (**Figure 4B**)
- The mean half-life of WTX-330 was 53 hours in NHPs
- Plasma-free IL-12 compared with total IL-12 INDUKINE™ was < 0.1% in NHPs
- 6 mg/kg of WTX-330 (single and repeat dose) was tolerated in cynomolgus monkeys. Favorable exposure multiples were achieved in monkeys compared with the target efficacious dose

Figure 4. Plasma PK of WTX-330 in mice and cynomolgus monkeys

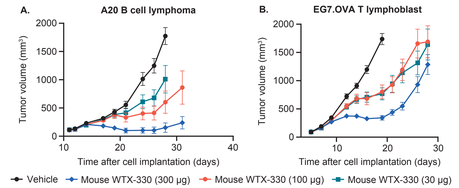


(**A**) PK analysis for total IL-12 (intact mouse WTX-330 and free IL-12 [ELISA]) after a single dose in mouse plasma.
(**B**) PK analysis for WTX-330 in NHPs.
IL-12, interleukin-12; LLOQ, lower limit of quantification; NHPs, non-human primates; PK, pharmacokinetics.

Anti-tumor efficacy in lymphomas supports clinical development

- Lymphoma is unresponsive to I/O drugs in mouse models and human patients
- Mouse WTX-330 at 300 µg/animal effectively controlled tumor growth in two lymphoma models (**Figure 5**)
- All dose levels were well tolerated in mice

Figure 5. Mouse WTX-330 inhibits B and T cell lymphomas in mouse tumor models



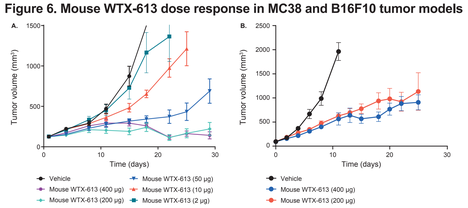
Anti-tumor efficacy using (**A**) A20 B cell lymphoma (BALB/c) and (**B**) EG7.OVA T lymphoblast (C57Bl/6) models. Tumor cells were injected subcutaneous and mice were dosed via intraperitoneal injection. Dosing started on Day 11 (A20) or on Day 5 (EG7.OVA).

RESULTS – WTX-613

Anti-tumor efficacy in syngeneic mice

- Mouse WTX-613 induced tumor stasis in MC38 tumors (**Figure 6A**)
  - Regrowth was prevented at 200 µg/animal after treatment stopped on Day 11
  - All dose levels were well tolerated in mice
- B16F10 is a less immunogenic, difficult-to-treat mouse melanoma model (**Figure 6B**)
  - Mouse WTX-613 inhibited tumor growth rate in B16F10 with no accelerated tumor regrowth rate after treatment stopped on Day 11
  - Both dose levels were well tolerated in mice

Figure 6. Mouse WTX-613 dose response in MC38 and B16F10 tumor models



(**A**) Anti-tumor efficacy using MC38 model in female C57Bl/6 mice. Mice were dosed via intraperitoneal injection starting on Day 1.
(**B**) Anti-tumor efficacy using B16F10 model in female C57Bl/6 mice. Mice were dosed via intraperitoneal injection starting on Day 1.

WTX-613 activated type I IFN immune responses in MC38 mouse tumors

- Mouse WTX-613 activated CD8+ T cells, natural killer cells, and dendritic cell (**Figure 7A–C**)
- NanoString™ analysis of MC38 tumors treated with mouse WTX-613 revealed 261/770 transcripts with statistically significant differences in expression (with count > 50) (**Figure 7D**)
- Pathway scores for broad immune activation were elevated after mouse WTX-613 treatment (**Figure 7E**)

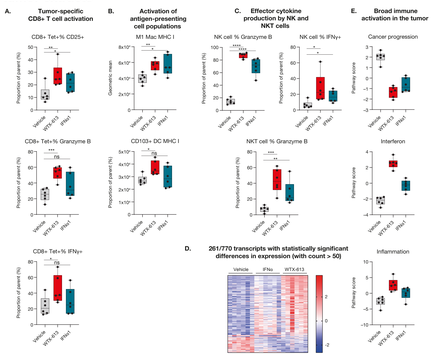
PK and tolerability

- There was an overall increase in exposure (C_{max} and AUC) with increasing dose of WTX-613 in naïve mice (**Figure 8A**) and in NHPs (**Figure 8B**)
- The mean half-lives of mouse WTX-613 and WTX-613 was 44 hours in mice and 172 hours in NHPs, respectively
- 10 mg/kg of WTX-613 was tolerated in cynomolgus monkeys
- Favorable exposure multiples were achieved in monkeys compared with target efficacious dose

Anti-tumor efficacy in lymphomas supports clinical development

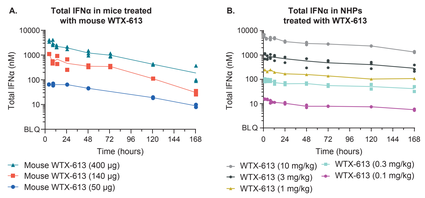
- Lymphoma is unresponsive to I/O drugs in mouse models and human patients
- Mouse WTX-613 at 133 µg/animal effectively controlled tumor growth in both models (**Figure 9**)
- All dose levels were well tolerated in mice

Figure 7. Immune profiling and NanoString™ analysis of MC38 tumor extracts



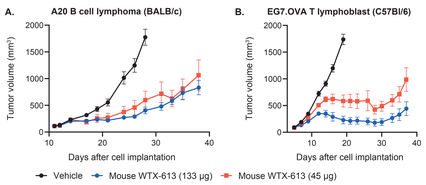
MC38 tumors were implanted and allowed to grow before mice were randomized into treatment groups on Day 0. Mice receiving mouse WTX-613 (400 µg) were dosed on Day 1 and Day 4. Mice receiving IFNα1 (144 µg) were dosed twice daily to give a similar exposure profile. Tumors were harvested on Day 5. (**A**) 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). WTX-613 = Mouse WTX-613. CD, cluster of differentiation; DC, dendritic cell; IFN, interferon; IFNα1, interferon alpha 1; IFNγ, interferon gamma; MAC, membrane attack complex; MHC I, major histocompatibility complex class I; NK, natural killer; NKT cell, natural killer T cell; Tet, tetramer.

Figure 8. Plasma PK of WTX-613 in mice and cynomolgus monkeys



(**A**) PK analysis for total IFNα (intact mouse WTX-613 and free IFNα) in mouse plasma. (**B**) PK analysis for WTX-613 in NHPs.
BLQ, below limit of quantification; IFNα, interferon alpha; NHPs, non-human primates; PK, pharmacokinetic.

Figure 9. Mouse WTX-613 inhibits B and T cell lymphomas in mouse tumor models



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Tumor cells were injected subcutaneous and mice were dosed via intraperitoneal injection.
Dosing started on Day 11 (A20) or on Day 5 (EG7.OVA).

CONCLUSIONS

- WTX-330 (an IL-12 INDUKINE™ molecule) and WTX-613 (an IFNα INDUKINE™ molecule) demonstrated anti-tumor activity in solid tumor and lymphoma syngeneic mouse tumor models
- Both INDUKINE™ molecules modulated immune profiles in mouse tumors with an MOA expected for IL-12 and IFNα but with much better tolerability
- PK profiles showed that tolerated exposures in NHPs exceeded those required for anti-tumor efficacy in mice
- Data support further exploration in mouse lymphoma models with focus on MOAs and αPD-1 combinations
- Preclinical development for both INDUKINE™ molecules is ongoing

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