WTX-330, a Conditionally Activated IL-12 INDUKINE™ Therapy, Releases IL-12 Selectively in the Tumor Microenvironment to Activate Anti-Tumor Immune Responses and Induce Regressions in Mouse Tumor Models

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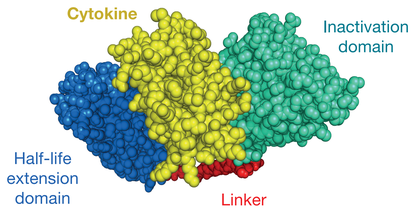
Werewolf THERAPEUTICS

INTRODUCTION

- Systemic therapy with proinflammatory immune modulators is a promising approach for treating cancer
  - The cytokine interleukin-12 (IL-12) is a potent inducer of innate and adaptive anti-tumor immunity, but there currently are no approved IL-12 therapies available
  - Unfortunately, high-dose IL-12 treatment is associated with inflammation and tissue damage, which has rendered IL-12 treatment strategies impractical
- WTX-330 is an inducible polypeptide (INDUKINE™ therapies) designed to be an inactive IL-12 pro-drug with a half-life extension domain to support infrequent systemic administration (**Figure 1**)
  - Kept inactive in the periphery via high-affinity antibody blockade tethered to IL-12 via a tumor protease-sensitive linker
  - Designed to minimize the severe toxicities seen with recombinant human IL-12 (rIL-12) therapy and maximize clinical benefit when given alone or in combination with immune checkpoint inhibitors
- WTX-330 is designed to be a first-in-class, systemically delivered, conditionally activated IL-12 INDUKINE™ molecule for the treatment of relapsed or refractory advanced or metastatic solid tumors or lymphoma

Figure 1. Key features of WTX-330



Key features of WTX-330 include peripheral blockade (green) of the IL-12–IL-12 receptor interaction to limit systemic toxicity, half-life extension (blue) for optimal exposure in tumors and conditional activation in the tumor microenvironment.

RESULTS

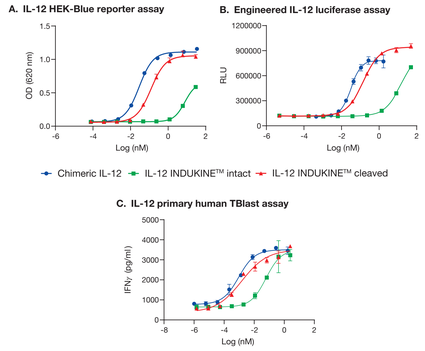
PROOF OF CONCEPT

- Three cell-based assays were used to demonstrate inducibility of the cleaved INDUKINE™ molecule, WTX-330, after *in vitro* activation by cleavage of protease-sensitive linkers (**Figure 2**)

RESULTS

- Activity was compared with recombinant chimeric IL-12 (mouse p35, human p40) to ensure cross-reactivity with the mouse/human IL-12 receptor

Figure 2. Cell-based assays show activity of the IL-12 INDUKINE™ molecule (WTX-330)

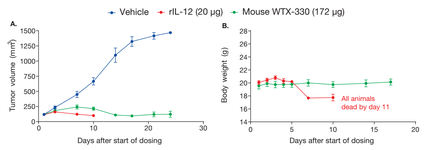


HEK, human embryonic kidney; IFNγ, interferon gamma; OD, optical density; RLU, relative light units.

ANTI-TUMOR EFFICACY

- WTX-330 mouse surrogate molecule (mouse WTX-330) vs rIL-12 were given to MC38 (colon) tumor mice at equivalent molar doses over the treatment period (**Figure 3**)
  - Both treatments were active, but rIL-12-treated animals were all deceased by Day 11
  - Conversely, mouse WTX-330-treated animals remained well and retained body weight

Figure 3. Mouse WTX-330 vs rIL-12 in MC38 mouse tumor model



*Anti-tumor efficacy was assessed in the MC38 tumor model. Tumor cells were injected subcutaneously. **A)** Tumor growth of MC38 mice treated with mouse WTX-330 (twice weekly for 2 weeks, via IP), vehicle (IP), or rIL-12 at equivalent molar doses over the treatment period (twice daily for 10 days, IP). **B)** Body weight of mouse WTX-330- or rIL-12-treated animals. IP, intraperitoneal injection; rIL-12, recombinant human interleukin-12.*

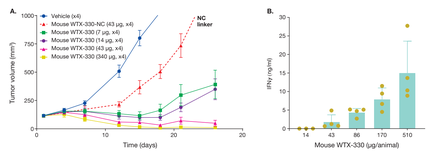
DOSE RESPONSE

- A dose response with mouse WTX-330 in the syngeneic MC38 tumor model showed:
  - mouse WTX-330 induced 100% complete regression in MC38 tumors (**Figure 4A**)
  - the non-cleavable surrogate is much less active than mouse WTX-330 that has a cleavable linker (**Figure 4A**)

RESULTS

  - all dose levels were well tolerated in mice
  - systemic interferon gamma levels remained low after treatment (**Figure 4B**)

Figure 4. Mouse WTX-330 dose response in MC38 mouse tumor model

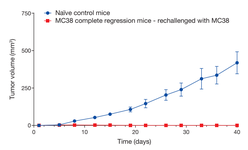


IFNγ, interferon gamma; NC, non-cleavable.

IMMUNE MEMORY

- Mouse WTX-330 was shown to induce immune memory in MC38 mice rechallenged with MC38 tumor cells after showing complete regression with WTX-330 (**Figure 5**)

Figure 5. Mouse WTX-330 induces immune memory in MC38 mice



Mice previously treated with mouse WTX-330 and displaying complete regression, alongside naïve age-matched control mice, were rechallenged with MC38 tumor cells.

ANTI-TUMOR EFFICACY IN OTHER MOUSE TUMOR MODELS

- Mouse WTX-330 shows a dose response in the mouse colon CT26 tumor model with no weight loss (**Figure 6A**)
- In the difficult-to-treat B16F10 melanoma model, mouse WTX-330 is efficacious with tumor regrowth only seen after treatment ended (**Figure 6B**)
- Mouse WTX-330 is efficacious and well-tolerated in the less immunogenic EMT-6 model (**Figure 6C**)

PLEIOTROPIC IMMUNE RESPONSES

- WTX-330 activates pleiotropic immune responses in MC38 mouse tumors (**Figure 7**)
- Mouse WTX-330 induces CD4+ T-cell, natural killer cell and tumor-specific CD8+ T-cell activation (**Figures 7A–C**)
- Pathway scores for antigen processing, dendritic cell functions and innate cell activity were elevated (**Figure 7E**)

RESULTS

Figure 6. Anti-tumor activity of mouse WTX-330 in other mouse tumor models

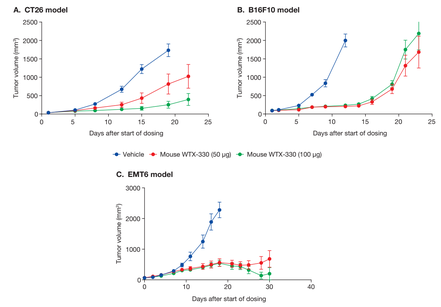
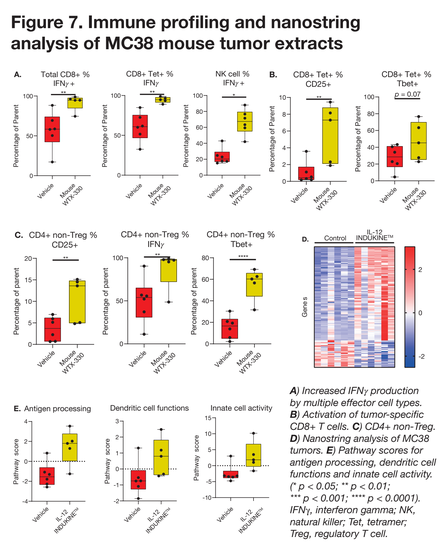


Figure 7. Immune profiling and nanostring analysis of MC38 mouse tumor extracts



***A)** Increased IFNγ production by multiple effector cell types. **B)** Activation of tumor-specific CD8+ T cells. **C)** CD4+ non-Treg. **D)** Nanostring analysis of MC38 tumors. **E)** Pathway scores for antigen processing, dendritic cell functions and innate cell activity. (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001). IFNγ, interferon gamma; NK, natural killer; Tet, tetramer; Treg, regulatory T cell.*

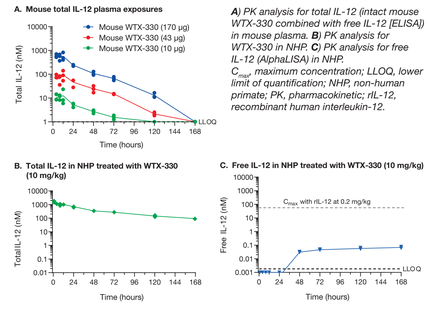
PHARMACOKINETICS AND TOLERABILITY

- There was an overall increase in exposure (maximum concentration [C_{max}] and area under the curve [AUC]) with increasing dose of mouse WTX-330 in naïve mice (**Figure 8A**)
- The mean half-life ($T_{1/2}$) of WTX-330 was 53 hours in non-human primates (NHP) (**Figure 8B**)
- Plasma free IL-12 compared with total IL-12 INDUKINE™ was < 0.1% (**Figure 8C**)
- 6 mg/kg WTX-330 (single and repeat dose) was well tolerated in Chinese and Mauritian cynomolgus monkeys (**Table 1**)

RESULTS

- Exposure (C_{max}, AUC) was higher in NHP (given at the tolerated dose of 6 mg/kg) compared with mice (given at the efficacious dose of 0.7 mg/kg) (**Table 1**)

Figure 8. Plasma PK of WTX-330 in mice and cynomolgus monkeys



***A)** PK analysis for total IL-12 (intact mouse WTX-330 combined with free IL-12 [ELISA]) in mouse plasma. **B)** PK analysis for WTX-330 in NHP. **C)** PK analysis for free IL-12 (AlphaLISA) in NHP. C_{max}, maximum concentration; LLOQ, lower limit of quantification; NHP, non-human primate; PK, pharmacokinetic; rIL-12, recombinant human interleukin-12.*

Table 1. PK of WTX-330 in mice and cynomolgus monkeys

	Dose	C_{max} (nM)	AUC (nM*h)	Findings
Mouse	0.7 mg/kg	22.5	811	MC38 tumor growth inhibition
NHP	6 mg/kg	876.8	33,694	Well-tolerated in monkeys
Target exposure multiples		39.0	41.6	

Exposure parameters expressed as C_{max} and AUC, and target exposure multiples comparing highest tolerated exposure in NHP divided by lowest efficacious exposure in MC38 tumor mice. AUC, area under the curve; C_{max}, maximum concentration; NHP, non-human primate; PK, pharmacokinetics.

CONCLUSIONS

- Proof-of-concept assays of WTX-330 (an IL-12 INDUKINE™ lead molecule) demonstrate anti-tumor activity in syngeneic mouse models and better tolerability compared with rIL-12
- Mouse WTX-330 potently inhibits tumor growth in MC38, CT26, B16F10 and EMT6 mouse tumor models
- Changes in immune profiles in mouse tumors after IL-12 INDUKINE™ therapy support a mechanism of action similar to rIL-12
- WTX-330 is well tolerated in NHP and reaches C_{max} and AUC exposures far higher than the efficacious exposures seen in mice

Poster presented at AACR Annual Meeting 2021; Virtual Meeting