mWTX-330, an IL-12 INDUKINE™ molecule, Selectively Activates Tumor Infiltrating Lymphocytes and Reprograms the Tumor Microenvironment in Murine Syngeneic Tumor Models

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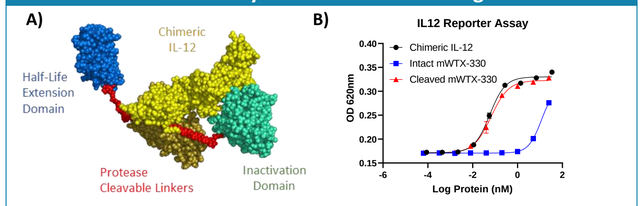
Werewolf THERAPEUTICS

Introduction

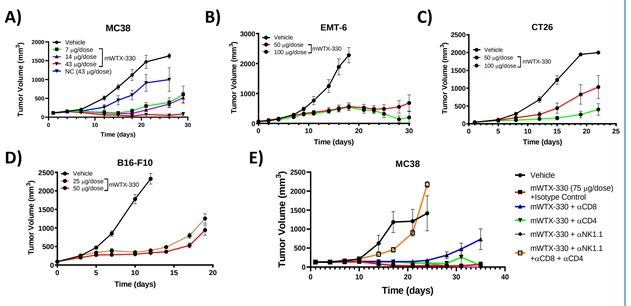
- Systemic therapy with proinflammatory immune modulators is a promising approach for the treatment of cancer
  - The cytokine interleukin-12 (IL-12) is a potent inducer of innate and adaptive anti-tumor immunity, but potentially lethal toxicity associated with systemic administration of IL-12 has prevented IL-12 treatment strategies from successful clinical application
- WTX-330 is an inducible protein (INDUKINE™) designed to be an inactive IL-12 pro-drug with a half-life extension domain to support infrequent systemic administration
  - The pro-drug is inactive in peripheral tissues due to a high-affinity antibody blockade tethered to IL-12 via a tumor protease-sensitive linker. This design will minimize the severe toxicities seen with recombinant human IL-12 (rIL-12) therapy while maximizing the potential clinical benefits
- 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
- Since human IL-12 is not active in mouse cells, an INDUKINE™ surrogate molecule containing chimeric IL-12 was designed (mWTX-330) to explore the therapeutic mechanism of action of this molecule in murine syngeneic tumor models (Fig.1)

Figure 1: Murine WTX-330 (mWTX-330) is a Selectively Inducible IL-12 Prodrug



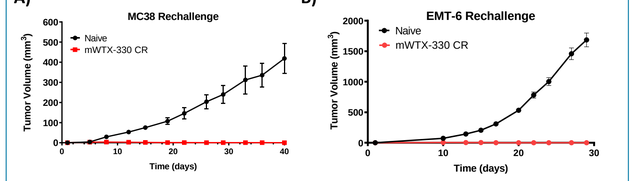
A) Key features of the INDUKINE™ molecule mWTX-330, including peripheral blockade (green) of the cytokine - receptor interaction to limit systemic toxicity, half-life extension (blue) for optimal exposure in tumors, and protease cleavable linkers (red) that results in conditional release of chimeric IL-12 in the tumor microenvironment. B) In vitro activity of mWTX-330 in the IL-12 HEK-Blue reporter assay, comparing intact mWTX-330 (blue squares) and cleaved mWTX-330 (red triangles) to recombinant chimeric IL-12 (black circles).

Figure 2: mWTX-330 Generates Robust Anti-Tumor Immunity in a Cleavage Dependent Manner



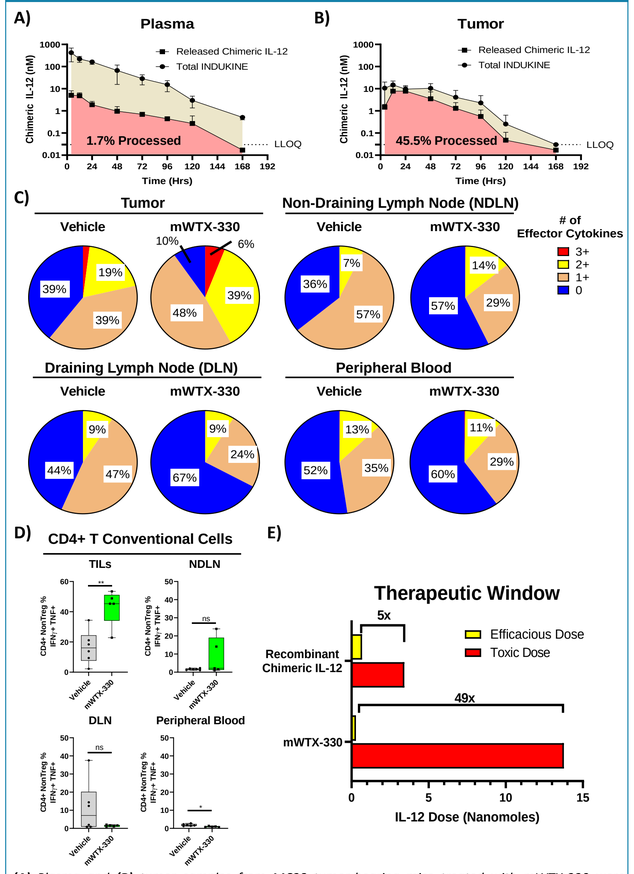
*Tumor growth curves of **(A)** MC38, **(B)** EMT-6, **(C)** CT26, or **(D)** B16-F10 tumors. Mice were dosed twice a week for 2 weeks, with the specific doses reported in the figure. In some experiments, mice were treated with a non-cleavable variant of mWTX-330 (Panel A, NC, Blue). **(E)** MC38 tumor bearing mice were dosed twice a week with depleting antibodies in conjunction with mWTX-330.*

Figure 3: mWTX-330 Induced Tumor Rejection Generates a Potent Memory Response Against Rechallenge



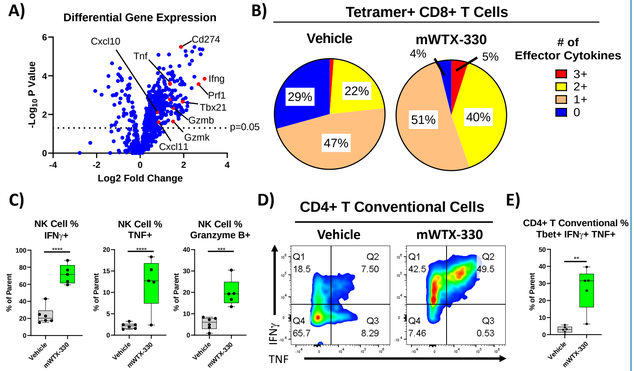
*Mice that completely rejected either **(A)** MC38 tumors or **(B)** EMT-6 tumors were rechallenged with the same tumor cell line at least 60 days after complete rejection (CR) of the primary tumor. As a control, age matched, tumor naïve animals were also challenged, and tumor growth was monitored over time.*

Figure 4: mWTX-330 is Preferentially Activated in the TME and Expands the Therapeutic Window Compared to Chimeric IL-12



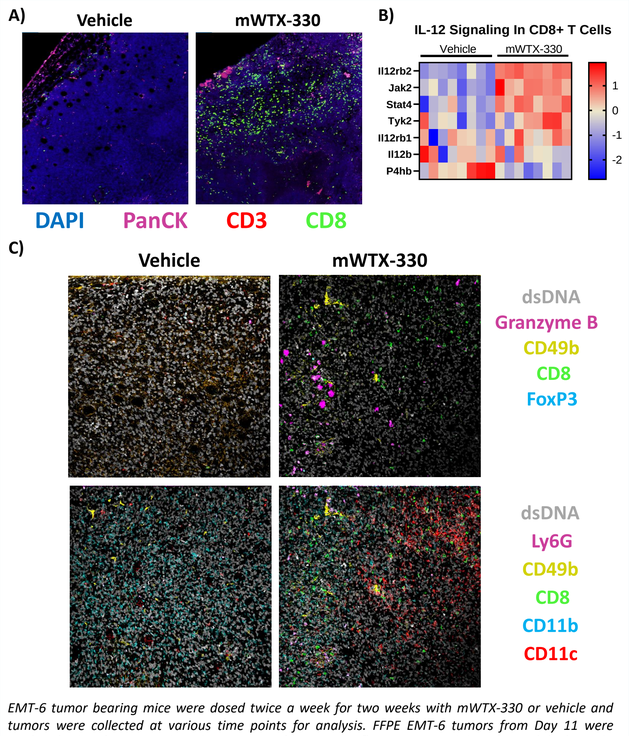
***(A)** Plasma and **(B)** tumor samples from MC38 tumor-bearing mice treated with mWTX-330 were analyzed at various timepoints for either the presence of the total INDUKINE™ protein (tan) or free chimeric IL-12 (red). The area under the curve was calculated, and the ratio of total INDUKINE™ molecule to free chimeric IL-12 was calculated. **(C)** MC38 tumor bearing mice were dosed twice with mWTX-330, and the frequency of polyfunctional CD8+ T cells in the tumor, peripheral blood, tumor draining or non-tumor draining lymph nodes was measured by examining co-expression of IFNγ, TNF, and Granzyme B. **(D)** The frequency of CD4+ T conventional cells producing IFNγ and TNF in the tumor, peripheral blood, non-tumor draining lymph node, or tumor draining lymph node was measured. **(E)** Representation of the therapeutic window of recombinant chimeric IL-12 or mWTX-330 in MC38 tumor-bearing mice based on multiple experiments identifying the minimally efficacious dose and the maximum tolerated dose of each molecule.*

Figure 5: mWTX-330 Activates Various Tumor Infiltrating Immune Cell Populations in the MC38 Syngeneic Tumor Model



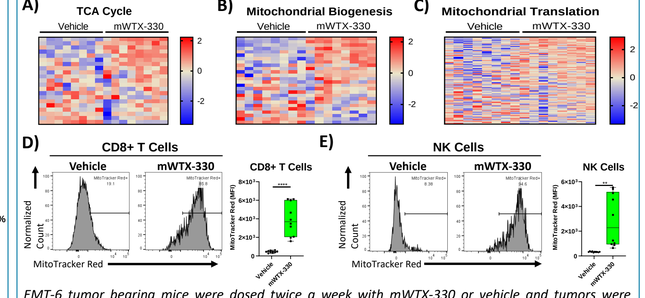
*MC38 tumor bearing mice were dosed twice a week with mWTX-330 or vehicle and tumors were collected 24 hours after the second dose. **(A)** Volcano plot of transcripts differentially expressed between mWTX-330 and vehicle-treated mice as determined by Nanostring analysis. **(B)** The frequency of polyfunctional, tetramer positive CD8+ T cells was measured by examining co-expression of IFNγ, TNF, and Granzyme B. **(C)** The frequency of tumor infiltrating NK cells producing IFNγ, TNF, or Granzyme B. **(D-E)** The frequency of CD4+ T conventional cells with a T_H1 phenotype (Tbet+IFNγ+TNF+).*

Figure 6: Systemic Treatment With mWTX-330 Induces IL-12 Signaling in the TME and Drives Substantial Tumor Infiltration



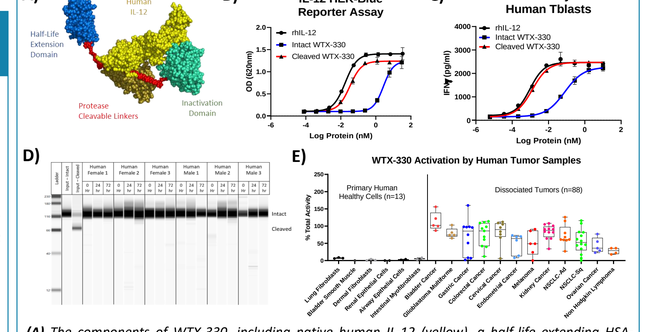
*EMT-6 tumor bearing mice were dosed twice a week for two weeks with mWTX-330 or vehicle and tumors were collected at various time points for analysis. FFPE EMT-6 tumors from Day 11 were analyzed using the Nanostring DSP system, and **(A)** immunofluorescence staining of DAPI (Blue), PanCK (pink), and CD8 (green) was performed. **(B)** Pathway analysis was performed using the Nanostring DSP software and heatmaps of genes associated with IL-12 signaling. **(C)** Multiplexed ion beam imaging was performed on FFPE tumors from Day 11, with various markers specified in the figure.*

Figure 7: mWTX-330 Treatment Metabolically Reinvigorates Tumor Infiltrating CD8+ T cells and NK Cells



*EMT-6 tumor bearing mice were dosed twice a week with mWTX-330 or vehicle and tumors were collected after three doses. Pathway analysis was performed on tumor infiltrating CD8+ T cells using the Nanostring DSP system and heatmaps of transcripts associated with **(A)** the TCA cycle, **(B)** mitochondrial biogenesis, or **(C)** mitochondrial translation were generated. Tumor infiltrating **(D)** CD8+ T cells or **(E)** NK cells from either vehicle or mWTX-330 treated animals were stained with Mitotracker Red to measure actively respirating mitochondria.*

Figure 8: WTX-330, a Fully Human IL-12 INDUKINE™ Molecule, Is Selectively Activated by Primary Human Tumor Samples



***(A)** The components of WTX-330, including native human IL-12 (yellow), a half-life extending HSA-specific single-domain antibody (blue), the activity blocking Ab domain (teal), and the protease-cleavable linkers (red). **(B)** In vitro activity of WTX-330 in the HEK-Blue IL-12 reporter assay comparing intact (blue), and protease-activated (cleaved) WTX-330 (red) to rhIL-12 (black). **(C)** In vitro activity of intact (blue) and cleaved (red) WTX-330 in primary human Tblasts compared with rhIL-12 (black). **(D)** WTX-330 was diluted into healthy human serum from n = 6 donors and incubated at 37°C for 24 or 72 hours before WTX-330 processing was measured by capillary western blot analysis. **(E)** WTX-330 was exposed to primary human tumor samples (n = 88) or primary human healthy cells (n = 13) for 48 hours before INDUKINE™ protein cleavage was measured. Processing activity was normalized to a non-cleavable control (0% activity) and a pre-cut control INDUKINE™ molecule (100% activity).*

Conclusions

- mWTX-330 generates potent anti-tumor immunity in syngeneic tumor models of varying immunogenicity in a cleavage dependent manner and generates protective memory against rechallenge.
- The INDUKINE™ design of mWTX-330 results in preferential activation of tumor infiltrating lymphocytes and significantly expands the therapeutic window of this molecule compared to recombinant IL-12.
- mWTX-330 activates various tumor infiltrating effector cells in murine syngeneic tumor models.
- Systemic administration of mWTX-330 results in increased tumor infiltration and drove robust IL-12 signaling in intratumoral CD8+ T cells.
- mWTX-330 treatment metabolically reinvigorates effector cells in the TME, significantly increasing mitochondrial respiration by tumor infiltrating CD8+ T cells as well as NK cells.
- WTX-330, a fully human IL-12 INDUKINE™ molecule, has inducible activity and is preferentially activated in the presence of primary human dissociated tumor samples.