WTX-330 is a Conditionally Activated IL-12 Prodrug that Fundamentally Reprograms Tumor Infiltrating CD8+ T Cells and Drives Tumor Regression

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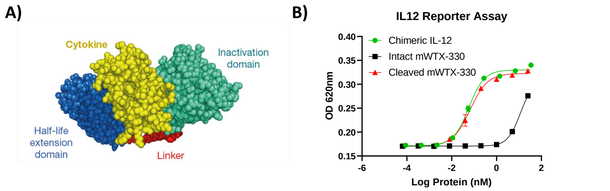
Introduction

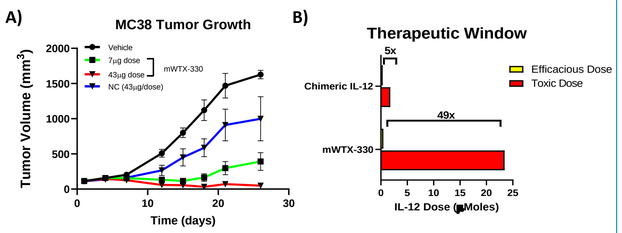
- Systemic therapy with proinflammatory immune modulators is a promising approach for the treatment of cancer
  - The cytokine interleukin-12 (IL-12) is a potent inducer of innate and adaptive anti-tumor immunity, but potentially lethal toxicity associated with systemic administration of IL-12 cytokine treatment has rendered IL-12 treatment strategies impractical
- 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 (Figure 1)
  - The pro-drug is inactive in peripheral tissues due to 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 containing a chimeric IL-12 molecule was designed (mWTX-330)

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



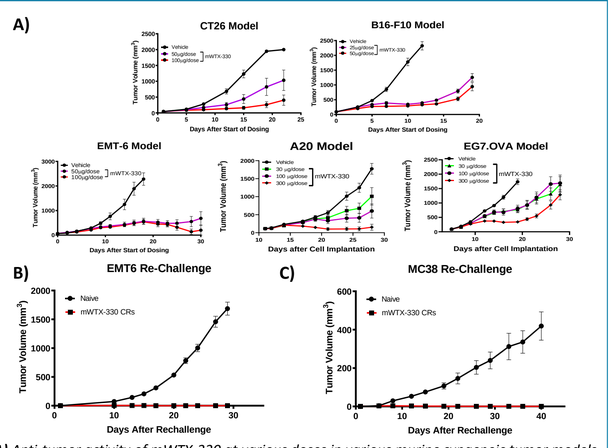
A) Key features of an INDUKINE™ molecule include peripheral blockade (green) of the cytokine-receptor interaction to limit systemic toxicity, half-life extension (blue) for optimal exposure in tumors and a protease cleavable linker that results in conditional release of a native cytokine in the tumor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

Figure 2: Murine WTX-330 is Well Tolerated and Induces Tumor Regression in a Cleavage Dependent Manner



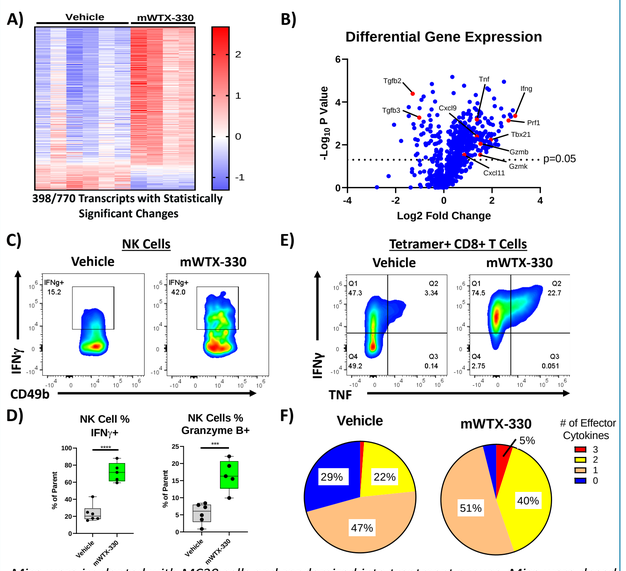
A) Anti-tumor activity of mWTX-330 at various doses in the MC38 mouse model. mWTX-330 was dosed intraperitonially twice a week for two weeks at 7 μg/dose or 43 μg/dose. A non-cleavable (NC) variant of mWTX-330 was also tested at 43 μg/dose. B) Graphic depiction of the calculated therapeutic window for chimeric IL-12 and mWTX-330 on a per molar basis using the same tumor model, based on the identification of the active and toxic dose level for both treatments.

Figure 3: Murine WTX-330 Generates Anti-Tumor Immunity and Protective Memory in Multiple Syngeneic Tumor Models



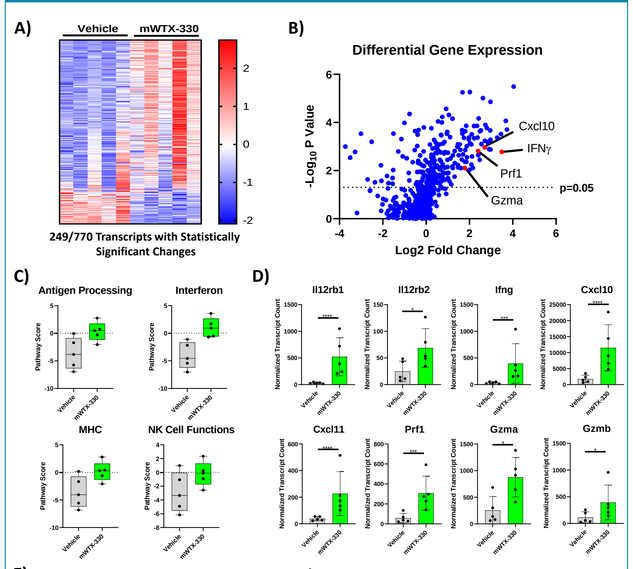
A) Anti-tumor activity of mWTX-330 at various doses in various murine syngeneic tumor models. Mice were dosed twice a week with the dose noted on the figure legends for a total of two weeks. Naïve mice or mice that rejected primary B) EMT6 or C) MC38 tumors (CRs) were re-challenged with the same tumor on the opposite flank and tumor volume was measured over time.

Figure 4: Murine WTX-330 Activates MC38 Tumor Infiltrating NK Cells and CD8+ T Cells



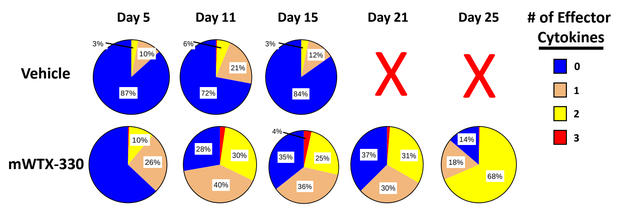
Mice were implanted with MC38 cells and randomized into treatment groups. Mice were dosed on Day 1 and Day 4, and tumors were harvested on Day 5. A) Heatmap of transcripts with statistically significant differences in expression between the two treatments derived from Nanostring analysis of bulk RNA from tumor samples. Transcripts were excluded from the heat map if they had average normalized counts below 50. Each lane represents an individual animal. B) Volcano plot of transcripts differentially expressed between mWTX-330 and vehicle-treated mice. C,D) The frequency of tumor infiltrating NK cells producing IFNγ or Granzyme B. E) The frequency of tetramer positive CD8+ T cells producing IFNγ and/or TNF. F) The frequency of polyfunctional tetramer positive CD8+ T cells was measured by examining co-expression of IFNγ, TNF, and Granzyme B

Figure 5: Murine WTX-330 Activates B16-F10 Tumor Infiltrating NK Cells and CD8+ T Cells



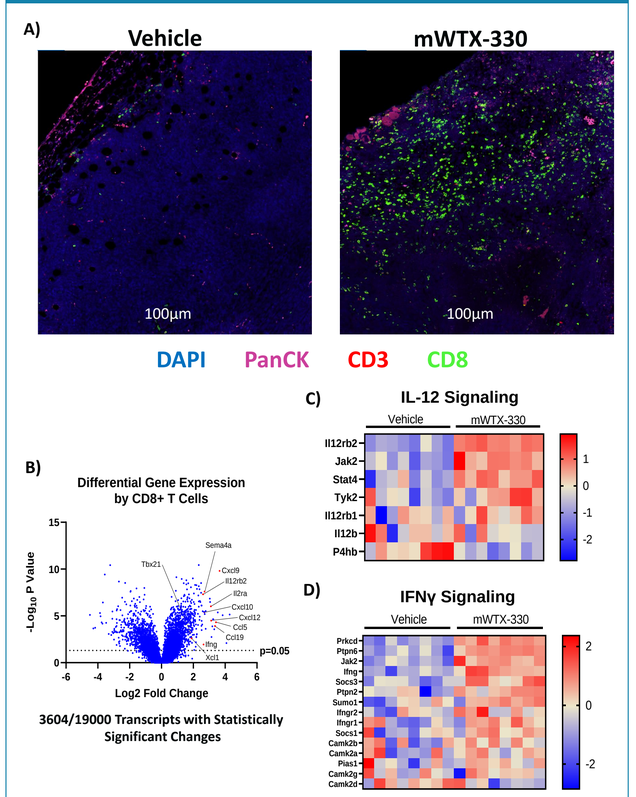
Mice were implanted with B16-F10 cells and randomized into treatment groups. Mice were dosed on Day 1 and Day 4, and tumors were harvested on Day 5. A) Heatmap of transcripts with statistically significant differences in expression between the two treatments derived from Nanostring analysis of bulk RNA from tumor samples. Transcripts were excluded from the heat map if they had average normalized counts below 50. Each lane represents an individual animal. B) Volcano plot of transcripts differentially expressed between mWTX-330 and vehicle-treated mice. C) Pathway scoring and D) normalized counts from individual transcripts. E) The frequency of tetramer+ CD8+ T cells producing IFNγ and/or Granzyme B. F) The frequency of polyfunctional tetramer positive CD8+ T cells was measured by examining co-expression of IFNγ, TNF, and Granzyme B by flow cytometry.

Figure 6: Murine WTX-330 Induces a Sustained Polyfunctional CD8+ T Cell Response



Mice were implanted with EMT6 cells and randomized into treatment groups. Mice were dosed twice weekly for two weeks, and tumors were harvested at the indicated timepoints. The frequency of polyfunctional tumor infiltrating CD8+ T cells was measured by examining co-expression of IFNγ, TNF, and Granzyme B by flow cytometry.

Figure 7: Systemic Administration of Murine WTX-330 Results In IL-12 and IFNγ Signaling by Tumor Infiltrating CD8+ T Cells



Mice were implanted with EMT6 cells and randomized into treatment groups. Mice were dosed twice weekly for two weeks, and tumors were harvested on Day 11. Nanostring GeoMX analysis was performed on FFPE tumor tissues. A) Immunofluorescence images of tumor infiltrating CD8+ T Cells. B) Differential gene expression analysis of tumor infiltrating CD8+ T Cells. Heatmap analysis of genes associated with C) IL-12 and D) IFNγ signaling by tumor infiltrating CD8+ T cells.

Conclusions

- The INDUKINE™ design of mWTX-330 simultaneously decreases the amount of drug required for anti-tumor activity while increasing the maximum tolerated dose, resulting in a significant expansion of the therapeutic window compared to recombinant IL-12
- mWTX-330 generates potent anti-tumor immunity in syngeneic tumor models of varying immunogenicity in a cleavage dependent manner.
- mWTX-330 induced tumor regression generates protective immunity against future rechallenge.
- mWTX-330 fundamentally shifts the transcriptional profile within the tumor and activates tumor infiltrating cytolytic effector cells in the MC38, B16-F10, and EMT6 tumor models.
- Systemic administration of mWTX-330 results in increased tumor infiltration as well as significantly increased IL-12 and IFNγ signaling by intratumoral CD8+ T cells in the EMT6 syngeneic tumor model.