Werewolf THERAPEUTICS

Shifting the Balance In Cytokine Therapeutics

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Development of WTX-712, a Conditionally Active IL-21 INDUKINE™ Molecule for the Treatment of Cancer

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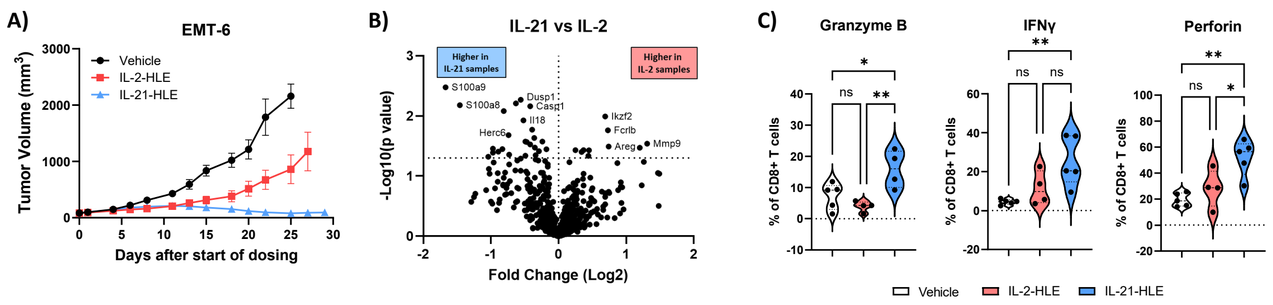
BACKGROUND

Development of an IL-21 Prodrug for Cancer Immunotherapy

In the past decade, great strides have been made in the development of novel immunotherapies, such as immune checkpoint inhibitors (ICI) to treat cancer. However, a large percentage of patients do not respond to ICI or acquire resistance after initial response, highlighting an unmet need for alternative immunotherapies. As potent immunomodulators, cytokines have been explored as treatments for cancer, but their use has been limited due to toxicity and poor pharmacokinetics (PK). One of these key cytokines, interleukin 21 (IL-21), is a pluripotent cytokine that activates anti-tumor T cell responses, induces B cell activation, and promotes generation and maintenance of germinal centers and tertiary lymphoid structures. IL-21 acts on a broader range of cells than IL-2 and does not induce vascular leak syndrome. Clinical activity of IL-21 has been hampered by poor PK and adverse events at dose levels associated with signs of efficacy. Werewolf Therapeutics has developed an IL-21 INDUKINE™ molecule, named WTX-712, which contains wildtype human IL-21, an inactivation domain, and a half-life extension domain tethered together by tumor selective protease cleavable linkers. In preclinical studies, WTX-712 has been shown to be peripherally inactive, releasing IL-21 selectively within the tumor.

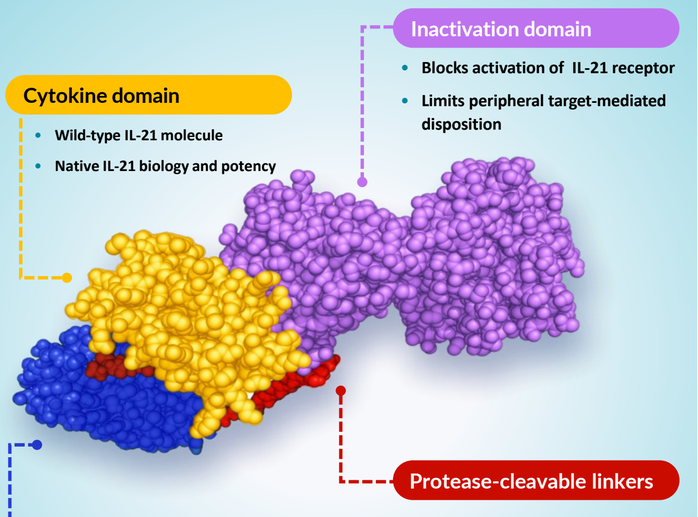
IL-2 and IL-21 Cytokines Trigger Different Responses in Tumor Models

Differential Efficacy of IL-2 and IL-21 in Anti-PD-1 Resistant Syngeneic Tumor Model



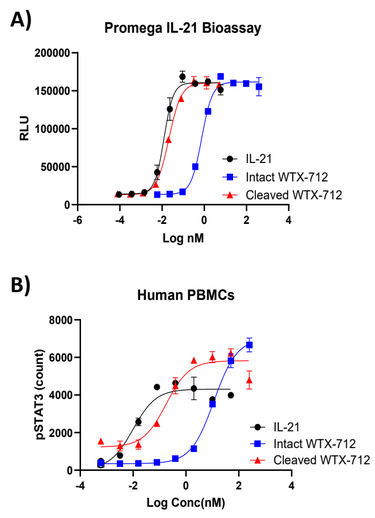
(A) EMT-6 tumor bearing mice were randomized into treatment groups, dosed twice a week for two weeks with vehicle (black), IL-2-HLE (red) or IL-21-HLE (blue). Tumor volumes were measured twice a week. (B) Nanostring analysis of RNA from EMT-6 tumors after dosing with IL-2-HLE or IL-21-HLE. Differentially expressed genes between IL-2-HLE and IL-21-HLE samples graphed as volcano plot comparing fold change and p value. C) Flow cytometry analysis of CD8 T cell effector proteins expression at day 12 after dosing initiation.

Key Features of INDUKINE Molecules



WTX-712 is an Inducible IL-21 Pro-Drug

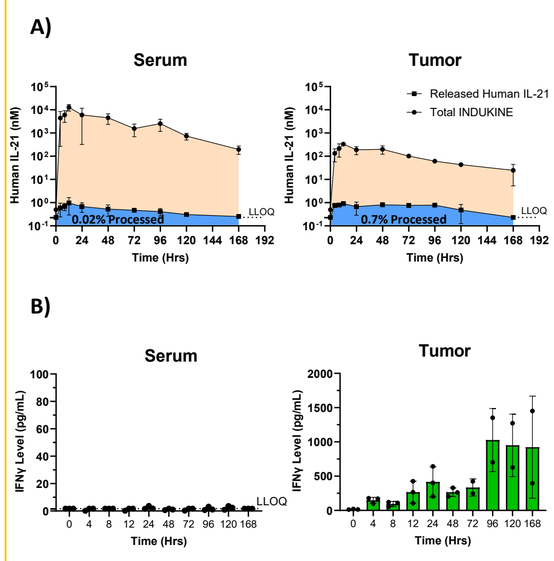
In Vitro Data



(A) In vitro activity of WTX-712 in the IL-21 Promega reporter assay or (B) measurement of pSTAT3 in human PBMCs after incubation comparing intact WTX-712 (blue squares) and in vitro cleaved/activated WTX-712 (red triangles) to recombinant IL-21 (black circles).

Favorable WTX-712 Exposure

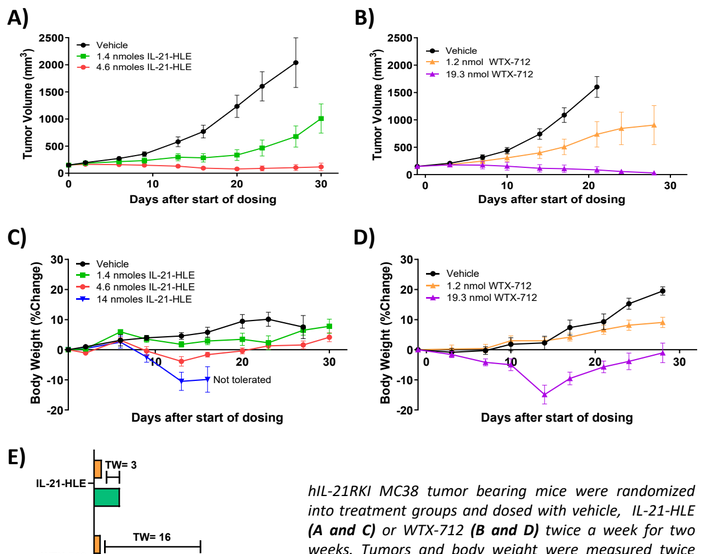
WTX-712 is Preferentially Processed in the Tumor Microenvironment



hIL-21RKI MC38 tumor bearing mice were randomized into treatment groups and dosed once with WTX-712. Mice were sacrificed at indicated timepoints, and serum and tumors were collected. (A) Total WTX-712 and free IL-21 exposure (B) IFNγ generation in serum and tumor after single dose of WTX-712.

Masking and Inducibility Improves Tolerability

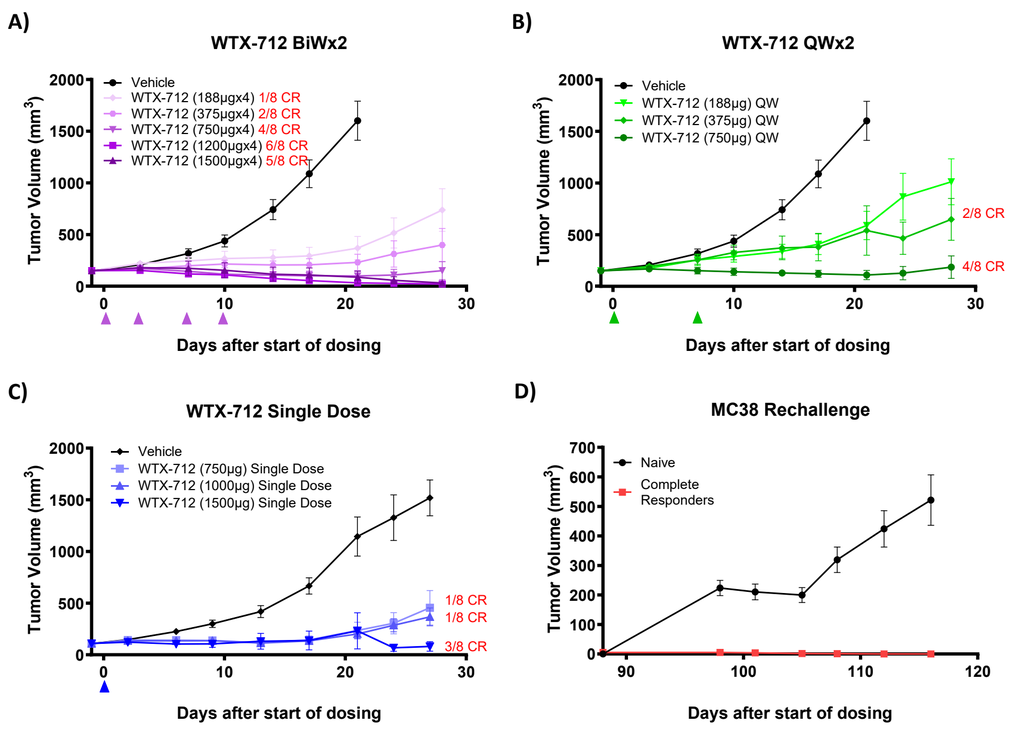
WTX-712 Improves the Therapeutic Window of IL-21



hIL-21RKI MC38 tumor bearing mice were randomized into treatment groups and dosed with vehicle, IL-21-HLE (A and C) or WTX-712 (B and D) twice a week for two weeks. Tumors and body weight were measured twice weekly. (E) Representation of the Therapeutic Window (TW) of IL-21-HLE or WTX-712 in MC38 tumor-bearing mice based on the minimum efficacious dose (MED) and maximum tolerated dose (MTD) of each molecule. Therapeutic Window calculated by dividing MTD by MED.

Dose Dependent Efficacy Leading to Regressions and Immunological Memory

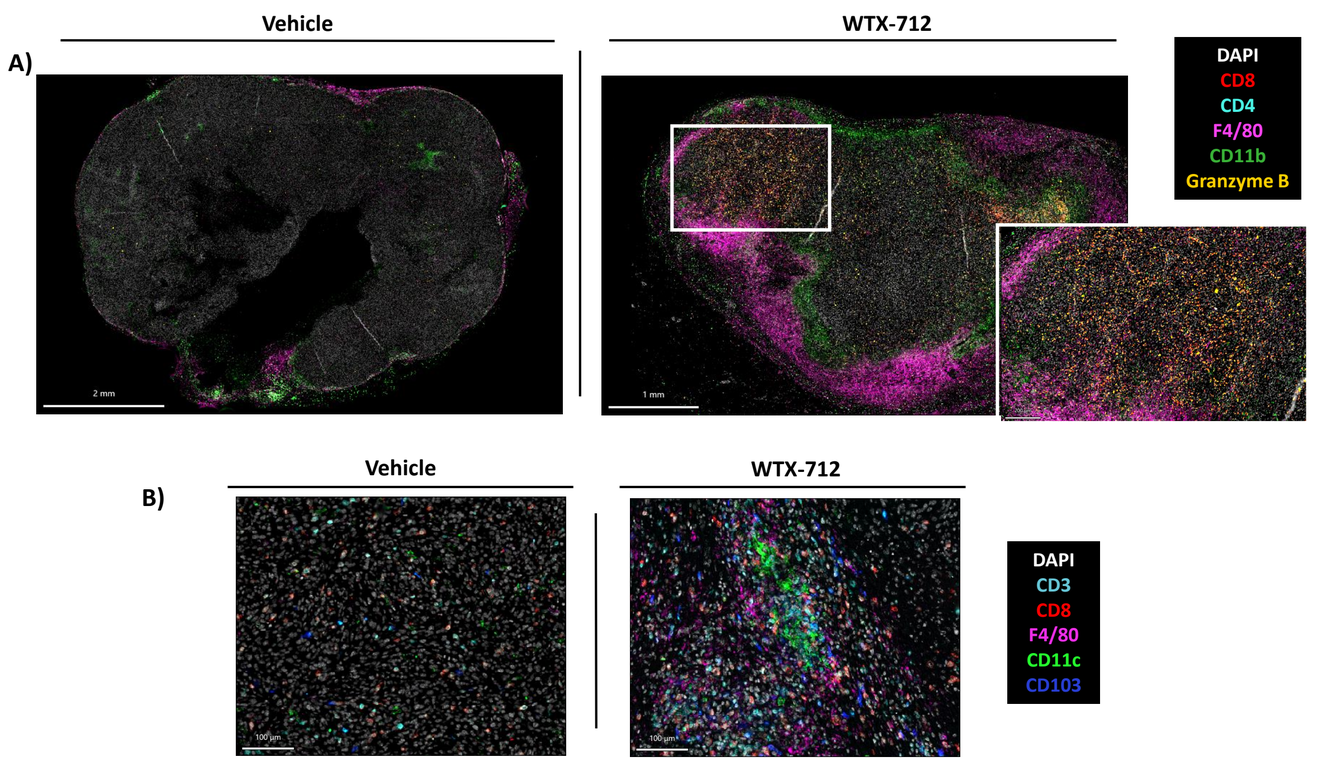
WTX-712 is Efficacious at Several Dosing Schemes and Protects Against Rechallenge



hIL-21RKI MC38 tumor bearing mice were randomized into treatment groups and dosed with WTX-712 either twice weekly for two weeks (A), once weekly for two weeks (B), or one single dose (C). Tumors and body weight were measured twice weekly. (D) WTX-712 treated mice with complete regressions (CRs) were rechallenged with MC38, naïve mice were used as control.

Spatial Profiling Demonstrates Deep Intratumoral Immune Infiltration

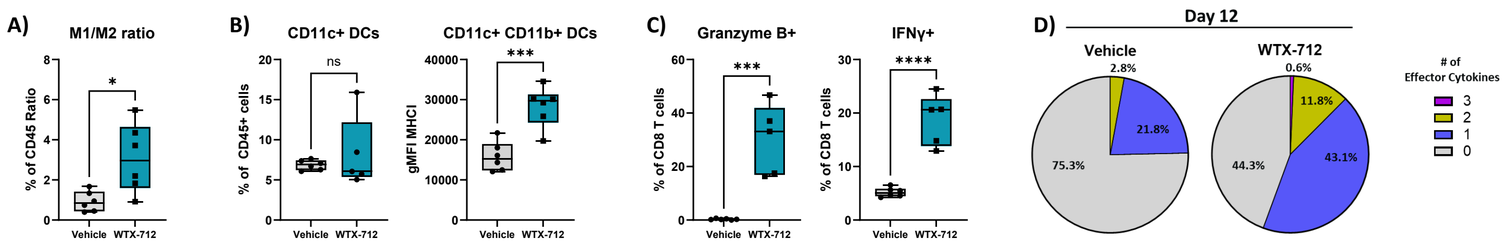
WTX-712 Treatment Results in Enhanced Effector Functions and Presence of Immune Hubs



hIL-21RKI MC38 tumor bearing mice were randomized into treatment groups and dosed with WTX-712 twice weekly for two weeks. Tumor were collected on day 10 after start of dosing and fixed in formalin. Tumors were embedded in paraffin, sectioned, dewaxed and antigen retrieved. Tumors were stained and imaged using a Lunaphore COMET multiplex immunofluorescence platform. A) DAPI, CD8, CD4, F4/80, CD11b, Granzyme B. B) DAPI, CD3, CD8, F4/80, CD11c, CD103.

TIL Profiling Reveals an Anti-Tumor Phenotype

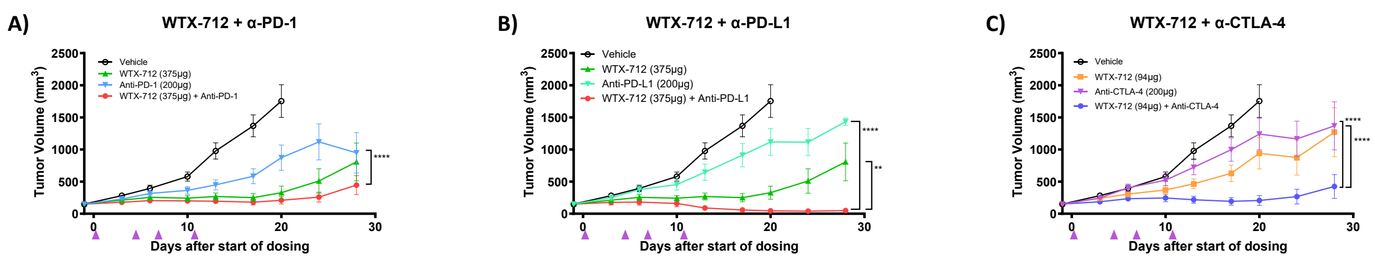
WTX-712 Treatment Results in Altered APC Compartment and Activates CD8+ T cells



*MC38 tumor bearing hIL-21RKI mice were randomized into treatment groups, dosed with WTX-712 twice a week for two weeks, and tumors were harvested for TIL analysis by FACS on day 12. (A) Ratio of M1 to M2 type macrophages in the tumor. (B) Frequency of CD11c+ DCs and mean fluorescent intensity of MCHI expression. (C) Frequencies of cytokine production from CD8+ T cells found in the tumor. Vehicle (grey) and WTX-712 (teal) treated mice (D) Frequency of polyfunctional CD8+ T cells in the tumor based on co-expression of IFNγ, TNF, and Granzyme B. p values represent the results of a students T test at individual timepoints: * = p<0.05, ***= p<0.005, ****= p<0.0001*

WTX-712 Preclinical Combination Data with ICI Demonstrates Improved Anti-Tumor Efficacy

Improved Efficacy of WTX-712 in Combination with Immune Checkpoint Inhibitors



hIL-21RKI MC38 tumor bearing mice were randomized into treatment groups and dosed twice weekly for two weeks. Mice were dosed with WTX-712 alone or in combination with (A) anti-PD-1, (B) anti-PDL-1 or (C) anti-CTLA-4. Tumors and body weight were measured twice weekly.

SUMMARY and CONCLUSIONS

- WTX-712 is a novel INDUKINE molecule engineered to enhance the therapeutic window of IL-21
- Anti-tumor efficacy driven by IL-21 differs from that of other potent pro-inflammatory cytokines such as IL-2
- WTX-712 demonstrates *in vitro* inducibility and activity
- WTX-712 is inactive in the periphery and shows selective release of free IL-21 in the TME linked to IFNγ release
- WTX-712 is efficacious at various dosing regimens and provides protection again tumor rechallenge
- WTX-712 treatment drives CD8+ T cell polyfunctionality, induces a favorable M1/M2 macrophage ratio, and promotes immune cell interactions.
- WTX-712 demonstrates enhanced efficacy when combined with ICI antibodies blocking the PD-1 /PD-L1 or CTLA-4 pathways
- Together these data support continued exploration of WTX-712, an IL-21 INDUKINE molecule, as a therapy for cancer

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