**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 (ICIs) to treat cancer. However, a large percentage of patients do not respond to ICIs 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 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 wild-type 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.

**Key Features of INDUKINE Molecules**

**Cytokine domain**
- Unique IL-21 sequence
- N-terminal GCFF cleavable protease

**Protease-dosed linkers**
- Stable protease suicide substrate
- Efficient cleavage in tumors and tumor microenvironment

**HALT® domain**
- Enables high tumor exposure while eliminating the drug from plasma
- Released IL-12 a short half-life for rapid clearance

**Dose Dependent Efficacy Leading to Regressions and Immunological Memory**

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

**Spatial Profiling Demonstrates Deep Intratumoral Immune Infiltration**

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

**SUMMARY and CONCLUSIONS**

- **WTX-712** is a novel INDUKINE™ molecule engineered to enhance the therapeutic window of IL-21.
- **Anti-tumor efficacy drivers** by IL-21 differ from that of other potent pro-inflammatory cytokines such as IL-4, IL-15, or IL-23.
- **WTX-712** demonstrates in vitro activity and scalability.
- **WTX-712** is inactive in preclinical screens but shows selective release of free IL-21 in the TMD linked to PK release.
- **WTX-712** is efficacious in various disease settings and provides protection against tumor rechallenge.
- **WTX-712** treatment drives CD8 T cell polyfunctionalities, induces a favorable M1/M2 macrophage ratio, and promotes immune cell interactions.
- **WTX-712** demonstrates enhanced efficacy when combined with ICIs, 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.

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

**Favorable WTX-712 Exposure**

**WTX-712 is Preferentially Processed in the Tumor Microenvironment**

**Masking and Inducibility Improves Tolerability**

**WTX-712 Improves the Therapeutic Window of IL-21**

**TIL Profiling Reveals an Anti-Tumor Phenotype**

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

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

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

**Confers high tumor exposure while eliminating drug from plasma, IL-21**