Background 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, despite these improvements, subsets 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 have 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 native human IL-21, an inactivation domain, and a half-life extension domain tethered together by protease sensitive linkers. WTX-712 has been shown in preclinical studies to be peripherally inactive, but due to dysregulation of the protease milieu in the tumor microenvironment, upon dosing and dissemination to the tumor, the linkers are cleaved, and IL-21 is released selectively within the tumor.

Methods Human IL-21 receptor knock-in (hIL-21R KI) mice bearing syngeneic tumors were treated with WTX-712 or half-life extended human IL-21, and tumor growth and body weight were monitored over time. In some experiments, tissues were harvested at different timepoints and analyzed by various techniques, including flow cytometry, tissue PK and high-plex immunofluorescence.

Results Our data demonstrate that WTX-712 showed in vitro and in vivo inducible activity. In mouse syngeneic tumor models, WTX-712 was efficacious with an expanded therapeutic window compared to half-life extended IL-21. Mice bearing MC38 tumors dosed with WTX-712 twice-weekly results in complete regressions and upon rechallenge showed complete protection against tumor growth. Anti-tumor efficacy was linked to expansion and activation of tumor infiltrating T cells and NKT cells, increased polyfunctionality in CD8+ T cells and alterations in the antigen presenting cell compartment. Additionally, WTX-712 demonstrated improved efficacy in combination with ICI in the MC38 tumor model. Utilizing multiplex immunofluorescence, we observed altered tumor architecture and activated immune infiltrates in tumors of WTX-712 treated mice compared to vehicle.

Conclusions Together, these data support continued exploration of WTX-712, an IL-21 INDUKINE™ molecule, as a therapy for cancer.

Ethics Approval All mouse in vivo work was performed in accordance with current regulations and standards of the U.S. Department of Health and Human Services, Public Health Service (PHS) and the NIH Office of Laboratory Animal Welfare (OLAW). All animal studies were conducted at Charles River Laboratories (Explora Biolabs Watertown, MA) or Biocytogen Boston Corporation (Wakefield, MA) with approval of the each institute’s Institutional Animal Care and Use Committee (IACUC).