Background Cytokine therapy could become a pillar of cancer immunotherapy given its potential to activate the immune system and promote antitumor activity. However, many cytokine therapies are limited in their antitumor effectiveness in patients due to dose limiting toxicities. WTX-124 is a conditionally activated prodrug that is designed to take advantage of the dysregulated protease milieu in the TME to deliver native IL-2 in a targeted fashion to tumor tissues. WTX-124 is stable in peripheral tissues where it remains in an inactivated state. Recent approaches to improve upon IL-2 therapy have focused on developing attenuated forms of the cytokine (non-alpha formats) that are unable to bind to the high affinity IL-2 receptor, aiming to reduce its off-target toxicity as well as minimizing potential Treg suppressive effects. However, non-alpha forms of IL-2 may also have reduced ability to activate tumor specific T effector cells that are important for achieving an optimal antitumor immune response. To test this, we created a version of WTX-124 containing a non-alpha IL-2 moiety and characterized their in vitro and in vivo comparative responses.

Methods Mice bearing MC38 or CT26 syngeneic tumors were treated with vehicle, WTX-124 (a wild type IL-2 containing INDUKINE molecule), or a non-alpha IL-2 version of the INDUKINETM molecule, and tumor growth and body weight were monitored over time. Tumor tissues were harvested at various timepoints for analysis by flow cytometry and highplex immunofluorescence to assess and characterize the mechanisms associated with response.

Results Systemic administration of WTX-124 but not the non-alpha IL-2 INDUKINETM molecule resulted in robust antitumor immunity in syngeneic tumor models, and preferentially activated tumor-infiltrating immune cells versus peripheral cells. Only WTX-124 activated tumor specific CD8+ T cells, increasing their polyfunctionality. Additionally, only WTX-124 stimulated natural killer (NK) cells and skewed conventional CD4+ T cells toward a T helper 1 (TH1) phenotype. Importantly, WTX-124 treatment induced regulatory T cells (Treg) fragility, driving these CD4+ FoxP3+ T cells to produce effector cytokines like IFNγ and TNF.

Conclusions Our data demonstrates that the full activity of IL-2 contained in WTX-124 is necessary to activate a potent antitumor response in mouse syngeneic tumor models. This data corroborates recent publications demonstrating that IL-2 non-alpha molecules fail to take full advantage of the positive antitumor biology generated by IL-2. WTX-124 is currently in Phase I clinical testing (NCT05678998).

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 Worcester, MA) with approval of the Explora Biolabs and the Charles River Laboratories Institutional Animal Care and Use Committees (IACUC).