**Background** Cytokines are powerful modulators of the immune system, making them a promising target for novel cancer immunotherapies. IL-12 is a pleotropic cytokine that acts on various immune cell populations, including professional APCs as well as adaptive and innate effector cells. Furthermore, recombinant IL-12 generates robust anti-tumor activity in murine models. However, its clinical use has been impeded by a poor pharmacokinetic profile and severe, sometimes fatal, adverse events following systemic administration. mWTX-330 is a selectively inducible IL-12 containing pro-drug (INDUKINE™ molecule) that is comprised of chimeric IL-12 (human p40, mouse p35) linked to a half-life extension domain and an inactivation domain by protease cleavable linkers. Following systemic administration, the inactivation domain keeps the molecule inactive in the periphery, while selective processing of the linkers in the tumor microenvironment results in the local release of chimeric IL-12.

**Methods** Mice bearing syngeneic tumors were treated with mWTX-330, and tumor growth and body weight were monitored over time. In some experiments tissues were harvested at various timepoints, and analyzed by flow cytometry, NanoString analysis, or other downstream techniques.

**Results** mWTX-330 generated robust anti-tumor immunity in multiple syngeneic tumor models, including MC38, CT26, B16-F10, and EMT-6. Antibody based depletion of the CD8+ T cells resulted in a loss of late tumor control, while depletion of CD8+ T cells, CD4+ T cells, and NK cells resulted in a complete loss of tumor control following mWTX-330 treatment. Furthermore, mWTX-330 treated mice that rejected primary tumors were protected against later rechallenge with the same tumor cell line. Systemic mWTX-330 treatment selectively activated tumor infiltrating immune cells, with little evidence of immune cell activation in the peripheral blood or secondary lymphoid tissues. Likewise, mWTX-330 was better tolerated than chimeric IL-12, while maintaining efficacy at a similar dose, resulting in a significant expansion of the therapeutic window due to the INDUKINE design of this molecule. Additionally, mWTX-330 treatment increased the frequency of polyfunctional CD8+ T cells, skewed CD4+ non-Tregs towards a TH1 phenotype, robustly drove NK cell production of effector cytokines within the tumor and triggered a metabolic re-invigoration of effector cells.

**Conclusions** These data highlight the preclinical potency and selective inducibility of mWTX-330 in various murine syngeneic tumor models. More importantly, they demonstrate the feasibility of the INDUKINE approach to address the clinical roadblocks seen with IL-12 therapy and support the ongoing clinical development of our IL-12 INDUKINE molecule containing a fully human payload, WTX-330.