SPATIAL ANALYSIS OF TUMOR INFILTRATING LYMPHOCYTE POPULATIONS IN SYNGENEIC MOUSE TUMOR MODELS AFTER TREATMENT WITH IL-12 (MWTX-330) AND IL-2 (WTX-124) INDUKINE™ MOLECULES


Background Cytokines are powerful modulators of the immune system, making them a promising class of targets 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, making it an attractive molecule for cancer immunotherapy. Likewise, IL-2 is a potent activator of NK and T cell proliferation and effector function that has been approved for clinical use in melanoma and renal cell carcinoma. However, the wider use of these cytokines in the clinic has been impeded by their poor pharmacokinetic properties and the serious adverse events associated with their systemic administration. To address these shortcomings, Werewolf Therapeutics has designed selectively inducible cytokines (INDUKINE™ molecules), consisting of a cytokine payload linked to a half-life extension domain and an inactivation domain by a protease cleavable linker. After systemic dosing, these molecules have been shown in preclinical models to remain inactive in peripheral tissues whereas the linkers are selectively cleaved in the tumor microenvironment to deliver potent native cytokines within.

Methods Mice bearing syngeneic tumors were treated with either mWTX-330 (a chimeric IL-12 containing INDUKINE™ molecule) or WTX-124 (a human IL-2 containing INDUKINE™ molecule), and tumor growth was monitored over time. Tissues were harvested at various timepoints and analyzed by various techniques, including high-plex immunofluorescence.

Results In the EMT6 model, treatment with mWTX-330 resulted in a substantial increase in density of tumor infiltrating dendritic cells, widespread deployment of Granzyme B, and an increase in the CD8/Treg ratio throughout the tumor tissue. Tumor infiltrating CD8 T cells from mice treated with mWTX-330 expressed lower level of exhaustion markers throughout the entire tumor tissue, demonstrating the widespread effects of INDUKINE™ treatment despite heterogeneity in the tumor microenvironment. Interestingly, PD-L1 was upregulated in tumor cells by mWTX-330 treatment, but this increased expression colocalized with areas of intense infiltration by immune cells. Likewise, INDUKINE™ treatment of CT26 tumor bearing mice resulted in substantial quantitative and qualitative changes to the tumor infiltrating immune cell populations, and widespread activation of effector cells. This phenomenon was further amplified by combination treatment with blockers of the PD-1 pathway, highlighting the potential for INDUKINE™ treatments to amplify the effects of checkpoint inhibitors.

Conclusions Treatment of murine tumor bearing mice with either mWTX-330 or WTX-124 resulted in significant remodeling of immune cell populations found within the tumor tissue, simultaneously increasing immune cell infiltration, and generating a potent activation of effector cells.

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).

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