252 THE COMBINATION OF ACT AND INDUKINE THERAPY LEADS TO IMPROVED ANTITUMOR IMMUNITY IN SOLID TUMORS

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Background Adoptive cell therapy (ACT) expands tumor-specific T cells obtained from tumor biopsies or genetically engineered peripheral T cells to treat a patient's malignancy. Even though there has been clinical success with chimeric antigen receptor (CAR) T cells in hematological malignancies, limited success has been demonstrated with ACT in solid tumors. One of the main limitations of ACT is the high cell number required to treat patients. The cells require repeated *in vitro* restimulation and long-term expansion leading to an exhausted, terminally differentiated phenotype, which results in a short-lived antitumor response. This has warranted the need for novel strategies to improve engraftment and functionality of cellular therapies against solid tumors.

Methods We have designed IL-2 and IL-12 INDUKINE^{TM} polypeptides [cytokine pro-drugs] to be masked from peripheral activation, via a blocking domain. They are designed to utilize the unique protease profile of tumors to deliver active cytokine specifically to the tumor microenvironment. In addition to a blocking domain, our INDUKINE molecules are engineered with a half-life extension domain to improve exposure with less frequent dosing. In the following studies, we tested whether our INDUKINE molecules could improve the engraftment and antitumor efficacy of ACT products. Our studies used the pmel-1 transgenic mouse model in which $CD8^+$ T cells express a T cell receptor specific for gp100 expressed on melanocytes and melanoma and the human CD19 CAR-T cell model against subcutaneous Raji tumors. Efficacy and tumor profiling using Flow Cytometry and multi IF will be presented.

Results Upon transfer of tumor-specific T cells into tumor bearing mice, mice were administered with the INDUKINE molecule or vehicle biweekly for 1.5 weeks. The combination of pmel-1 ACT and INDUKINE polypeptides enhanced antitumor activity and animal survival compared to either pmel-1 or INDUKINE treatment alone. Antitumor activity was positively correlated with increased donor cell engraftment and long-term persistence of effector memory T cells in the periphery and the tumor microenvironment. The combination of CD19 CAR-T cells and INDUKINE polypeptides showed improved antitumor responses against Raji tumors compared to CAR-T cells alone. INDUKINE polypeptide administration enhanced engraftment and persistence of CAR-T cells. The IL-2 INDUKINE molecule preferentially expanded $CD4^+$ CAR-T cells whereas the IL-12 INDUKINE molecule expanded $CD8^+$ CAR-T cells.

Conclusions Current clinical ACT practices can result in short-lived immunity due to heavily differentiated and exhausted cell products in solid tumors. Administration of INDUKINE proteins with ACT could reinvigorate donor cell function leading to improved engraftment and long-term clinical responses.

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) with approval of the Explora BioLabs Institutional Animal Care and Use Committee (IACUC).

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