SUPERKINE IL-2 AND IL-33 ARMORED CAR T CELLS RESHAPE THE TUMOR MICROENVIRONMENT TO UNIVERSALLY REDUCE SOLID TUMORS

Shannon Ferry, Rachel Brog, Courtney Shiebout, Cameron Messier, W Cook, Charles Sentman, H Frost, Yina Huang*. Dartmouth College, Lebanon, NH, USA

**Background** CAR T cell efficacy against solid tumors is challenged by key obstacles present within the tumor microenvironment (TME). These include tumor-intrinsic expression of inhibitory ligands that induce T cell exhaustion, the heterogeneous expression of tumor antigens that contribute to immune evasion, the absence of essential nutrients required for T cell survival, and the presence of tumor associated immunosuppressive cells.

**Methods** To increase CAR T cell resistance to immunosuppression and broaden tumor recognition, we armored CAR T cells with a combination of the IL-2 superkine (Super2) and the alarmin IL-33. Super2+33 armored CAR T cells were transferred to mice with established primary or metastatic B16F10 melanoma or intradermal MC38 colon cell carcinoma without preconditioning regimens. Tumor growth, overall survival, and changes to tumor infiltrating leukocytes were assessed.

**Results** We show that a single dose of CAR T cells armored with Super2 and IL-33 promoted the control of solid tumors in immune competent mice without lymphodepletion or preconditioning regimens. Super2 and IL-33 synergized to expand adoptively transferred CAR T cells and shifted leukocyte proportions in the TME by recruiting and activating a broad repertoire of endogenous innate and adaptive immune cells. Tumors treated with Super2 and IL33 CAR T cells had significantly increased infiltration of endogenous CD8 T cells, including an effector subset with low expression levels of PD-1 and TIM-3. Super2 and IL33 synergy also resulted in reduced proportion of regulatory T cells and a phenotypic switch from M2-like to M1-like macrophages that expressed increased MHC class II. However, depletion of endogenous CD8 T cells or NK cells did not disrupt tumor control, suggesting that broad immune activation compensates for loss of individual subsets. Additionally, IFNg, perforin, and CAR expression by transferred T cells were dispensable for the observed therapeutic effect of Super2 and IL33 expressing T cells, underscoring the contribution of endogenous immune cells in mediating tumor control.

**Conclusions** Super2 and IL-33 CAR T cells promoted antitumor immunity in multiple solid tumor models and was impervious to antigen loss, highlighting its potential as a universal CAR T cell platform for treatment of solid tumors. CAR T cells harnessing Super2 and IL33 synergy represent a novel strategy for improving the efficacy of CAR T cells in solid tumors by promoting the activation of endogenous immune cells.