ENHANCING ANTI-CANCER ACTIVITY OF THERAPEUTIC T-CELLS WITH A SYNAPSE-STABILIZING RECEPTOR

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Background CAR T-cells show remarkable efficacy against B-cell malignancies with uniformly high expression of target antigens. In contrast, tumors with low/heterogenous antigen expression often evade CAR-T cytotoxicity and necessitate multi-antigen targeting which often broadens off-tumor toxicity. We hypothesized cytotoxicity of CAR T-cells targeting low/heterogeneous antigens can be selectively augmented with an additional non-cytotoxic receptor specific to another abundantly expressed tumor antigen. This synapse-stabilizing receptor (SSR) would facilitate formation of a cytotoxic immune synapse and CAR-mediated killing of tumor cells without directly eliciting T-cell degranulation therefore limiting additional toxicity against healthy tissues.

Methods To model suboptimal antigen targeting, we used CLL1-specific CAR and survivin (Sur)-specific TCR which produce limited cytotoxicity against acute myeloid leukemia (AML) cells with low/intermediate antigen expression. Using gammaretroviral transduction, we armed these T-cells with an SSR targeting CD38, an antigen highly expressed in leukemia but also in critical hematopoietic progenitors. SSR-armed CAR- and TCR-T cells have been evaluated in preclinical models of human AML and in coculture assays with normal CD38+ mature and progenitor cells.

Results SSR arming significantly increased cytotoxicity of CLL1.CAR T-cells against antigen-low AML line MOLM-13. Integrating into the SSR a modified signaling endodomain of LAT (modLAT177) further enhanced secretion of IFN-γ, IL2 and TNF-α as well as T-cell degranulation (measured by CD107a/granzymeB staining) in both CD4+ (P=0.0066), and CD8+ T-cells (P=0.0189). Similar results were observed with SurTCR where SSR arming enabled killing THP-1 AML cells that were otherwise resistant to Sur.TCR cytotoxicity. SSR expression effectively masked CD38 on T-cell surface and protected them from daratumumab (CD38 mAb) in vitro thus enabling combinatorial immunotherapeutic approaches. In vivo, SSR-armed CLL-1.CAR T-cells mounted greater systemic expansion and produced a >30-fold reduction of circulating leukemic blasts in a mouse xenograft model of AML significantly extending animal survival compared to a group receiving unarmed CLL-1 CART (P=0.0039). Because SSR design precluded heterodimerization with the CAR, we observed no CD38-directed toxicity of SSR-armed CLL-1.CAR T-cells against normal CLL-1-negative CD38-positive mature (NK-cells) and hematopoietic progenitor cells in cord-blood CFU assays.

Conclusions Arming engineered T-cells with a non-cytotoxic SSR with integrated modLAT177 signaling augmented their selective tumor cytotoxicity and expansion without broadening off-tumor cytotoxicity against normal tissues. This approach can be utilized to enhance immunotherapy against tumors with suboptimal levels of antigen expression while preserving specificity of targeting through a quasi-Boolean ‘AND’ gate.

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