

IL-2 VARIANT IMPROVES CAR-T FUNCTIONALITY AND EFFICACY AGAINST SOLID TUMORS

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Background Solid tumors remain a challenge for chimeric antigen receptor T (CAR-T) cell therapy due to lack of tumor-specific antigens, often-hard-to-penetrate tumor structure, and hostile tumor microenvironment (TME) for T-cell activation and survival. Interleukin (IL)-2 is an essential cytokine central to the initiation and maintenance of T-cell-mediated immune responses, which is usually downregulated in TME. IL-2 has been shown to improve the effectiveness of various T-cell-based therapies for solid tumors. However, systemic administration of IL-2 has been shown to elicit immunosuppression through regulatory T cells and cause capillary leak syndrome, both of which limit its use in T-cell immunotherapies, such as CAR-T. To improve the resistance of CAR-T to TME and to enhance its persistence, expansion and efficacy *in vivo*, we designed novel second generation mesothelin (MSLN)-specific CAR constructs (MSLN-CAR-T-IL-2tb) that incorporate secretory form of IL-2 variants (IL-2tb). IL-2tb produced by MSLN-CAR-T-IL-2tb improves cell viability, expansion, and potency, and reduces immunosuppression which could also potentially stimulate endogenous polyclonal tumor-infiltrating lymphocytes in solid tumors.

Methods MSLN-CAR-T-IL-2tb and MSLN-CAR-T cells were generated by lentiviral transduction. To assess *in vitro* proliferation, CAR-T cells were repeatedly cocultured with MSLN-expressing tumor cell lines and CAR⁺ T cells were enumerated. CAR-T cell apoptosis, memory phenotype and exhaustion were monitored by flow cytometry at various time points. MSLN-CAR-T-IL-2tb and MSLN-CAR-T cell resistance to TME was tested *in vitro*. Cytotoxicity was determined using RTCA- or luciferase-based assays. CAR-T tumoricidal activity *in vivo* was evaluated in human cell line-derived xenograft models using severe immunodeficient mice.

Results MSLN-CAR-T-IL-2tb and MSLN-CAR-T demonstrated comparable efficacies in short-term tumor killing assays *in vitro* against multiple tumor cell lines expressing varying levels of MSLN in the presence of exogenous IL-2. However, when cultured in the absence of IL-2, MSLN-CAR-T-IL-2tb was much longer-lasting than MSLN-CAR-T in terms of CAR-T cell viability, proliferation, and persistence. MSLN-CAR-T-IL-2tb was more cytotoxic against multiple MSLN-expressing tumor cells, including MDA-MB-231 and HCC70 (triple-negative breast cancers) and OVCAR-3 (ovarian cancer). Moreover, in multiple xenograft mouse models, MSLN-CAR-T-IL-2tb showed very potent and durable anti-tumor responses.

Conclusions MSLN-CAR-T cells expressing a secretory form of IL-2 variant were able to maintain long-term proliferation and cytotoxicity which could be partly due to the reduced immunosuppression in the TME. Autocrine and paracrine loops of IL-2 can further improve CAR-T functionality in solid tumor and could be a promising strategy for clinical application.

Ethics Approval All animal experiments were conducted in facilities accredited by the service providers' Institutional Review Boards.

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