Background Receptor tyrosine kinase-like Orphan Receptor 1 (ROR1) is widely expressed in various hematologic and solid tumors. A clinical trial employing ROR1-CAR T-cells with scFv-R12 binder showed poor tumor infiltration and dysfunctionality of CAR T-cells in NSCLC & TNBC patients, suggesting that further CAR-binder/construct optimization may be required to improve CAR T-cell persistence and control of T-cell inhibitory factors in the tumor microenvironment.

Methods We designed new ROR1-CAR constructs, either non-boosted (CAR-1), or boosted with TGFbRII Dominant Negative fragment (TGFbRIIDN), or membrane-bound IL-7 (mbIL7), to overcome the TGFb1 inhibitory effect in the tumor microenvironment, and to enhance T-cell persistence, respectively. We also designed ROR1-CAR with R12 binder (CAR-2) as control. The ROR1-CARS were transduced into human primary T-cells using lentiviral vectors. In vitro cytoxicity was assessed by co-culture with hematologic mantle cell lymphoma (MCL, Jeko-1), or solid ovarian (OVCAR-3), pancreatic (CAPAN-2) or lung (NCI-H226) tumor cell lines; TNF-α, IFN-γ and IL-2 cytokine release was assessed by ELISA. To simulate tumor microenvironment, CAR T-cells were challenged with TGFb1 or cultured without exogenous IL-2. In vivo xenograft studies were performed in NSG mice bearing Jeko-1 or OVCAR-3 xenografts; tumor progression was monitored, and CAR T cells expansion in mouse peripheral blood was analyzed by flow cytometry.

Results CAR-1-transduced T-cells showed enhanced activation and cytotoxicity against Jeko-1 MCL as compared to CAR-2 in vitro, and eliminated tumors in the Jeko-1 model in vivo. CAR-1 also mediated potent killing and enhanced cytokine release as compared to CAR-2 upon incubation with OVCAR-3, CAPAN-2, and NCI-H226 solid tumor cell lines in vitro. Surprisingly, in vivo ovarian OVCAR-3 xenograft model, CAR-2 failed to reject tumors, whereas CAR-1 mediated remissions; analysis of CAR T-cells in peripheral blood revealed rapid expansion of CAR’T-cell population and enrichment for the central memory phenotype in CAR-1 as compared to CAR-2. In addition, we successfully boosted CAR-1 with TGFbRIIDN, which attenuated the inhibitory effect of TGFb1 on CAR T-cell cytotoxic activity in vitro. We also equipped CAR-1 with mbIL7, which enhanced cytotoxic activity and mediated extended functionality of the CAR T-cells without exogeneous IL-2 for up to 18 days.

Conclusions The novel fully human ROR1-CAR1 T-cells effectively eliminated both hematologic and solid tumors in vivo, and were superior to ROR1-CAR-2 T cells. Furthermore, the boosting elements TGFbRIIDN and mbIL7 are promising tools to overcome the inhibitory effects of tumor microenvironment and sustain CAR T-cell persistence.

REFERENCE