260 NOVEL CLL-1-DIRECTED CAR-T CELLS MEDIATE POTENT ANTIGEN-SPECIFIC CYTOLYTIC ACTIVITY IN MOUSE MODELS OF ACUTE MYELOID LEUKEMIA

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Background Acute myeloid leukemia (AML) is the most common form of leukemia in adults. However, the clinical outcome for high-risk patients remains poor, highlighting the urgent need for the development of new therapeutic strategies [1]. Chimeric antigen receptor (CAR) T cell therapy holds promise as an immunotherapeutic strategy and targeting C-type lectin-like molecule-1 (CLL-1, CD371) represents an attractive approach, as CLL-1 is highly expressed on AML blasts and leukemic stem cells [1]. Here, we present preclinical data detailing the development and functional characterization of novel CLL-1-directed CAR-T cells to identify top candidates from a panel of 24 CLL-1 binders.

Methods CLL-1-directed binders were identified by phage display technology and evaluated by flow cytometric, ELISA and Octet analyses. Selected binders were used to generate second generation CLL-1-directed CAR constructs with a 4–1BB co-stimulatory domain. CAR constructs were transduced into primary T cells using lentiviral vectors and investigated for antigen-specific cytolysis by flow cytometry-based assays. Co-culture experiments were conducted for 24 and 48 hours with CLL-1-expressing WT and CLL-1 knockout (KO) HL60 target cells. CAR candidates showing robust antigen-dependent activity were further evaluated for potency by bioluminescent-based assays at low effector to target (E:T) ratios, for long-term persistence in repeated stimulation assays, and for avidity measurements in acoustic force microscopy assays. The top CLL-1 CAR candidates were further studied in an *in vivo* murine xenograft model using HL60 AML cells in NSG mice.

Results We completed an in-depth *in vitro* characterization of 24 CLL-1-directed CAR-T cells and identified top candidates that exhibited 1) potent and specific cytotoxicity of CLL1-expressing targets with minimal nonspecific killing of CLL1 KO targets, 2) high levels of antigen-dependent activation, and 3) significant antigen-dependent cytokine secretion. Importantly, top candidates effectively killed CLL1-expressing targets at low E:T ratios, demonstrated superior persistence after repeated stimulation with target cells, and displayed similar high binding avidity. Lead CLL-1 CAR-T cell candidates significantly reduced *in vivo* tumor growth as assessed by IVIS imaging and flow cytometric analyses, which also indicated tumor cell clearance and CAR T cell expansion.

Conclusions Altogether, our preclinical data demonstrate highly efficacious and antigen-specific CLL-1-directed CAR-T cells, with potent *in vitro* and *in vivo* cytolytic activity. These results support further clinical development of the lead CLL-1 CAR candidate either as a stand-alone treatment or in combination with the VOR eHSC platform to eliminate on-target off-tumor toxicity to fully benefit high-risk AML patients in need.

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