FUNCTIONAL VALIDATION OF SINGLE DOMAIN ANTI-BODY-DERIVED CD33 SPECIFIC CAR-T CELLS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA

Brikena Gjeci*, Salar Khan, Julian Scherer, Tirtha Chakraborty. Vor Biopharma, Cambridge, MA, USA

Background Acute myeloid leukemia (AML) is an aggressive bone marrow malignancy with relapse rates >50% in treatment-responsive patients. One promising new class of AML treatment is chimeric antigen receptor (CAR) T cell therapy, which has demonstrated success for certain leukemias, lymphomas, and myelomas. Conventional CAR-T cells are generated using single-chain variable fragments (scFv) derived from two domains (VL and VH) of monoclonal murine antibodies, potentially leading to misfolding and anti-CAR immunogenicity. In contrast, camelid-derived single domain antibodies (sdAbs) have high thermal stability and refolding capacity due to their reduced size. Single domain antibodies can access novel epitopes and exhibit reduced immunogenic potential, suggesting their suitability as binders in CARs. Focusing on CARs targeting the AML-relevant antigen CD33, we here validate novel sdAb-derived CARs (sdCD33CAR) in a semi-automated multi-step process including cell-cell avidity and compare them to traditional scFv-derived CARs.

Methods A phage-display library containing $10^{11}$ vicuña sdAbs was panned against soluble and cell-associated CD33 to identify distinct sdAb binders by ELISA and FACS. Ten binders were selected, expressed as soluble human Fc tagged molecules, and screened for affinity by Octet. Five binders were used for sdCARs with 4-1BB costimulatory domains. Lentiviral vector (LVV) was generated with a liquid handler to express CARs in an IL-2 Reporter System (CAR-IRS cells). CAR-IRS cells enable functional CAR validation by activation-dependent induction of a fluorescent reporter. CAR-IRS activation was assessed by flow cytometry or continuous IncuCyte live cell imaging. CAR-IRS cells were also tested for cell-cell avidity against CD33-expressing target cells using the Z-Movi platform.

Results Octet analysis of sdAbs indicated affinity values ($K_D$ range 2-13 nM) and binding kinetics favorable for CAR development. In the CAR-IRS assay, sdCD33CARs exhibited higher surface expression (up to 3-fold) and normalized activity (up to 70-fold) compared to scFv-based CD33CARs. Fluorescent reporter results were supported by CD69 staining (up to 15-fold increase). CAR-IRS flow cytometry was validated by IncuCyte imaging. Z-Movi analysis showed comparable avidity between the sdAb and scFv CARs, suggesting that sdCD33CARs represent a promising therapeutic approach.

Conclusions We have undertaken extensive in vitro validation of sdCD33CARs in an objective CAR-IRS background. Automated liquid handling allows for high-throughput LVV transduction to generate CAR-IRS cells, which enables reliable, fast, and economical comparisons across CAR constructs. We have identified one sdCD33CAR construct, which out-performs the traditional scFv-derived CD33CARs in our initial in vitro screens. These results provide promising in vivo screening candidates for future CAR-T cell therapies against AML.

REFERENCES