237 EPITOPE ENGINEERED HEMATOPOIETIC STEM CELLS TO ENABLE MULTI-SPECIFICITY CAR-T CELLS FOR ACUTE MYELOID LEUKEMIA

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Background Acute myeloid leukemia (AML) is associated with an unfavorable outcome for >50% of patients. Whereas novel immunotherapies, such as CD19-CAR-T, demonstrated striking efficacy when targeting dispensable antigens (Ag), the same approach cannot be exploited for AML, due to lack of actionable leukemia-restricted Ags. AML targets are shared with progenitors (HSPCs) or mature myeloid cells, leading to on-target/off-tumor toxicity. We reasoned that precise modification of target epitopes in donor HSPCs used in hematopoietic stem cell transplantation (HSCT) would result in loss of recognition by CAR/mAbs, without affecting protein expression and function. Epitope-editing allows targeting genes essential for leukemia survival regardless of expression in HSPC, minimizing the risk of immune-escape.[1]

Methods We selected the cytokine receptors FLT3, CD123 and KIT, found in >85% of AML cases. By library screenings, we identified substitutions in their extracellular-domain that avoid detection by therapeutic Abs. We validated the functionality of mutated receptors (ligand affinity, western-blot, proliferation, RNAseq, phospho-proteomics) and their resistance to on-target killing (mAb-affinity, CAR-T co-culture). We optimized a base-editing protocol to introduce these mutations in CD34+HSPCs. We exploited advanced in vivo models with co-engraftment of healthy HSPCs, patient-derived AML xenografts (PDX) and CAR-T to assess selective elimination of leukemia and protection of healthy hematopoiesis.

Results Epitope variants were resistant to in vitro CAR-T killing and did not induce CAR activation. Electroporation of ABE8e mRNA+sgRNAs into CD34+HSPCs achieved 90%, 85% and 75% editing efficiency on FLT3, KIT and CD123. After xenotransplant into NBSGW mice, epitope-edited HSPC sustained long-term multi-lineage hematopoiesis, similar to AAVS1 controls. Upon treatment with FLT3-CAR-T, we observed sparing of HSPCs, granulo-mono progenitors and B-cell subsets derived from FLT3-edited HSPCs, while treatment with CD123-CAR showed protection of epitope-edited myeloid lineages, compared to AAVS1. Concomitantly, co-engrafted PDXs were eradicated by either FLT3- or CD123-CAR-T. Due to AML intra-tumoral heterogeneity and plasticity, targeting several Ags might be required to eradicate leukemia stem cells. To this end, we optimized high-efficiency multiplex-editing to enable targeting of multiple Ags without overlapping toxicities. We confirmed resistance of dual-FLT3/CD123 epitope-edited HSPCs and the superior efficacy of dual-target CAR-T in mice co-engrafted with a PDX partially resistant to FLT3-targeting alone. Additionally, our approach was able to protect HSPCs from the combination of FLT3-CARs with FLT3-tyrosine kinase inhibitor Crenolanib, while controls showed additional toxicity.

Conclusions In conclusion, transplantation of epitope-engineered HSPCs endowed with selective resistance to multi-specifc CAR-T-cells is a novel approach to enable more effective and safer immunotherapies for difficult-to-target tumors such as AML.

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REFERENCE

1. Epitope Editing Enables Targeted Immunotherapies for Acute Myeloid Leukemia, Casirati G, *et al. Nature* (*provisionally accepted*)

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