155 CD5 KNOCKOUT ENHANCES THE POTENCY OF MULTIPLEX BASE-EDITED ALLOGENEIC ANTI-CD5 CAR T-CELL THERAPY FOR THE TREATMENT OF T-CELL MALIGNANCIES

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Background T-cell lymphomas and leukemias are a class of diseases lacking durable effective therapies, where median survival for patients suffering from relapsed/refractory disease is often measured in months. Translation of B-cell targeting CAR-T therapeutic success to T-cell malignancies comes with significant challenges. Notably, the shared expression of target antigens on malignant T-cells and in the T-cell product itself results in CAR-T activation and fratricide during manufacturing. To overcome the challenges associated with creating CD5-targeting CAR-Ts, we developed a process to simultaneously base edit five target genes, including CD5 and PD1, to produce potency-enhanced allogeneic anti-CD5 CAR T-cells for use as an off-the-shelf treatment for T-cell malignancies.

Methods Anti-CD5 CAR-Ts were produced in a GMP-compatible process using T-cells isolated from healthy human donors. T-cells were modified using base editing technology to simultaneously knock-out five target genes in a single electroporation step. Edited T-cells were transduced with a lentivirus encoding a second-generation anti-CD5 CAR. Knockout frequencies were evaluated by flow cytometry and next-generation sequencing. Anti-CD5 CAR-Ts were then characterized for their specificity in vitro and potency in in vivo xenograft tumor models.

Results Simultaneous base editing at five genomic loci resulted in anti-CD5 CAR-Ts edited with 94–98% efficiency at each target gene, greatly diminishing the likelihood of GvHD, CAR-T rejection, fratricide, and checkpoint inhibitor activation. In addition, CD5 has an established role as a negative regulator of TCR signaling, and T cells lacking CD5 have enhanced proliferative capacity.[1] Anti-CD5 CAR T-cells with or without CD5 KO demonstrated equally potent cytotoxicity and cytokine production in vitro against CD5 expressing tumor lines. However, CD5 KO greatly improved in vivo efficacy of anti-CD5 CAR-Ts in a murine model of T-ALL against established tumor xenografts. Mice previously cleared of tumor underwent a second tumor challenge to assess the persistence of anti-CD5 CAR-T cells and were cleared of tumor a second time, indicating extended persistence of functional anti-CD5 CAR-T cells in vivo.

Conclusions Our approach addresses current technological limitations in developing and applying CAR-Ts that target T-cell malignancies and demonstrates that simultaneous multiplex base editing of up to five targets can create universally compatible, fratricide-resistant, therapeutically active anti-CD5 CAR-Ts. We further demonstrate that CD5 knockout produces CAR-T cells with enhanced potency capable of clearing multiple tumor challenges in vivo. We are progressing this CD5-targeting CAR-T cell product towards potential clinical development for the treatment of T cell malignancies and other CD5+ hematological tumors.

REFERENCE

1. Guillaume V, Peredo G, Romain R. CD5, an Undercover Regulator of TCR Signaling. *Frontiers in Immunology* 2018;**9**:2900.

Ethics Approval All animal studies were performed according to the guidelines and approval of the Institutional Animal Care and Use Committee.

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