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CAR-T CELL THERAPY WITH CRISPR/CAS9 ENGINEERING FOR RELAPSED/REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA, WITH UNIVERSAL DUAL TARGET CD19/CD22.

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INTRODUCTION

Autologous chimeric antigen receptor T (CAR-T) cell therapy is a successful approach for treating relapsed/refractory acute lymphoblastic leukemia (r/r ALL). Nevertheless, certain attributes of autologous CAR-T cells can cause delays in treatment availability. Another concern is the relapse caused by antigen evasion after single-targeted CAR-T therapy. Therefore, our goal is to develop CRISPR-modified universal off-the-shelf CAR-T cells that target both CD19 and CD22 (dual-targeted) as an innovative therapy for r/r ALL.

TRANSLATIONAL RELEVANCE

Limitations associated with autologous chimeric antigen receptor T (CAR-T) cells, including an undesirable waiting period between leukapheresis and CAR-T cell infusion, possible manufacturing failures, low viability and inferior quality of CAR-T cells due to prior chemotherapy, and high cost, might delay their availability for use in treatment of acute lymphoblastic leukemia. $CD19^-$ relapse after CD19 CAR-T therapy is another limitation. In this study, we developed CRISPR-edited allogeneic off-the-shelf CD19/CD22 dual-targeted CAR-T cells as a treatment strategy for relapsed/refractory acute lymphoblastic leukemia. Potent antileukemia activities were identified, and no CRISPR gene editing–associated genotoxicity, immunogenicity, or GvHD were observed. Moreover, all patients received infusion within 8 days of enrollment. Our data demonstrate that CRISPR-edited allogeneic CD19/CD22 dual-targeted CAR-T cells may improve the availability and accessibility of CAR-T therapy.

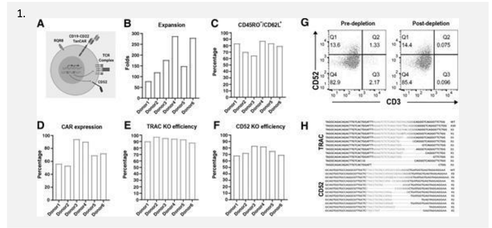
METHOD

In this phase I study with dose escalation and an open-label design, we administered universal CAR-T cells (CTA101) targeting CD19 and CD22 to patients with r/r ALL. These CAR-T cells were engineered using CRISPR/Cas9 to disrupt the TRAC region and CD52 gene, thus avoiding rejection mediated by the host immune system. We assessed safety, effectiveness, and the cellular kinetics of CTA101.

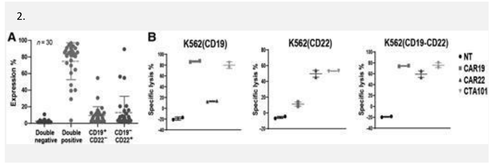
RESULTS

The utilization of CRISPR/Cas9 technology facilitated highly efficient and precise gene editing, resulting in the production of universal CAR-T cells. No genotoxicity or chromosomal translocation associated with gene editing was observed. Six patients received infusions of CTA101 at doses of 1 (3 patients) and 3 (3 patients) × 106 CAR+ T cells per kilogram of body weight. Cytokine release syndrome was observed in all patients. Thus far, no adverse events related to dose-limiting toxicity, graft-versus-host disease (GvHD), neurotoxicity, or genome editing have been reported. On day 28 after CTA101 infusion, the complete remission (CR) rate was 83.3%. With a median follow-up of 4.3 months, three out of five patients who achieved complete remission or complete remission with incomplete hematologic recovery (CR/CRi) remained free of minimal residual disease (MRD).

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1. Characteristic of CTA101. **A,** Schematic illustration of CD19/CD22 dual-targeting CTA101 design. **B–F,** CTA101 was manufactured by electroporation of *TRAC*- and *CD52*-targting Cas9 RNP, followed by lentiviral transduction of the CAR transgene. Expansion, phenotype, CAR expression, and *TRAC* and *CD52* knockout efficiency are shown. **G,** TCR/CD3 and *CD52* expression before and after CliniMACS magnetic bead depletion. **H,** Gene editing eve

2. CTA101-mediated $CD19^-$ escape mitigation. **A,** CD19 and CD22 expression profile in B-ALL patient samples. **B,** Cytotoxicity of CD19/CD22 single-antigen targeting and CTA101 CAR-T cells against CD19/CD22 single- and double-positive tumor cells was measured using a luciferase-based assay. NT, nontransduced T cell.

CONCLUSIONS

CRISPR/Cas9-engineered universal CD19/CD22 CAR-T cells exhibited a manageable safety profile and prominent antileukemia activity. Universal dual-targeted CAR-T cell therapy may offer an alternative therapy for patients with r/r ALL.

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