Preclinical Results of an Allogeneic, Universal CD19/CD7-Targeting CAR-T Cell Therapy (GC502) for B Cell Malignancies

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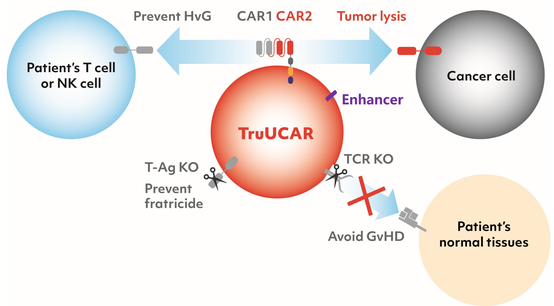
Abstract # 1722

BACKGROUND

Autologous CD19 CAR-T therapies show very promising clinical efficacy, but are limited in their applicability by several factors including cost, time to manufacture, and other factors involving patients own T-cell qualities. GC027, a CD7 targeting allogeneic, universal CAR-T (UCAR-T) currently in development for the treatment of T-cell acute lymphoblastic leukemia (T-ALL) has demonstrated robust expansion and anti-leukemia efficacy with a manageable safety profile in an investigator-initiated trial in China. These data suggest that, a single CD7 targeting CAR-T therapy is able to generate a therapeutic window by suppressing host vs graft (HvG) rejection of UCAR-T cells by patients' own NK and T cells, and achieve efficacy in patients with T-ALL. Based on these findings we have developed GC502, a CD19/CD7 dual-targeting, allogeneic CAR-T therapy for B-cell malignancies, in which the CD19 CAR moiety targets malignant cells while CD7 CAR moiety suppresses HvG in variety of preclinical models.

TruUCAR™ PLATFORM



METHODS

- **GC502 was manufactured using leukopaks from HLA-unmatched healthy donors.**
- **GC502 contains a 4-1BB-based, 2nd generation dual targeting CAR, comprising an anti-CD19 and an anti-CD7 single-chain variable fragments (scFvs).**
- **TRAC and CD7 loci were disrupted to avoid graft vs host disease and fratricide, respectively.**
- **The expression and function of dual CAR candidates with different CAR designs were evaluated by in vitro assays and mouse xenograft tumor models.**
- **A T-cell enhancer was included to achieve optimal anti-tumor efficacy.**

RESULTS

Screening and characterization of CD19/CD7 dual CAR candidates

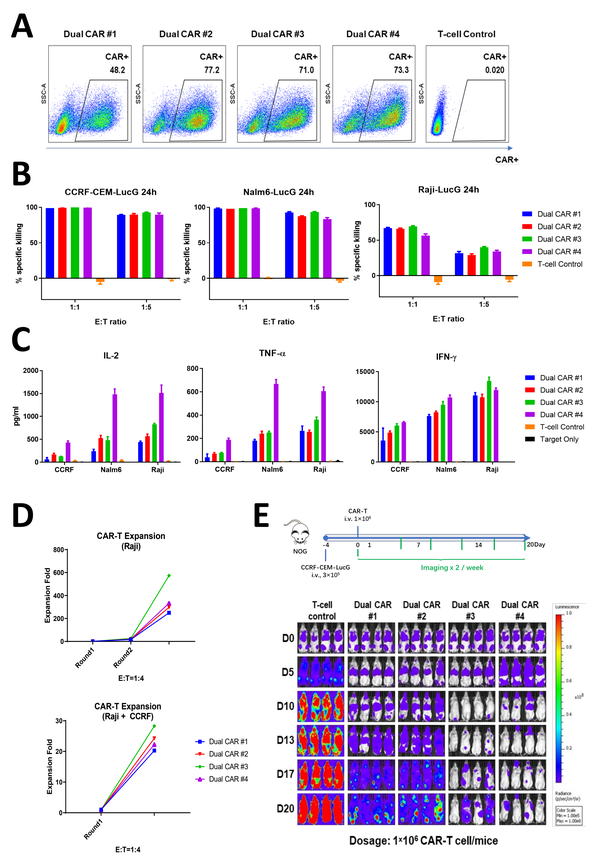


Figure 1.
A. CAR expression was examined by FACS analysis. All candidates displayed stable surface CAR expression.
B. Single CAR functionality was assessed by *in vitro* killing of CD7+ (CCRF-CEM) or CD19+ (Nalm6 and Raji) tumor cells at indicated E:T ratios. Tumor killing efficiencies was determined by enumerating residual target cells via luciferase assay.
C. Cytokine secretion during killing assay was evaluated by CBA kit.
D. CAR-T cell was challenged with Raji for 2 rounds or Raji-CCRF mixture for 1 round at E:T 1:4. CAR-T cells were count each round and cell proliferation were compared between different candidates.
E. *In vivo* efficacy of dual CAR candidates were evaluated in murine xenograft model using CCRF-CEM. 10^6 CAR-T cells (low dose) per mice was infused as a stress test for better separation of difference in CAR efficacy between different candidates.

Superior expansion and anti-tumor efficacy

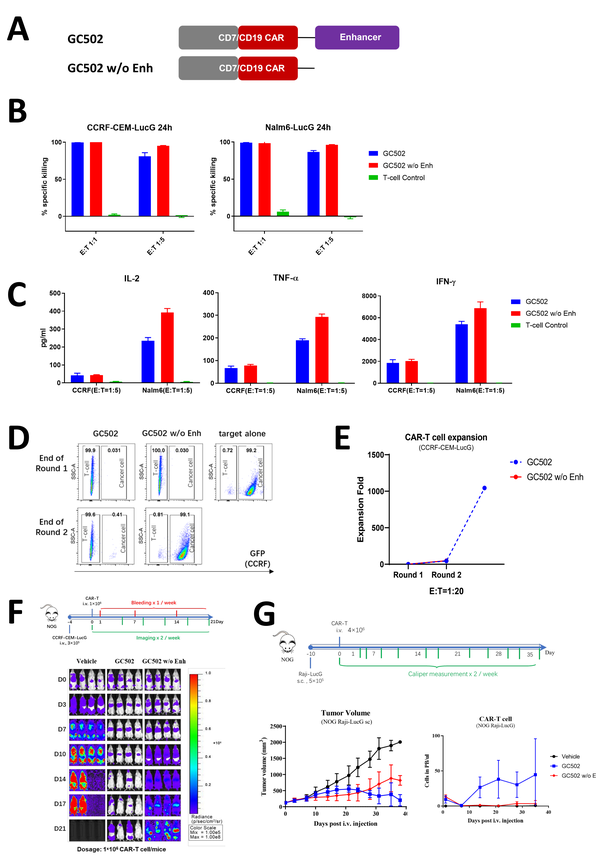


Figure 2.
A. Schematic illustration of GC502 design.
B-C. *In vitro* tumor killing of CD7+ CCRF-CEM or CD19+ Nalm6 cells (B) and cytokine secretion during the killing (C) were compared between GC502 and GC502 without enhancer.
D-E. Capacities of target killing (D) and CAR-T expansion (E) were evaluated in a stress test by serial killing assay with E:T at 1:20. Target killing was analyzed by flow cytometry (D); CAR-T cell number and fold of expansion was measured at each round of killing (E).
F. *In vivo* efficacy of CD7 CAR were compared in CCRF-based murine xenograft model. Tumor burden was monitored by bioluminescence.
G. *In vivo* efficacy of CD19 CAR were assessed in a Raji-based murine lymphoma model. Tumor burden measured by caliper and CAR-T cell expansions were measured by flow cytometry.

Robust anti-allogeneic T/NK-cell activity

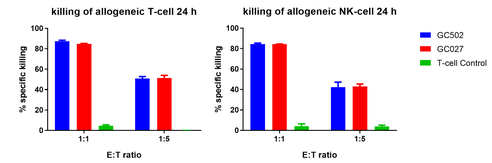


Figure 3. CAR-T cells were incubated with allogeneic T or NK-cell at indicated E:T ratio. Killing of allogeneic T or NK cells was examined by flow cytometry at 24 hour of co-incubation.

Potent *in vivo* functionalities comparing to single CAR products with proven clinical efficacies

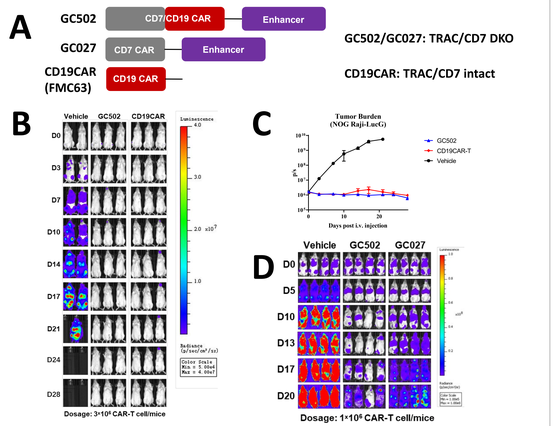


Figure 4.
A. Schematic illustration of GC502, GC027 and CD19CAR.
B-C. CD19 CAR activities of GC502 and TCR intact CD19 CAR were compared in a Raji-based murine xenograft model.
D. CD7 CAR activities of GC502 and GC027 were compared in a CCRF-CEM based murine xenograft model. Tumor burden in B-D was analyzed by bioluminescence.

CONCLUSIONS

- **GC502 demonstrated potent CD19/CD7 dual CAR functionalities comparing to single CARs with proven clinical efficacies.**
- **GC502 displayed superior CAR-T cell expansion and persistence.**
- **GC502 exhibited robust anti-allogeneic T/NK activity to suppress host vs graft (HvG) rejection.**
- **A promising "off-the-shelf" product for B-malignancies**