CD7 targeted “off-the-shelf” CAR-T demonstrates robust in vivo expansion and high efficacy in the treatment of patients with relapsed and refractory T-cell malignancies

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Article

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Abstract

T-cell acute lymphoblastic leukemia (T-ALL) represents an area of high unmet medical needs. Once relapsed, patients have limited treatment options and usually a poor prognosis. T-ALL antigens such as CD7 is extensively expressed in normal T cells and natural killer (NK) cells, and extending the success of CAR-T therapy to T cell malignancies was challenged by CAR-T cell fratricide, the high production cost, long lagging time and potential product contaminations. GC027 is an “off-the-shelf” allogeneic CD7 targeted CAR-T therapeutic product for T cell malignancies. It demonstrated superior cell expansion and anti-leukemia efficacy in mouse xenograft model. In our previous study, we observed promising efficacy result in the first two r/r T-ALL patient treated with GC027. Deep and durable response in these two patients suggests the potential of a “off-the-shelf” allogeneic CAR-T product as a promising standalone therapy. In the expanded study, 11 out of 12 patients had extensive GC027 cell expansion, rapid eradication of T-lymphoblasts, and reached complete response within 1-month post infusion. At data cut-off of Nov. 30, 2022, 1 patient had progression free survival of > 3 years. With manageable toxicity profile, GC027 demonstrated superior clinical efficacy and durability in T cell malignancies.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a type of highly aggressive hematological malignancies. It accounts for 25% of patients in adult ALL and 15% of pediatric ALL. Similar to B-ALL, it is potentially curable in adult patients, and the 5-year overall survival (OS) rates is 48%. However, adult patients with relapsed or refractory (r/r) disease have poor disease outcomes and the long-term survival rate was reported to be less than 10%1,2. Novel agents, such as targeted antibody therapies and CAR-T therapies are in demand to improve the OS in R/R T-ALL patients1.

Over the last decades, great progress of targeted autologous CAR-T therapies for the treatment of malignancies arising from the B-cell lineage were achieved. There are 6 autologous CAR-T therapies approved by the US FDA; 2 are BCMA targeted CAR-T products for treating multiple myeloma (MM), and 4 are CD19 targeted CAR-T products for treating r/r DLBCL or B-ALL. Transmembrane protein CD7 is highly expressed in 95% T-ALL cases, making it an ideal target for T-ALL treatment3,4,5,13. However, it’s been reported that the widespread of antigen overlap in healthy T cells results in fratricide among CAR-T cells during the manufacture process, which compromises the product yields and effectiveness3,7. Genetic disruption of CD7 locus, downregulate surface CD7 expression using endoplasmic reticulum (ER) retention methods or epitope masking are all effective approaches to eliminate cell surface CD7 expression and avoid fratricide4,6,8,13,19. Sorting out the naturally occurring CD7- T cells, which accounts for 3–12.5% of patient T cells, is another feasible strategy to make autologous CD7 CAR-T7,14, but it may burden CAR-T manufacture process.

The applications of autologous CAR-T therapies were also limited by major challenges such as the lengthy production time, the potential leukemia cell contamination as well as the high manufacturing cost4,13. Previously, we have reported a clinical study showing the initial safety and efficacy for GC027, a CD7 targeted “off-the-shelf” allogeneic CAR-T product4. The study showed rapid, deep and durable response to GC027 therapy in two r/r T-ALL adult patients4. Here we report updated result from an expanded patient group treated with GC027 as a stand-alone treatment option for r/r T-ALL adult patients which represent a high unmet medical need patient population.

Materials And Methods

Culture of cancer cell lines

Human T-ALL cell line CCRF-CEM (ATCC CCL-119) and B-lymphoma cell line Raji (ATCC CRL-1582) were purchased from ATCC. Cells were cultured in RPMI-1640 medium (Gibco, Cat: 21870-076) supplemented with 10% FBS (Gibco, Cat:10099-141C) and 100IU/ml penicillin-streptomycin (Gibco, Cat:15140-122). Both cell lines were transduced with firefly luciferase-GFP expressing lentivirus. Luciferase-GFP stable expression cells lines were maintained in the same culture media.

Lentivirus packaging

Lentivirus stocks were generated by transfection of 293-FT cells with pFUW-CD19CAR-41bbz, pFUW-CD7CAR-41bbz or GC027 (pFUW-CD7CAR-41bbz-P2A-C7R) plasmid together with packaging vectors pMDLg/pRRE, pRSV-Rev and pMD2.G. One day before transfection, 30 million 293FT cells were seeded in 30ml antibiotic free complete DMEM medium (Gibco) in 15cm dish. Culturing medium was replaced 6 hours after transfection. Transfected cells were incubated in 2% FBS DEME medium for another 48-72 hours before harvest virus supernatant.

Generation of CD7 CAR T Cells

Primary human T cells were stimulated with Dynabeads<sup>™</sup> Human T-Activator CD3/CD28 (ThermoFisher Scientific, Cat#11132D) and culture at 37°C in X-vivo 15 (Lonza, Cat:04-418Q) supplemented with 300IU/ml IL-2 (Peprotech, Cat:AF-200-02-1MG). 2 days post T cell activation, CRISPR RNP were prepared by mixing Cas9 protein (IDT, Cat:1081061) with sgRNAs (Genscript) targeting TRAC and CD7 at 1:1 molar ratio at 37°C for 15 min and stored at -80°C until use. CRISPR RNP were delivered into pre-activated primary human T cells with Lonza 4D-nucleofactor (Lonza, 4D-Core) per manufacturer's protocol. Cells were recovered and cultured in X-vivo 15 media (Lonza, Cat:04-418Q) at 37°C for 8 days. Lentivirus was introduced after nucleofection if CAR transgene expression was desired.

Fluorescence-activated cell sorting (FACS) analysis

All FACS analysis were performed on Canto II or Fortessa flow cytometer (BD Biosciences, FacsCanto). Cells were incubated with fluorochrome conjugated antibodies at 4°C for 30mins, washed 2 times with FACS buffer (DPBS+2% FBS), and re-suspended in 250ul FACS buffer. CD34-APC (Biolegend, Cat:343510),
Q-PCR analysis of CAR copy number

PE-His (Biolegend, Cat:362603), human CD7-his tag antigen (R&D, Cat:7579-CD-050), CD3-BV421 (BD, Cat:562526), CD4-PE-Cy7 (BD, Cat:557852), CD8-APC-Cy7 (BD, Cat:460179) and live/dead dye 7-AAD (BD, Cat:51-68981E) or DAPI (BD, Cat:564907) were used for cell staining. Data were analyzed with FlowJo 10 (FlowJo, LLC).

Intracellular staining

T cells were cultured with or without IL2 for 3 days before incubation with anti-CD34-APC 30mins at 4°C. After being washed 2 times with washing buffer (DPBS+1% HSA), cells were re-suspended in 300uL Perm Buffer III on ice and then stained with anti pSTAT5-PE (BD, Cat:612567) at 4°C overnight. Before FACS analysis, cells were washed 2 times with washing buffer (DPBS+1% HSA), and re-suspended in 250ul FACS buffer.

Cytotoxicity assay & Cytokine release assay

Luciferase expressing CCRF-CEM (CD7+) or Raji (CD7-) cells (target) were co-cultured with CD7-CAR-T cells or GC027 cells (effector) at effector: target ratio range from 1:9 to 3:1 for 7 or 24 hours. Remaining target cell were measured by luciferase-based luminescence (Promega, Cat: E6120). Percent of target cell killing was calculated by comparing to target cells only group.

Culture supernatant was collected after 24hr of co-culture. The amount of released cytokine during the 24hr killing were detected with Human Th1/Th2 Cytokine Cytometric Bead Array (CBA) Kit (BD Biosciences, Cat:551809).

Cell expansion during repeated target killing

Every 2-4 days, CD7-CAR-T cells or GC027 cells were stimulated with luciferase-GFP expressing CCRF-CEM at 1:3 effector: target ratio and cultured till target cells were completely eliminated. Cells were cultured in different cytokine conditions as indicated. CAR-T cell concentration were measured by NC-200. Total cell number were cultured by cell concentration and total volume.

T-ALL mouse xenograft model

5 to 7-week-old NOG mice (Taconic, NOD/SCID/yc-/-) were engrafted with 2x10^6 CCRF-CEM-Luciferase-GFP cells by intravenously (IV) injection. 6 days later, cancer bearing mice were randomly grouped and injected IV with 3x10^6 (High Dose) or 1x10^6 (Low Dose) CAR-T cells. Tumor burden were monitored by bioluminescence (BLI) in an IVIS Imaging system (Caliper Life Sciences) and Living Image software (PerkinElmer) was used for imaging and calculating total luminescence. 100 μL of blood sample was collected weekly and analyzed for CAR-T cell expansion in the peripheral blood. After red blood cell lysis, cells were washed in FACS buffer and incubated with anti-human CD45, CD2, CD4, CD7 and CD8 antibodies for subsequent flow cytometric analysis. Mice weight were measured 2 times per week. Mice were euthanized when weight loss exceeding 20% of baseline. All animal experiments were conducted in compliance with laboratory animal welfare and ethics committee.

Study design and oversight of clinical trial

This phase I/phase II clinical trial was approved by the Ethics Committee of the Second Affiliated Hospital of the Army Medical University Medical. The trial was registered at the Chinese Clinical Trial Registry (http://www.chictr.org.cn/) as ChiCTR1900025311. The trial was designed and conducted with the Declaration of Helsinki. All the participants and/or their guardians were well informed and provided written informed consent.

The primary objective of the study is to study the safety and the secondary objective is to study the objective response rate (ORR) within 3 months post infusion. The trial design includes an initial dose stage and a dose expansion stage. The initial dose escalation stage plans to enroll 3 patients. At dose 1, each patient receives GC027 product at 6x10^4 CAR+ cells/kg, dose 2 at 1-1.5x10^7 CAR+ cells/kg.

Key patient inclusion criteria included adult R/R T-ALL patients between 18-70 years of age, with CD7+ leukemia blasts as analyzed by FACS or IHC at time of enrollment. Patients needed to have a morphologically confirmed disease via bone marrow biopsy with an ECOG performance status of 0-2. Patients with active infections or major organ dysfunctions were excluded from this trial.

Assessment of treatment emergent adverse events & patient responses

CRS and ICAN were evaluated according to the ASTCT consensus. Minimal residual disease (MRD) was conducted by flow cytometry according to Brent L Wood's principles. Patient response was assessed according to National Comprehensive Cancer Network Guidelines for Acute Lymphoblastic Leukemia (2019.V2). For patients only with bone marrow involvements, response assessment includes morphological analysis and flow cytometry evaluations. For patients with extra-medullary involvements, response assessment will also include imaging analysis of the lesions via PET-CT.

Flow cytometry analysis of peripheral blood samples

Peripheral blood samples were collected in EDTA tubes for CAR-T cells analysis by flow cytometry using a FACSCantoll Plus platform (BD Biosciences, San Jose, CA, USA). 100μL of peripheral blood was stained with CD2-FITC (Biolegend 309206), CD34-PE (Biolegend 343506), CD7-APC (Biolegend 343108), CD4-PE-Cy7 (BD Bioscience 557852), CD8-APC-Cy7 (BD Bioscience 560179), CD3-BV421 (BD Bioscience 562426), CD45-BV510 (BD Bioscience 563204), CD56-BV605 (Biolegend 362538). Erythrocytes were lysed using red blood cell lysing solution (BD, 349202) and white blood cells were collected by centrifugation and washed with 2ml of PBS before data acquisition.

Q-PCR analysis of CAR copy number
Cellular genomic DNA was extracted from 1 ml of peripheral blood using the QiAamp DNA Blood Midi kit (Qiagen, Redwood City, CA, United States,51185). gDNA was amplified using the TB Green Premix Ex Taq (Tli RnaseH Plus) Kit (Takara Biotechnology, Shiga, Japan, RR420A). The amplification was detected in real time using the Applied Biosystems 7500 Real-Time PCR System (Life Technologies, MA, USA). Primer pair targeting WPRE region was used to detect CAR copy number, and primer pair targeting RPP30 gene was used as reference. The primer pairs were experimentally validated under the following criteria: (i) a single, gene-specific product was generated; (ii) the amplification efficiency ranged between 90% and 110%; (iii) the cycle threshold (Ct) value of the no-template control was more than 40.4.

**Analysis of serum cytokines**

After centrifugation, serum samples were aliquoted and kept at −70℃. Cytokines were analyzed using CBA kit (RAISECARE, 12 Cytokine detection kit) on FACSCantoII Plus platform (BD Biosciences, San Jose, CA, USA).

**Statistics**

cytokine data were analyzed using the Kruskal-Wallis test method. Other comparisons were performed using the Student's t-test or ANOVA. Mouse survivals in xenograft model were analyzed by the Kaplan-Meier curves with Mantel-Cox log-rank test method. GraphPad Prism 8 software (GraphPad software) was used for statistical analysis. P values <.05 were considered statistically significant.

**Results**

**Generation of GC027 CAR-T cells**

GC027 construct comprises a CD7 targeted CAR and C7R, an IL7Ra-derived T cell enhancer that contains CD34 etco domain and a mutated IL7Ra transmembrane and intracellular domain9 (Figure 1A, 2A). The CD7 CAR was generated by fusing a CD7-specific scFv to a second-generation CAR backbone, which includes CD8 hinge and transmembrane region, 4-1BB co-stimulation and CD3 primary stimulation domain (Figure 1A, 1B). Besides targeting T-ALL blasts, the CD7 CAR also targets patient's own T and NK cells, which suppresses graft rejection mediated by alloreactive killer cells (Figure 1B). To avoid GvHD and CAR-T cells fratricide, TCR and CD7 expression were efficiently eliminated from CAR-T cell surface by disrupting TRAC and CD7 loci with CRISPR/Cas9 RNP complex (Figure 1C)13.

**An IL7Ra-derived T cell enhancer C7R improves CAR-T cell expansion by activating JAK/STAT5**

GC027 displayed comparable anti-leukemia activity to a control CAR7 construct, which doesn't contain C7R (Figure 1A, 2B). The production of pro-inflammatory cytokine IL6, TNFα and IFNγ weren't affected by the addition of C7R either (Figure 2C). IL2 secretion was downregulated in GC027, however, C7R mediated constitutive active pSTAT5 signal in deprivation of IL2 or any other cytokine that activates Jak/STAT5 pathway (Figure 2D). In a repeated tumor killing assay, CAR-T cells were challenged each 2-4 days by CD7+ CCRF-CEM cells. GC027 showed superior CAR-T cell expansion comparing to control CD7 CAR-T cells (Figure 2E). This finding is in consistent with an earlier study, that co-expressing the C7R with a GD2-directed CAR increased T-cell proliferation, survival, and antitumor activity during repeated exposure to tumor cells, without disturbing T-cell function.9

**Anti-leukemia activity and in vivo CAR-T expansion in xenograft T-ALL model**

The anti-leukemia efficacy of GC027 was tested in a xenograft model. We IV engrafted human T-ALL cells CCRF-CEM cells into NOG mice (Figure 3A). 6 days after tumor engraftment, treatment with high dose of CD7 targeted CAR-T cells significantly increased mouse survival by 2 weeks, compared with control mice treated with vehicle control or T cells expressing an irrelevant CD19 CAR (Figure 3C); however, leukemia cells started to grow back around 2.5-3 weeks post CAR-T infusion (Figure 3B). These mice also experienced > 20% weight dropping along with increasing tumor burden (Figure 3D). In contrast, in mice receiving GC027, leukemia cells were rapidly cleared (Figure 3B) and mouse body weight was well maintained around baseline levels (Figure 3D). CAR-T cell expansion was monitored every week in mouse peripheral blood. GC027 demonstrated significantly enhanced cell expansion and persistence in vivo comparing with the control CD7 CAR T cells (Figure 3E).

**Patient characteristics and treatment protocol**

Total of 18 r/r T-ALL patients were screened, 6 patients were excluded: 1 patient with CNS involvement, 4 patients with severe infection, and 1 patient with severe agranulocytosis. A total of 12 patients, including 11 patients with r/r T-ALL and 1 patient with r/r T-LBL, were enrolled to the study and received GC027 infusion. The media age is 28 years (ranges 19-47) and the median bone marrow tumor burden is 24.5% (ranges 0-66.28%, by FACS analysis). At enrollment, 4 patients had extra-medullary lesions (EMD), and 3 patients experience disease relapse post allo-HSCT (Figure 4, Table 1).

3 patients received 6.2-8.6×10⁶ cells/kg of GC027, and 9 patients received 1-1.5×10⁷ cells/kg (Figure 4). Before GC027 infusion, all patients received 4-6 days of lympho-depletion regimen. Chemotherapy agents include enhanced Fludarabine plus Cyclophosphamide, combined with melphalan, etoposide, or prednisolone. Detailed pre-conditioning regimen for each patient and CAR-T dosage were shown in Table 2.

**Safety profile**

No ICANS were observed. CRS of any grade occurred in 83% of patients (10 out of 12): 2pt experienced grade 2 CRS and 8 experienced grade 3 CRS. CRS onset was on the day of infusion (Figure 5A). CRS in all patients resolved after timely management.
CRS related pro-inflammatory cytokines were analyzed up to 3 weeks post CAR-T infusion. Significant elevated levels of IFN-γ, IL-2, IL-5, IL-6, IL-8, IL-10 were observed in peripheral blood samples from patients experienced CRS (Figure 4C). Among the elevated cytokines, the peak of IFN-γ emerged 1st on day 4. Peak of IL-8 emerged between day 4-6. IL-5 and IL10 levels in some patients reached a 2nd peak around day 14 (Figure 5C).

**Efficacy of GC027**

Out of 12 patients who received CAR-T cell infusion, 11 achieved complete response (CR) or complete response with incomplete hematological recovery (CRi) within 1-month post CAR-T infusion, with CR rate 91.7% (Figure 5B, Table 1). 3 out of 4 patients with EMD had complete remission of their lesions (Table 1). In patients who achieved CR, only 1 received subsequent allo-HSCT, none of the rest 10 patients received other anti-leukemia therapies after remission. 5 patients exit the trial within 2-5 months post CAR-T treatment due to various reasons. 3 patients lost follow-up, 1 patient died of transplantation-related syndrome and 1 patient died of severe infection. 4 patients experienced relapses within 5-10 months with full or partial CD7 expression. At data cut-off of Nov. 30, 2022, 2 patients were still in remission, and P03 had been in remission for over 3 years (Figure 5B).

**Pharmacokinetics of GC027**

CAR-T cell expansion in the peripheral blood was assessed by flow cytometry and qPCR. Except for the only one non-respondent patient, abundant GC027 expansion were observed in peripheral blood from all 11 responded patients (Figure 6A). As measured by flow cytometry, a maximal expansion of CAR-T cells at a median of 1314 cells/μL (6-9,716) was observed, and the median time for maximal cell expansion was day 9 post CAR-T infusion (day 6-13) (Figure 6A). Measured by qPCR, the maximal copy number with median of 365310.5 copies/μg DNA (98,460-1,923,666) was observed, and the median time to reach peak copy number was day 8.5 post CAR-T infusion (day 6-21) (Figure 6B). In 11 patients with robust CAR-T expansion, GC027 cannot be detected via flow cytometry or qPCR in most patients (9/11) 4 weeks post infusion (Figure 6B). The CD7+ and CD3+CD7- lymphocytes were detected using flow cytometry (Figure 6C/D). The CD7+ lymphocytes were quickly eliminated after GC027 infusion and began to recover at 3 weeks after infusion, but still remained below the level of baseline within 6 months after infusion. The CD3+CD7- lymphocytes expanded to peak level at 3 weeks after infusion and remain at a high level over baseline with 6 months after infusion.

**Discussion**

Comparing to the rapid progress and remarkable success of CAR-T therapies for B-ALL, B-NHL and multiple myeloma, the development of CAR-T therapy for T lineage malignancies are facing more challenges. Shared antigens between healthy T cells and T-malignant cells causes extensive fratricide, significant yield drop, and potential tumor cell contaminations during CAR-T manufacturing, which all compromise the applications of CAR-T therapy in treating T cell malignancies. In 2021, we reported the preliminary safety and efficacy result of GC027 for the treatment of two patients with r/r T-ALL, and discussed the feasibility of treating T-malignancies with an "off-the-shelf", allogeneic CAR-T therapy with genetic ablations of TCR and CD74. In this article, we report the updated study result from an extended patient cohort, which demonstrated the potential of GC027 for the treatment of r/r T-ALL/LBL as a standalone treatment option.

Development of CD7 targeted CAR-T therapy through different strategies are of extensive interests in the recent years. Pan, et al reported preparation of CD7 CAR-T product using allo-HSCT donor derived apheresis product, which is a viable approach to avoid cancer cell contaminations and reduce the risk of GvHD. Zhang, et al have applied an ER retention method to sequester CD7 protein at the intracellular compartment, and effectively solve the problem of fratricide in autologous products. Lu et al described a CD7 CAR that can mask the CD7 epitope on CAR expressing T cells; this approach effectively avoided fratricide and the expansion of naturally selected fratricide-resistant CD7 CAR T cells for 20 patients with T-ALL or T-LBL. A recent study by Velasquez, et al. reported a novel approach that utilized the nature selection during production to enrich the fratricide resistant, naturally occurring CD7-T cells and generated CD7-CARCD7 – T cells.

Comparing to allogeneic, universal CAR-T therapies, autologous approaches are usually considered to be more advantageous to expand and persist in patients without the risk of GvHD. However, due to the heterogeneity of leukemia blasts, GMP manufacturing from patients' own apheresis products is at risk of contamination by leukemia cells. So far, there is no report for finding CAR transgene in T leukemia cells yet, but transducing CD19 CAR into B-ALL cells patients without the risk of GvHD. However, due to the heterogeneity of leukemia blasts, GMP manufacturing from patients' own apheresis products is at risk of contamination by leukemia cells. So far, there is no report for finding CAR transgene in T leukemia cells yet, but transducing CD19 CAR into B-ALL cells.

"Off-the-shelf", allogeneic CAR-T targeting CD7 are not expected to have a long lifespan because they will be eliminated upon immune reconstitution of CD7+ T cells in the host. In the two reported CD7 targeting "off-the-shelf" approaches, CAR-T cells persist less than 4 weeks in majority of the patients. The short persistence is considered to be an advantage to prevent T-cell aplasia but also a disadvantage to prevent cancer recurrence. Interestingly, less than 4 weeks of CAR-T cell persistence is adequate for majority of patients to reach MRD-CR in both studies. 3 patients treated with GC027 maintained PFS > 6 months with a single infusion. Notably, serious infection and patient death were observed in both autologous and allogeneic studies, which suggest that short term deficiencies in CD7+ T and NK cells potentially lead to life threatening infections, and intensified patient care with prophylactic use of anti-viral medicine and antibiotics should be considered in the future studies.
Disease relapse with antigen loss was often seen in clinical studies with CD19 targeted therapies for B-ALL. We had similar observations in GC027 treated patients with T malignancies. Although 25% of patients maintained PFS > 6 months and the longest for > 36 month, more than half of the patient experienced relapse of disease with detectable CD7 expression in leukemia blast. Two recent reports on CD7 targeted auto- and allo-therapies supports that treatment outcomes can potentially be further enhanced by bridging to allo-HSCT.

In conclusion, the allogeneic, "off-the-shelf" CAR-T therapy GC027 showed promising early efficacy as a standalone therapy in heavily pretreated patient with T cell malignancies. A single infusion of GC027 was able to induce deep and durable responses within 4 weeks of infusion. Clinical toxicities are manageable. Intensified patient care for infections and bridging to allo-HSCT should be considered to achieve better clinical outcomes.

Declarations

Acknowledgement

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Authorship

Contributions: Sanbin Wang and Xi Zhang designed and leaded the clinical trial; Shiqi Li and Le Luo provided patient care and analyzed data. Xinxin Wang and Shiqi Li wrote the manuscript. Wei Cao and Xinxin Wang designed the CAR-T product and in vitro experiments, interpreted data and edited the manuscript. Jiaping He supervised CAR-T production. Zhongtao Yuan, Lin Liu and Yu Li provided clinical assistance. Jia Liu, Gui Li, Duanpeng Wang and Shiqi Li wrote the manuscript. Wei Cao and Xinxin Wang designed the CAR-T product and in vitro experiments, interpreted data and edited the manuscript. Jia Liu, Gui Li, Duanpeng Wang and Sheng Li coordinated the study and data interpretation. Lyzhe Chen, Ping Yin, Lianjun Shen and Wenling Li conducted experiments. Li Gao, Jun Rao, Lihua Fang and Yining Chen provided nursing support.

Competing Interests: The authors declare no competing interests.

References


**Tables**

**Table 1. Patient baseline characteristics and treatment outcome**

| Patients No. | ECOG | Diagnosis | EM | Sex | Age(year) | HLA match/Ab | Prior lines of therapy | Prior SCT | Prior best response | BM Tumor Burden Baseline | | After FC | CR | Risk Stratification |
|--------------|------|-----------|----|-----|-----------|--------------|------------------------|-----------|---------------------|-----------------------| |         |   |                           |
| 1            | 1    | T-ALL     | Y  | M   | 21        | 0%/N        | 2                      | N         | MRD+CR              | 35.38%                | | - | - |                           |
| 2            | 1    | T-ALL     | Y  | M   | 23        | 17%/B44 dim| 7                      | N         | CR                  | 6.97%                 | | 6.27% | Yes | - |                           |
| 3            | 2    | T-ALL     | N  | M   | 24        | 17%/N       | 7                      | N         | CR                  | 45.84%                | | 16.74% | Yes | - |                           |
| 4            | 1    | T-ALL     | N  | M   | 28        | 8.3%/-      | 5                      | N         | MRD-CR              | 6.64%                 | | 0.30% | Yes | NOTCH1                |
| 5            | 1    | T-ALL     | N  | F   | 28        | 8.3%/HLA II ++| 2                      | Allo-SCT  | MRD-CR              | 1.76%                 | | 1.47% | Yes | WT1/ABL               |
| 6            | 1    | T-LBL     | Y  | M   | 37        | 16.7%/-     | 2                      | Auto-SCT  | MRD-CR              | 0.00%                 | | 0.00% | Yes | - |                           |
| 7            | 1    | T-ALL     | N  | M   | 47        | 0%/N        | 16                     | N         | MRD-CR              | 66.28%                | | 82.23% | Yes | FBXW7/KRAS/Nl          |
| 8            | 1    | T-ALL     | N  | F   | 28        | 0%/N        | 3                      | Allo-SCT  | MRD-CR              | 23.30%                | | 2.48% | Yes | NOTCH/SH2B3/          |
| 9            | 1    | T-ALL     | Y  | M   | 34        | 17%/A34dim  | 1                      | N         | PR                  | 28.02%                | | 3.85% | Yes | - |                           |
| 10           | 1    | T-ALL     | N  | F   | 23        | 17%/N       | 8                      | N         | MRD-CR              | 25.85%                | | 6.64% | Yes | - |                           |
| 11           | 1    | T-ALL     | N  | M   | 35        | 17%/N       | 9                      | N         | CR                  | 4.00%                 | | 2.70% | Yes | SIL-TAL1              |
| 12           | 2    | T-ALL     | N  | M   | 19        | 33%/N       | 1                      | N         | MRD-CR              | 38.20%                | | 1.50% | Yes | - |                           |

**Table 2. Pre-conditioning scheme and Dosage**

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<table>
<thead>
<tr>
<th>Patients No.</th>
<th>Fludarabine</th>
<th>Cyclophosphamide</th>
<th>Etoposide</th>
<th>Melphalan</th>
<th>Prednisone</th>
<th>Dose Level</th>
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<tr>
<td>1</td>
<td>30mg/m²×6 days</td>
<td>600mg/m²×5days</td>
<td>N</td>
<td>25mg×1 day(0.5mg/kg)</td>
<td>N</td>
<td>4.3×10⁶/kg+4.3×10⁶/kg (day 8)</td>
</tr>
<tr>
<td>2</td>
<td>30mg/m²×6 days</td>
<td>600mg/m²×6days</td>
<td>N</td>
<td>50mg×1 day(0.6mg/kg)</td>
<td>N</td>
<td>6.7×10⁶/kg</td>
</tr>
<tr>
<td>3</td>
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<td>900mg/m²×4days</td>
<td>N</td>
<td>37mg/m²×2days(1.2mg/kg)</td>
<td>60mg/m²×4 days</td>
<td>6.2×10⁶/kg</td>
</tr>
<tr>
<td>4</td>
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<td>1000mg/m²×4days</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1×10⁷/kg</td>
</tr>
<tr>
<td>5</td>
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<td>1000mg/m²×4days</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1.2×10⁷/kg</td>
</tr>
<tr>
<td>6</td>
<td>30mg/m²×4 days</td>
<td>1000mg/m²×4days</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1×10⁷/kg</td>
</tr>
<tr>
<td>7</td>
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<td>600mg/m²×6days</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>1.2×10⁷/kg</td>
</tr>
<tr>
<td>8</td>
<td>30mg/m²×6 days</td>
<td>600mg/m²×5days</td>
<td>N</td>
<td>50mg×1day(0.93mg/kg)</td>
<td>N</td>
<td>1×10⁷/kg</td>
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<td>600mg/m²×5days</td>
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<td>50mg×1day(0.71mg/kg)</td>
<td>N</td>
<td>1×10⁷/kg</td>
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<tr>
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<td>600mg/m²×5days</td>
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<td>50mg×1day(0.83mg/kg)</td>
<td>N</td>
<td>1×10⁷/kg</td>
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<td>11</td>
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<td>600mg/m²×6days</td>
<td>100mg/m²×6 days</td>
<td>50mg×1 day(0.7mg/kg)</td>
<td>N</td>
<td>1.2×10⁷/kg</td>
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<tr>
<td>12</td>
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<td>70mg/m²×5 days</td>
<td>N</td>
<td>N</td>
<td>1.5×10⁷/kg</td>
</tr>
</tbody>
</table>

**Figures**

**A**

**CAR7**

**GC027**

**B**

Patient's T cell or NK cell

Cancer cell

CAR7 Tumor lysis

CAR7

Prevent HvG

Patient's normal tissues

**T-Ag KO**

Prevent fratricide

**TCR KO**

Avoid GvHD

**Figure 1**

Schematic illustration of GC027 product design.

A. Illustration of GC027 product components.

B. Illustration of the allogeneic approach of GC027 and strategies to address GvHD and HvG issues.
Figure 2

In vitro characteristics of GC027.

A. CD7-CAR and Enhancer (CD34 ecto) expression levels in CD7 CAR-T, GC027 and TRAC/CD7 double knockout T cell control (DKO).

B. pSTAT5 expression (y-axis) and Enhancer (CD34 ecto)-expression (x-axis) levels in cells cultured 3 days with or without IL-2 stimulation.

C. Proliferations of CD7 CAR-T or GC027 cells during repeated stimulation by CCRF-CEM cells.

D. CAR-T killing against CCRF-CEM cells (CD7+) or Raji cells (CD7-) during 7-or 24-hour co-culture at indicated Effector: Target ratios.

E. The release of cytokines levels during 24-hour co-culture of CAR-T cells with CCRF-CEM cells at 1:1 or 1:9 Effector: Target ratios.
Figure 3

In vivo efficacy in a human T-ALL xenograft murine model.

A. Scheme of the murine study. 2x10^6 human T-ALL cell line CCRF-CEM were engrafted via IV 6 days before CAR-T cell infusion. Tumor burden were measured 2 times per week via bioluminescence. 100 μL of blood sample was collected weekly and analyzed for CAR-T cell expansion.

B. Tumor burden in different treatment groups as shown by bioluminescence.

C. Survival curves of tumor bearing mice in different treatment groups.

D. Weight measurement in each treatment group.

E. CAR-T cell expansion in mouse peripheral blood as measured by flow cytometry.
Figure 4

Trial design and patient enrollment flow chart.

After signing informed consent, patients were evaluated for qualifications of enrollment into the dose escalation study. Total of 12 patients were enrolled in the study.
Figure 5
Safety and efficacy evaluations of patients with r/r T-ALL after GC027 treatment.

A. Incidence of CRS and ICANS.

B. Efficacy of patients after GC027 treatment and response duration. Response of individual patients infused with different dose levels of GC027 was shown in swimmer plot. 5 patient withdraw: 3 patients lost follow-up(P02,P05 and P07), 1 patient died of transplantation-related syndrome(P08) and 1 patients died of severe infection(P10).

C. Kinetics of serum concentrations of pro-inflammatory cytokines IFN-γ, IL-6, IL-8, IL-5, IL-10 and IL-2.

Figure 6
Detection of GC027 and target cells.

A. Absolute counts of GC027 in patient peripheral blood samples were measured using flow cytometry and numerated for cell concentrations per uL of blood.

B. Expansion and persistent of GC027 in peripheral blood were analyzed using qPCR and copy numbers per ug/DNA were calculated.

C. Detection of CD7+ target cells using flow cytometry and numerated for cell concentrations per uL of blood.

D. Detection of CD3+CD7- cells using flow cytometry and numerated for cell concentrations per uL of blood.