**CRISPR/Cas9 genome-edited universal CAR T cells in patients with relapsed and refractory lymphoma**

**Contents:**



## 1 Materials and methods

### 1.1 Study design

This study was designed to evaluate the safety and potential of administering allogeneic universal CAR T cells to patients with relapsed and refractory DLBCL. This study was approved by the ethics committee of the Chinese PLA General Hospital and registered at www.clinicaltrials.gov (identifier no: NCT03166878). All patients provided informed consent according to the Declaration of Helsinki. The patients were administered a preconditioning regimen of fludarabine and cyclophosphamide before universal CAR T cell infusion. Two days after preconditioning, the patients received universal CAR T cells infused intravenously. Therapy was terminated if uncontrollable toxicity occurred, the disease progressed, or the patients abandoned any treatment.

Adverse events during and after therapy were assessed and graded according to the National Cancer Institutes of Common Terminology Criteria for Adverse Events Version 4.0 (http://ctep.cancer.gov/). The response assessment for lymphoma was based on recommendations by the International Conference on Malignant Lymphomas Imaging Working Group (Cheson Response Criteria and The Lugano Classification 2014) [1].

### 1.2 Lentivirus production

CAR consisted of an anti-CD19 scFv derived from the FMC63 monoclonal antibody [2], the human CD8a hinge and transmembrane region, and the intracellular signalling domains of CD137 and CD3zeta, which were cloned into the pRRLSIN lentiviral backbone. The CAR construct was verified by DNA sequencing. The pRRLSIN vector plasmid, ps-PAX2 packaging plasmid and pMD2.G envelope plasmid were transfected into 293T cells according to the manufacturer's

instructions with Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA, USA). The lentiviral supernatants were collected and stored at -80°C.

**1.3 Universal CAR T cell manufacture**

Universal CAR T cells were manufactured under Good Manufacturing Practice condition as previously described [3,4]. Peripheral blood mononuclear cells (PBMCs) were collected from healthy male adult non-HLA-matched donors by leukapheresis, activated with Dynabeads Human T-Activator CD3/CD28 magnetic beads (Invitrogen) at a 1:1 bead:cell ratio and cultured in X-VIVO 15 medium (Lonza) supplemented with 300 U/mL recombinant human IL-2 (PeproTech). After culture for 2 days, activated T cells were added to the viral-loaded flasks and centrifuged. The transduced cells were transferred to new flasks the following day.

The Cas9 protein and sgRNA were electroporated into virus-transduced T cells to knock out the expression of endogenous TCR and B2M as previously described [5,6]. For preparation of the ribonucleoprotein mixture, Cas9 protein (Integrated Device Technology, Inc.) and sgRNA targeting T cell receptor α chain (TRAC) and B2M (Integrated Device Technology, Inc.) were incubated together at room temperature for 20 minutes immediately before electroporation. The beads were removed by CliniMACS magnetic depletion, and T cells were resuspended in Opti-MEM medium (Gibco) containing the ribonucleoprotein mixture and transferred into a 2 mm cuvette (Harvard Apparatus BTX). Then, the cells were electroporated using a BTX Gemini System (Harvard Apparatus BTX) at 250 V for 5 ms. Following electroporation, the cells were immediately transferred into prewarmed medium and cultured at 37°C and 5% $CO_2$ for 15 days. The cell products were cryopreserved after depletion of residual TCR-expressing cells by EasySep™ Human CD3 Positive Selection kit II (STEMCELL Technologies). The final product-release criteria included negative bacterial, fungal and *Mycoplasma* cultures, an endotoxin level ≤ 5 EU/mL, a negative Gram stain, ≥ 80% cell viability, ≥ 99% CD3-negative cells, ≥ 85% CD3 and B2M-double negative cells, and immunophenotype and detection of CAR expression. Batches of universal CAR T cells generated from a donor were used for the trials and manufactured before patient enrolment.

**1.4 Depleted of residual TCR-expressing cells**

Depletion of residual TCR-expressing cells was performed according to the manufacturer's instructions with an EasySep™ Human CD3 Positive Selection kit II (STEMCELL Technologies). Briefly, the cells were collected and incubated with CD3-positive selection cocktail II, followed by

incubation with RapidSpheres™. Then, CD3-negative cells were collected after magnetic separation.

**1.5 Flow cytometry**

Universal CAR T cells were incubated with a biotin-SP-AffiniPure F(ab)'2 fragment-specific goat anti-mouse IgG antibody (Jackson ImmunoResearch) to assess CAR expression, followed by incubation with streptavidin-PE (BD Biosciences). In addition, these cells were washed and stained with anti-human B2M, CD3, CD4, CD8, CD45RO, CD62L and CCR7 antibodies (BD Biosciences).

Universal CAR T cells were cocultured with Raji, Daudi or K562 cells at an effector-to-target ratio of 5:1 together for 8 hours to assess their abilities. Tumour lysis assays were performed according to the manufacturer's instructions with BD Pharmingen™ FITC Annexin V Apoptosis Detection Kit I.

Universal CAR T cells were cocultured with Raji (human Burkitt's lymphoma cell line), Daudi (human Burkitt's lymphoma cell line) or K562 (human chronic myelogenous leukaemia cell line) cells at an effector-to-target ratio of 5:1 together with an anti-human CD107a antibody (BD Biosciences) for 1 hour to assess degranulation, followed by incubation with a Golgi Plug protein transport inhibitor (BD Biosciences) for 3 hours.

Universal CAR T cells were cocultured with Raji, Daudi or K562 cells at an effector-to-target ratio of 5:1 in the presence of the Golgi Plug protein transport inhibitor (BD Biosciences) for 4 hours. The CAR T cells were subsequently washed, fixed, and permeabilized according to the manufacturer's instructions using a Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Biosciences). Intracellular cytokine staining was performed with APC-conjugated anti-human IFN-γ or APC-conjugated anti-human IL-2 antibodies (BD Biosciences).

T cell proliferation was quantified by staining universal CAR T cells with a 5 μM solution of carboxyfluorescein succinimidyl amino ester dye (CFSE) (Dojindo Laboratories) according to the manufacturer's instructions and incubating the CAR T cells with Raji, Daudi or cells at an effector-to-target ratio of 5:1 for 72 hours.

Data acquisition and analysis were performed using FACSCalibur flow cytometry. Samples were assessed using FACSCalibur (BD) and DxFLEX flow cytometry (Beckman Coulter). The data were analysed with FACSCalibur, Kaluza Analysis 2.0 (Beckman Coulter) and FlowJo software v.10 (FlowJo LLC).

**1.6 Quantitative real-time PCR (Q-PCR)**

The persistence of universal CAR T cells was assessed using Q-PCR to detect the level of CAR transgenes in peripheral blood. Genomic DNA was extracted from PBMCs with a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) before and at serial time points after universal CAR T cell infusion, at occasional time points when unexplained fever/chill or suspected cytokine release symptom (CRS) or any other events that may have been correlated with universal CAR T cells occurred, and during the follow-up period until the progression of disease. A 7-point standard curve that consisted of 100 to $10^8$ copies/μL CAR plasmid DNA was prepared, with each sample containing 100 ng of preinfusion PBMC genomic DNA to control for background signal. A 153-bp (base pair) fragment containing portions of the CD8a chain and adjacent 4-1BB chain sequences was amplified using the forward primer 5′-GGTCCTTCTCCTGTCACTGGTT-3′ and the reverse primer 5′-TCTTCTTCTTCTGGAAATCGGCAG-3′. Amplification of β-actin was used as an internal control and for normalization of DNA quantities. Q-PCR was performed with 100 ng of DNA in each reaction using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, USA).

**1.7 Enzyme-linked immunosorbent assay (ELISA)**

Serum from each patient was collected before and at serial time points after universal CAR T cell infusion, at occasional time points when unexplained fever/chill or suspected CRS or any other events that may have been correlated with universal CAR T cells occurred, and during the follow-up period until the progression of disease. The levels of serum IL-2, IL-6, IL-8, IL-10, TNF-α, and CRP were assessed by ELISA at scheduled time points.

**1.8 Analysis of gene editing efficiency**

The genomic disruption of TRAC and B2M in universal CAR T cells was measured as previously described [5,7,8]. The genomic DNA was extracted from the universal CAR T cells. Sequencing data derived from the areas around the gene-edited region were analyzed with Tracking Indels by Decomposition (TIDE) at a website of https://tide.nki.nl/. The PCR primers used for the amplification of target loci are listed in Supplemental Table 3.

**1.9 Evaluation of the off-target sites of TRAC and B2M sgRNAs**

The off-target sites of TRAC and B2M sgRNA were analyzed as previously described[5,7,9]. The 10 potential off-target sites for each sgRNA were predicted in CRISPOR (crispor.tefor.net) and

amplified by PCR and subjected to Sanger sequencing. The sequencing results were analyzed using TIDE method.

**1.10 Chromosomal translocation detection**

Chromosomal translocation events between the TRAC and B2M loci were analyzed by Q-PCR using combinations of the TRAC and B2M locus specific primers as previously described [7,10]. TRAC and B2M locus specific primers were presented in Supplemental Table 4. The reaction was performed using a MightyAmp™ for Real-Time kit (Takara) and a LightCycler 480 (Roche) [7]. Four plasmids containing the expected translocation events were used to determine the amplification efficiencies and copy numbers.

**1.11 Alloreactivity analysis**

Alloreactivity of universal CAR T cells was measured as previously described [6]. Allogeneic PBMCs obtained from healthy donors were first labelled with 5 μM carboxyfluorescein succinimidyl ester (CFSE) (Dojindo Laboratories) at room temperature for 10 minutes and then cocultured with CAR T or universal CAR T cells pre-exposed to 10 μg/mL mitomycin C at a PBMC-to-CAR T cell ratio of 5:1. After cocultured for 48 and 72 hours, the percentage of $CFSE^+CD3^+$ cells among the PBMCs was determined by flow cytometry, and the total number of viable cells was determined by 0.4% trypan blue staining. The number of $CD3^+$ cells among the PBMCs was determined by the following equation: $CD3^+$ cell number = percentage of $CFSE^+CD3^+$ cells × total number of viable cells.

**1.12 Statistics**

Statistical analyzes were performed with GraphPad Prism version 5 for Windows. The results are shown as the means ± SDs. The significance of differences between groups was determined by t tests, and P values less than 0.05 were considered statistically significant. Full clinical data are presented for the two patients, and testing of statistical significance was not applicable.

**References**

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lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood*. 2008;111(12):5446-5456.

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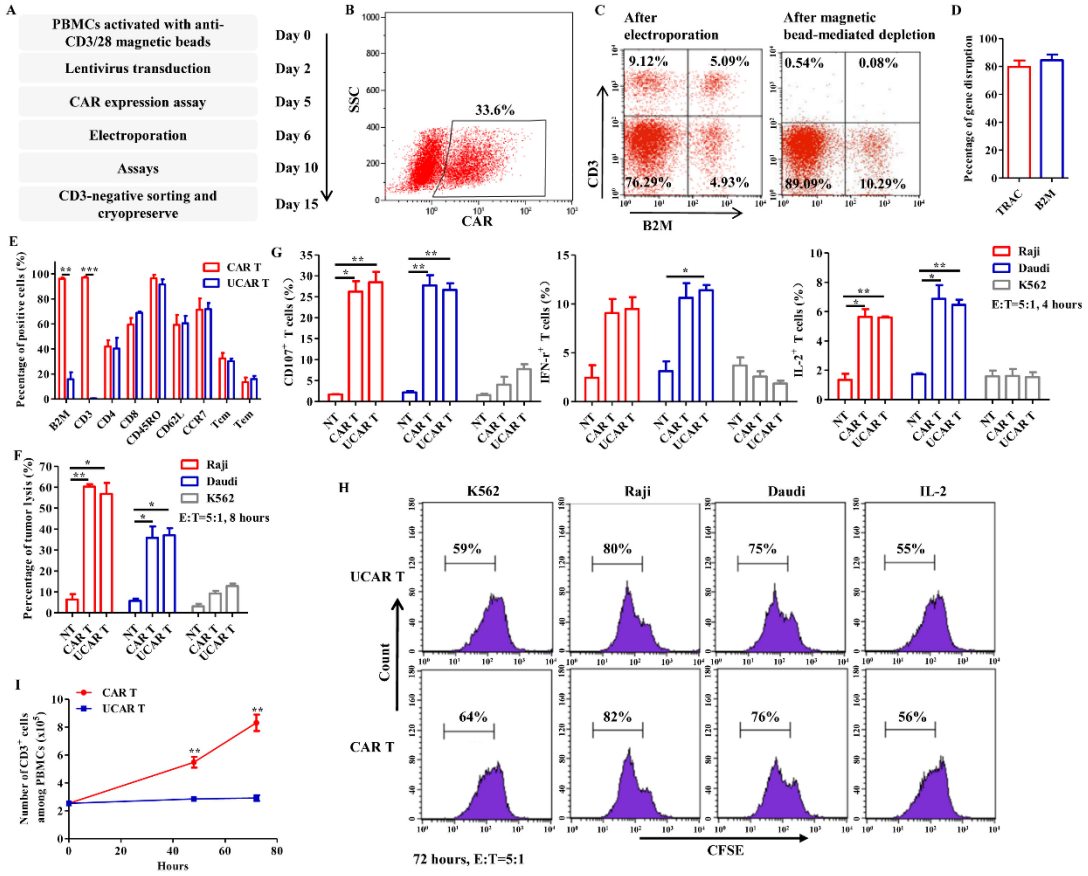
## 2. Supplemental Figures

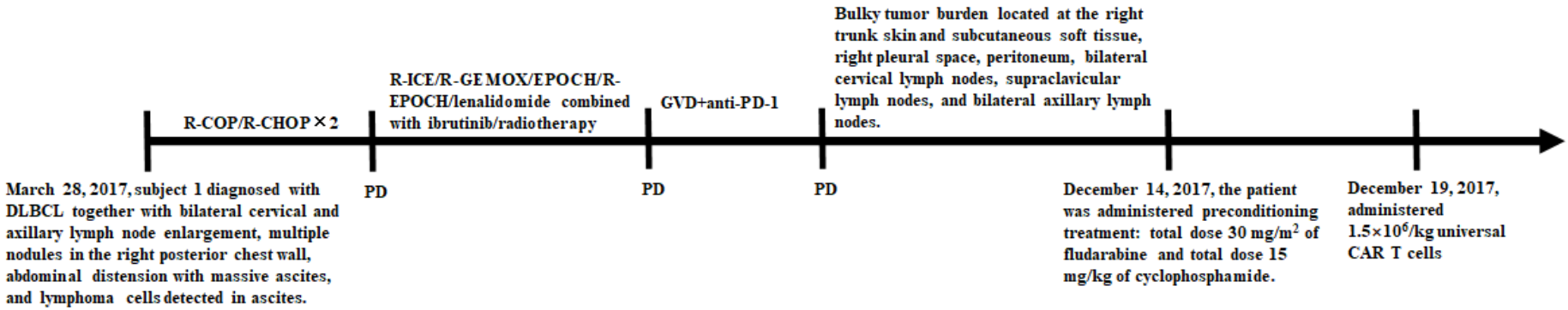
### Supplemental Figure 1




**Supplemental Figure 1 Generation, characteristics and specific cytotoxicity of universal CAR T cells.** (A) Universal CAR T (UCAR T) cells were successfully generated from healthy male adult non-HLA-matched volunteers' PBMCs according to standard operating procedures under Good Manufactured Practice and cryopreserved after 15 days of culture. Off-target mutations sites of TRAC and B2M sgRNA were predicted in CRISPOR (crispor.tefor.net), and 10 potential sites of each sgRNA in universal CAR T cells were chosen and genotyped and no any off-target mutations was detected (Supplemental Table 1 and 2). In addition, translocation events in infused universal CAR T cells were detected, with translocations occurring the least frequently (Supplemental Figure 7). (B) Representative CAR expression was detected after lentivirus transduction for 3 days. (C) Representative CD3 and B2M expression was detectable after electroporation and magnetic bead-mediated depletion of residual TCR-expressing cells, respectively. (D) TRAC and B2M gene-editing efficiencies in universal CAR T cells before depletion of residual TCR-expressing cells were measured by TIDE analysis. (E) Phenotypes of the universal CAR T cells. Tcm, central memory T

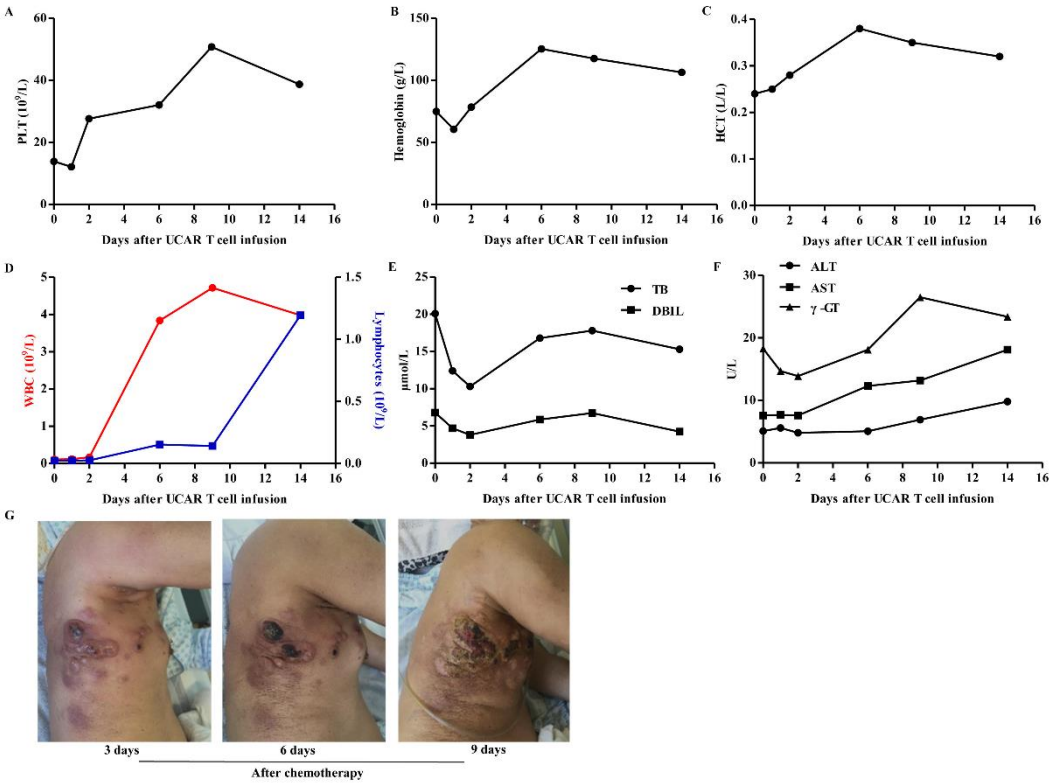
cells; Tem, effective memory T cells. CAR T represents cells from the same donor transduced for CAR and manufactured with no gene editing. (F) Specific tumor lysis of universal CAR T cells. The results of an 8-hour apoptosis analysis for $CD19^+$ Raji and Daudi cells and $CD19^-$ K562 cells after coculture with universal CAR T cells at an effector-to-target ratio of 5:1. NT represents cells from the same donor manufactured with no CAR transduction and with no gene editing. CD19 expression in Daudi, Raji and K562 cells is presented in Supplemental Figure 6. (G) CD107a and intracellular IFN-γ and IL-2 expression in universal CAR T cells after coculture with Raji, Daudi and K562 cells at an effector-to-target ratio of 5:1 for 4 hours. The numbers represent the percentages of positive cells. (H) Proliferation of universal CAR T cells. CFSE-labelled universal CAR T cells cocultured with Raji, Daudi and K562 cells at an effector-to-target ratio of 5:1 for 72 hours. The numbers represent the percentages of proliferating cells. (I) Alloreactivity of universal CAR T cells. Allogeneic PBMCs cocultured with mitomycin C-pretreated CAR T and universal CAR T cells at a PBMC-to-CAR T cell ratio of 5:1 and the number of $CD3^+$ cells among allogenic PBMCs were calculated for 48 and 72 h. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, t-test, GraphPad Prism version 5.

**Supplemental Figure 2**



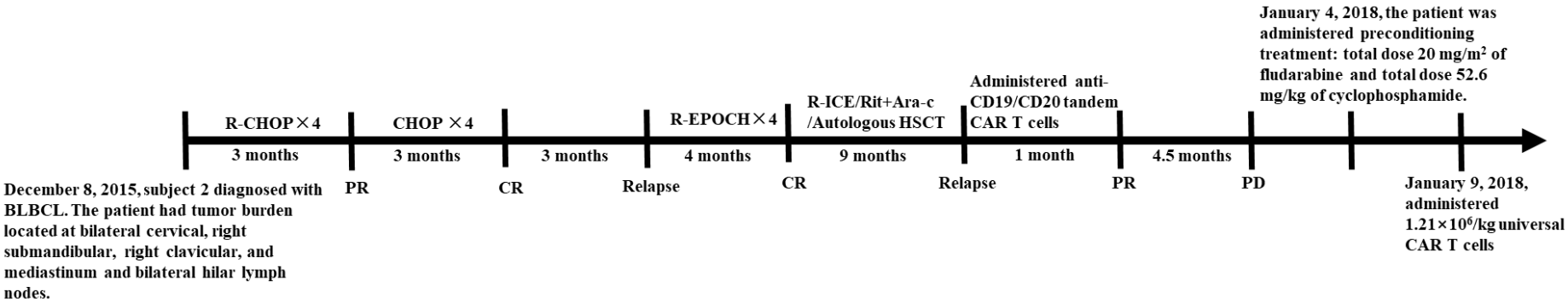

**Supplemental Figure 2** Schema of the clinical protocol for subject 1 before and after universal CAR T cell infusion. EPOCH, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin; GVD, gemcitabine, vinorelbine and liposomal doxorubicin; R-CHOP, rituximab, cyclophosphamide, hydroxydaunomycin, oncovin and prednisone; R-COP, rituximab, cyclophosphamide, vincristine and prednisone; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin; R-GEMOX, rituximab, gemcitabine and oxaliplatin; R-ICE, rituximab, ifosfamide, cyclophosphamide and etoposide.

**Supplemental Figure 3**



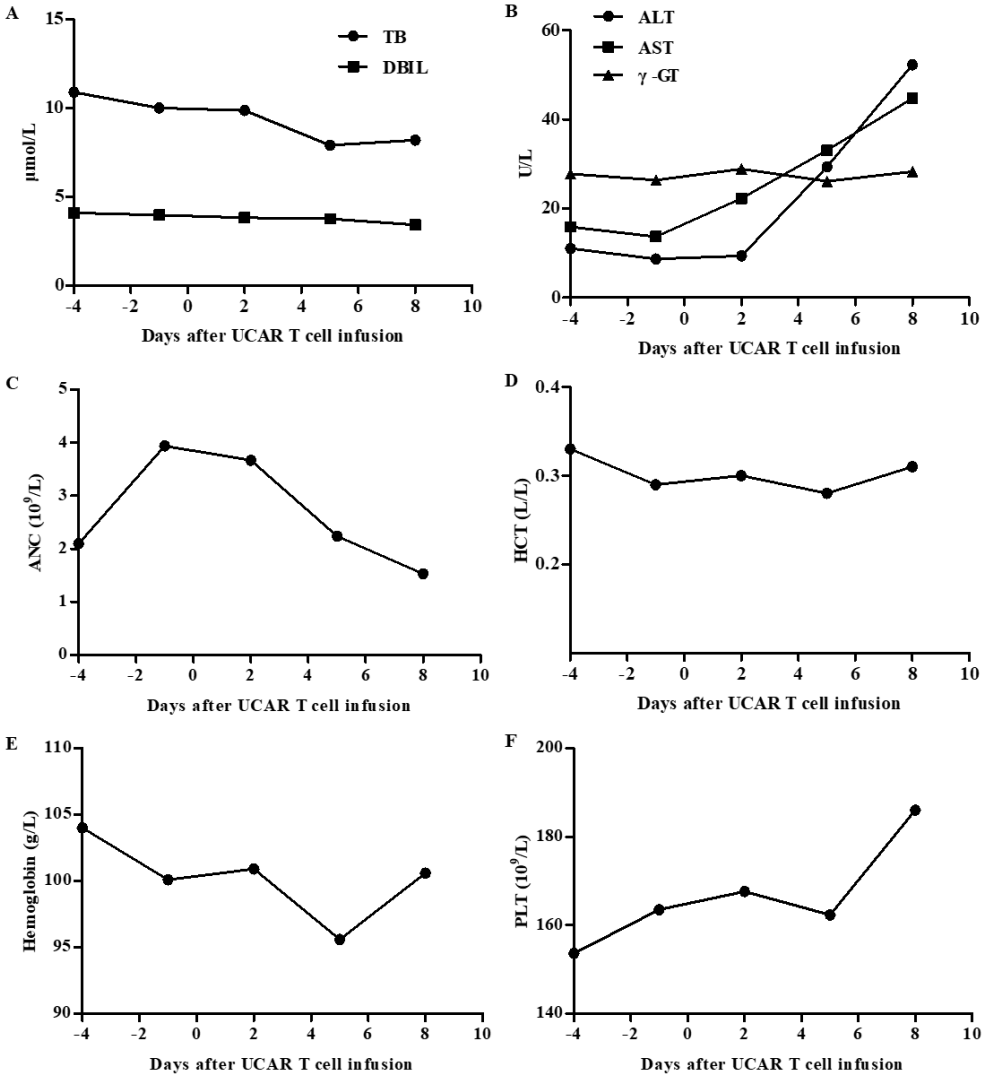

**Supplemental Figure 3 Toxicities in subject 1 after administration of universal CAR T cells.** (A-D) The change in platelets (PLT), hemoglobin, hematocrit (HCT), white blood cells (WBC, red line) and lymphocytes (blue line) after universal CAR T (UCAR T) cell infusion. (E) The changes in total bilirubin (TB) and direct bilirubin (DBIL) after universal CAR T cell infusion. (F) The changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (γ-GT) after universal CAR T cell infusion. (G) Skin damage was observed after that the patient received a previous chemotherapy treatment before universal CAR T cell infusion.

**Supplemental Figure 4**



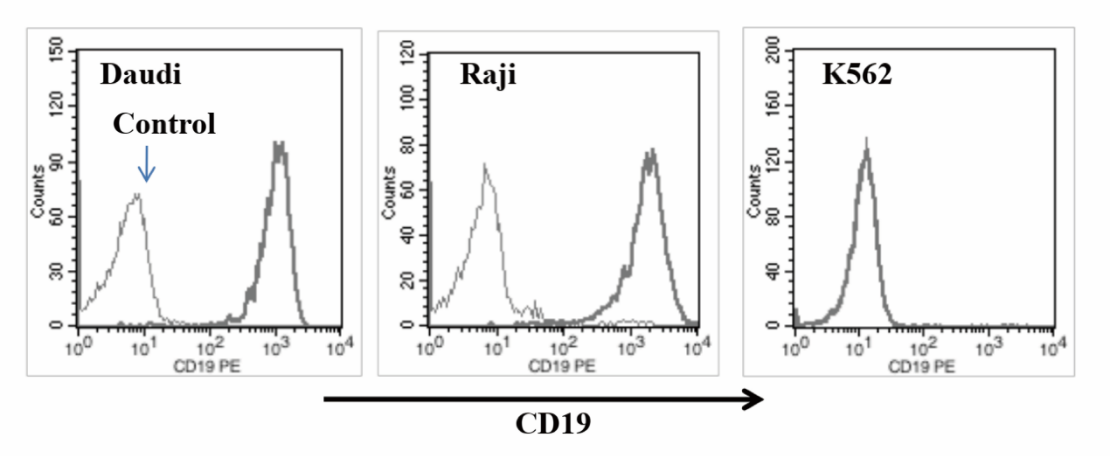

**Supplemental Figure 4** Schema of the clinical protocol for subject 2 before and after universal CAR T cell infusion. The patient achieved partial response within 1 month after tandem CAR T cell infusion. His disease relapsed with CD19-positive DLBCL within 5.5 months after tandem CAR T cell infusion. In addition, his disease was still CD19-positive before universal CAR T cell infusion. Ara-c, aracytine; CHOP, cyclophosphamide, hydroxydaunomycin, oncovin and prednisone; R-CHOP, rituximab, cyclophosphamide, hydroxydaunomycin, oncovin and prednisone; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin; R-ICE, rituximab, ifosfamide, cyclophosphamide and etoposide; Rit, radioimmunotherapy.

**Supplemental Figure 5**



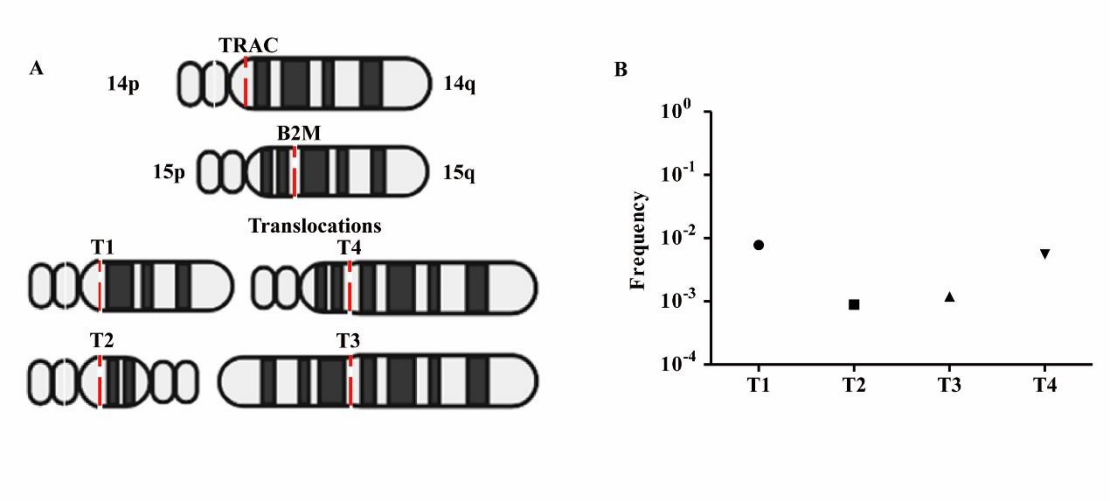

**Supplemental Figure 5 Toxicities in subject 2 after administration of universal CAR T cells.** (A) The changes in total bilirubin (TB) and direct bilirubin (DBIL) before and after universal CAR T (UCAR T) cell infusion. (B) The changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (γ-GT) before and after universal CAR T cell infusion. (C-F) The changes in absolute neutrophil value (ANC), hematocrit (HCT), hemoglobin, and platelets (PLT) before and after universal CAR T cell infusion.

**Supplemental Figure 6**




**Supplemental Figure 6** CD19 expression in Daudi (human Burkitt's lymphoma cell line), Raji (human Burkitt's lymphoma cell line) and K562 (human chronic myelogenous leukemia cell line) cells as determined by flow cytometry. These tumor cells were incubated with an anti-human CD19 antibody (BD), and gates were established by using FMO controls (CD19).

**Supplemental Figure 7**




**Supplemental Figure 7** Chromosomal translocation events. (A) Schematic representation of four expected translocations (Chromosome images created with BioRender.com). (B) Translocation frequency in universal CAR T cells before cell infusion. To evaluate the probability of such translocation events, the translocation frequencies of all four possible events that could be derived from the simultaneous editing of TRAC on chromosome 14 and B2M on chromosome 15 were quantified using Q-PCR.

**3. Supplemental Tables**

**Supplemental Table 1 Off-target candidates genotyping**

| Locus name | Chromosome | Position | Off-target sequence | LocusDesc |
|---|---|---|---|---|
| TRAC-sgRNA on target | chr14 | 22547526 | AGAGTCTCTCAGCTG GTACA CGG | exon_TRAC |
| Offset 1 | chr20 | 50274872 | AGAGTCATGCAGCTG GTACA TGG | intron_LINC01272 |
| Offset 2 | chr4 | 10325337 | ACAGTTTTTCAGCAG GTACA AGG | intergenic_WDR1\|ZNF 518B |
| offset 3 | chr1 | 96020939 | ATAGTCACTTAGCTG GAACA GGG | intron_RP11-147C23.1 |
| offset 4 | chr3 | 40585566 | AGAGTCTCTCAACA GATGCA GGG | intergenic_ZNF621\|RP 11-528N21.1 |
| offset 5 | chr5 | 179597791 | AGGGCCACTCAGCT GGTAAA GGG | intron_RP11-1379J22.2 |
| offset 6 | chr17 | 51198084 | ACAGTCTCTGAGTTA GTACA GGG | intron_MBTD1 |
| offset 7 | chr4 | 896864 | AGGGTGTCTCACCTG GCACA AGG | intron_GAK |
| Offset 8 | chr3 | 133976680 | AGAGTCTCACAGCA AGTCCA TGG | intron_SLCO2A1 |
| Offset 9 | chr6 | 65654636 | ATGGACTCTCAGCTG GTATA GGG | intron_EYS |
| Offset 10 | chrX | 53604954 | ATGGTCTCTCACCTG GTAAA TGG | intron_HUWE1 |
| B2M-sgRNA on target | chr15 | 44711556 | CGCGAGCACAGCTA AGGCCA CGG | exon_B2M |
| Offset 11 | chr19 | 2269912 | CGAGAGCACTGCTA AGGCCG GGG | intergenic_OAZ1/LLfo s-48D6.2\|OAZ1 |
| Offset 12 | chr14 | 94314414 | GGACAGCATAGCTAA GGCCA TGG | exon_SERPINA6 |
| Offset 13 | chr12 | 12716487 | CCCAAGCACAGTTAA GGCCA TGG | intergenic_GPR19\|CD KN1B |
| Offset 14 | chr5 | 177093209 | CAGGAGCACAGCCA AGGCCA GGG | exon_FGFR4 |
| Offset 15 | chr17 | 80649746 | GGAGAGCACTGCTG AGGCCA AGG | intron_RPTOR |
| Offset 16 | chr17 | 3930329 | CACGAGCACGGACA AGGCCA TGG | exon_ATP2A3 |
| Offset 17 | chr2 | 29216330 | CCCTAGCACAGCTAG GGCCA GGG | intron_ALK |
| Offset 18 | chr4 | 3823530 | CCCAAGCACAGCGA AGGCCA AGG | intergenic_ADRA2C\|A C226119.5 |

| | | | | |
|---|---|---|---|---|
| Offset 19 | chr12 | 122207858 | TGTGAGGACAGCTG AGGCCA AGG | exon_B3GNT4/DIABLO/RP11-512M8.5 |
| Offset 20 | chrX | 130117894 | GGGGAGATCAGCTA AGGCCA GGG | intergenic_ELF4\|AIFM1 |

Note: Ten potential off-target sites hit for each sgRNA were predicted in CRISPOR (crispor.tefor.net).

**Supplemental Table 2 Genotyping primers for off-target analysis**

| | Forward | Reverse |
|---|---|---|
| Offset 1 | TCGTCGGCAGCGTCCGCACCCTGG CAGTGACT | GTCTCGTGGGCTCGGAACTGTTCCG CCTGTTCTCC |
| Offset 2 | TCGTCGGCAGCGTCGCGCTGAAGG TCATACTTGC | GTCTCGTGGGCTCGGGGTATATTGA GTGAAACAGGTTTGT |
| Offset 3 | TCGTCGGCAGCGTCAGGCAAACAG TTCTCCATAGGT | GTCTCGTGGGCTCGGTCAAAGATGA GAGTGATTTAGGCA |
| Offset 4 | TCGTCGGCAGCGTCCTTCTGAGAG GGCCCAAAGG | GTCTCGTGGGCTCGGATTTGTTGCC ACAGACCACC |
| Offset 5 | TCGTCGGCAGCGTCAGAGAGAGC AGACTCCAGGC | GTCTCGTGGGCTCGGACAATGAAG GATCCTGGAGGG |
| Offset 6 | TCGTCGGCAGCGTCCAGTGTCTCG GTCCAGCATT | GTCTCGTGGGCTCGGTGGCAAGTTG AGGTGGAGTT |
| Offset 7 | TCGTCGGCAGCGTCCAAGTCCGCT TGCTGAGGA | GTCTCGTGGGCTCGGTGTCACAGA AGCTGCTCTGG |
| Offset 8 | TCGTCGGCAGCGTCCTGGAGCAGC CCCTAAAACA | GTCTCGTGGGCTCGGTGAGATGTTG AAGGGCAGGG |
| Offset 9 | TCGTCGGCAGCGTCACTGGGTGTC AAAAGGTTACAGA | GTCTCGTGGGCTCGGGTGAGAACA GAAGTGAGTGACCT |
| Offset 10 | TCGTCGGCAGCGTCTGGAAGCCTC TCAGATTTTGGA | GTCTCGTGGGCTCGGTGGCTCACCA AGTTTGAAAGT |
| Offset 11 | TCGTCGGCAGCGTCAGATCCGCCC CTTTGTCAAG | GTCTCGTGGGCTCGGAAATCCAACT CCGCACCCTT |
| Offset 12 | TCGTCGGCAGCGTCACCCTGGTGG ATCTCAGTCT | GTCTCGTGGGCTCGGTCATCTCCCC TGTGAGCATC |
| Offset 13 | TCGTCGGCAGCGTCGACGAGTTCC CACACGCAG | GTCTCGTGGGCTCGGTGTAGCTGTC TCAGACACGT |
| Offset 14 | TCGTCGGCAGCGTCGGACATCATC CTGTACGCGT | GTCTCGTGGGCTCGGGGAGAGCTT CTGCACAGTGG |
| Offset 15 | TCGTCGGCAGCGTCACTGCTTACT GCCCAGAAAGT | GTCTCGTGGGCTCGGCGTGGAAAA GCTTTCAGGCG |
| Offset 16 | TCGTCGGCAGCGTCGCCTACCTGT TGAGGGCATT | GTCTCGTGGGCTCGGTGTGAGGTGT TCGAGTCACG |
| Offset 17 | TCGTCGGCAGCGTCGCATGCTCTC TGTCCCTACC | GTCTCGTGGGCTCGGGAGACATCTA TGGCCCTCGC |
| Offset 18 | TCGTCGGCAGCGTCTGCCAATTCTT CCTCAAAGGC | GTCTCGTGGGCTCGGCTCCTTCCTC GCAGTGACAA |
| Offset 19 | TCGTCGGCAGCGTCGTCCTGTTGA GAGCACCAGG | GTCTCGTGGGCTCGGTGTGTTCTCT GATCTGCCCC |
| Offset20 | TCGTCGGCAGCGTCGTCCTCAGAG TGAAGCTGGG | GTCTCGTGGGCTCGGTCACTTTGTG CTCACTCCGG |

Note: data acquisition from CRISPOR. Offset 1 to 10 for TRAC-sgRNA, offset 11 to 20 for B2M-sgRNA.

**Supplemental Table 3 Genotyping primers for TIDE sequence**

| | Forward | Reverse |
|---|---|---|
| TRAC | TCGTCGGCAGCGTCACCCTGATCC TCTTGTCCCA | GTCTCGTGGGCTCGGTCGGTGAATA GGCAGACAGAC |
| B2M | TCGTCGGCAGCGTCATATAAGTGG AGGCGTCGCG | GTCTCGTGGGCTCGGAGGAGAGAC TCACGCTGGAT |

**Supplemental Table 4 TRAC and B2M locus specific primers**

| | Forward | Reverse |
|---|---|---|
| TRAC | AATCCTCCGGCAAACCTCTG | AGAGAGCCCTTCCCTGACTT |
| B2M | ACAGCAAACTCACCCAGTCT | GGTGCTAGGACATGCGAACT |