

Supplemental Methods

Preparation of CAR T cells

Lymphocytes were obtained from the patient through lymphocyte apheresis under protocols approved by the Ethic Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Written informed consent was obtained from the patient, in accordance with the Declaration of Helsinki. Peripheral blood mononuclear cells (PBMCs) were obtained using lymphocyte separation solution by the density gradient centrifugation at 700 g for 20 min. CD3⁺ T cells were isolated from the PBMCs by magnetic bead-conjugated anti-CD3 antibody (MiltenyiBiotec) and stimulated with anti-CD3/ anti-CD28 monoclonal antibody-coated magnetic beads (Thermo Fisher 11132D) in modified CTS™ OpTmizer™ T-Cell Expansion SFM (Thermo Fisher A10221-01) at 37 °C, 5% CO₂ for 18-24 h. Cells were then separately transduced with different lentiviral CAR vectors, and cultured for 10-14 days in vitro. The medium was renewed every 2-3 days and cell concentration was adjusted to 1.5-2.0×10⁶ /ml. The transduction efficiency and tumor cytotoxic effects of CAR T cells were monitored prior to infusion.

Phenotypic analysis by flow cytometry

Bone marrow mononuclear cells were stained with the following fluorescently-labeled monoclonal antibodies: BV421 anti-CD19 (Biolegend), PE-CY7 anti-CD10 (BDIS), Percp5.5 anti-CD34 (BDIS), Percp5.5 anti-CD38 (BD Pharmingen), V500 anti-CD45 (BDIS), PE anti-CD79b (Biolegend), PE anti-CD79a (BDIS), APC anti-CD22 (BDIS), FITC anti-TdT (Dako) and FITC anti-Igκ/Igλ (Dako). For intracellular proteins (TdT and CD79a) analysis, permeabilization process should be performed.

Cell-free DNA extraction

Peripheral blood (PB) samples (10 mL) were collected from patient using EDTA K2 anticoagulation tubes. Plasma cfDNA was extracted using the QiaAmp Circulating Nucleic Acid kit (Qiagen) with carrier RNA added before lysis.

Droplet digital PCR

MGB probes were designed using Primer Express 3.0.1 (supplementary table 1) and were synthesized by Invitrogen Company (Shanghai, China). ddPCR analysis was performed in a total reaction volume of 20 μ l. Droplets were generated in 8-well cartridges, using the QX200 droplet generator (Bio-Rad). Droplets were amplified and then read with a two-fluorescence detector (QX200, Bio-Rad). QuantaSoft version 1.7.4 (Bio-Rad) enabled determination of copy number of the samples. Samples were run in triplicate.

Copy number calculation of CAR

Genomic DNA of peripheral blood mononuclear cells was isolated. Absolute quantification of CAR gene copy number per microgramme genomic DNA was determined by ddPCR before and after infusion of CAR T cells. The sequences of the primers and probes were listed in supplementary table 1.

Supplementary table 1.		Sequences of primers used in ddPCR
<i>TP53</i> c.818G>A	Forward	TCTACTGGGACGGAACAGCTTT
	Reverse	CTCTGTGCGCCGGTCTCT
	Probe	AGGTGCATGTTTGTGCC
CAR22	Forward	CTGCCGCGCCAGTCA
	Reverse	CGGGCCGTTGCTGGTA
	Probe	ACCATCTGGTCTTATCTG
CAR19	Forward	CCGGCTGACCATCATCAAG
	Reverse	GGTCTGCAGGCTGTTCATCTT
	Probe	CAACAGCAAGAGCCAGG
Hu-CAR22	Forward	GGACACGGCGGTGTACTACTG
	Reverse	TGACCCCATATGTGCGAAAGCT
	Probe	CAGACTTCCTGGATACGAG