Distribution Measurements of Chimeric Antigen Receptor-Modified T Cells Against CD19

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Research article

Keywords: CAR-T, qPCR, Biodistribution, Tissue, Blood

Posted Date: September 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-63462/v1

Version of Record: A version of this preprint was published on February 25th, 2021. See the published version at https://doi.org/10.1186/s12885-021-07934-1.

Abstract

Backgroud: The unprecedented efficacy of chimeric antigen receptor (CAR) T-cell immunotherapy of $CD19^+$ B-cell malignancies has opened a new and useful way for the treatment of malignant tumor. Nonetheless, there are still formidable challenges in the field of CAR-T cell therapy, such as the biodistribution of CAR-T cells in vivo.

Methods: We demonstrated the distribution of CAR-T cells in the absence of target cells or with target cells in the mice and the dynamic changes in the patient blood over time after infusion were deteced by qPCR and FACS.

Results: CAR-T cells still proliferated in the mice without target cells and peaked at 2 weeks. However, CAR-T cells did not increase significantly in the presence of target cells within 2 weeks after infusion, but expanded at 6 weeks. In the clinical trial, we found that CAR-T cells peaked at 7-21days after infusion and can last for as long as 510 days in the peripheral blood of patients. Simultaneously, mild side-effects were noted which can be effectively controlled within two months in these patients.

Conclusions: CAR-T cells can expand themselves with or without target cells in mice. CAR-T cells can persistence for a long time in patients.

1 Background

Chimeric Antigen Receptor T (CAR-T) cell therapy is drawing more and more attention for the cure of relapsed or refractory (r/r) B cell malignancies, including acute lymphoblastic leukemia (B-ALL) and non-Hodgkin lymphoma (NHL) [1, 2]. The approval of two CAR-T cell products by the US Food and Drug Administration (FDA), Yescarta and Kymriah, have paved the way for the clinical availability of CAR-T cell therapy[3]. CAR-T cell therapy is currently being used in at least 270 active clinical trials worldwide[4].

Despite its successful use in patients with B-cell malignancies, there is a lack of substantive understanding of the actions of CAR T-cells in the human body. A typical multiphasic disposition profile of CAR-T cells consists of a rapid distribution phase leading to a time-restricted expansion phase, followed by contraction and prolonged persistence phases.

To date, there are no available standardized methods for monitoring in vivo behaviors of injected CAR T-cells. Although imaging methods such as the use of radioisotope-labeled cells, genetically engineered cells (e.g., green fluorescent protein expression), and nanoparticle-labeled cells (e.g., iron-dextrannanoparticles) have been used recently to characterize the distinct pharmacokinetic (PK) profiles of CAR-T cells[5], the most commonly used techniques to identify CAR-T cells in the body, such as flow cytometry, biopsy/immunohistochemistry (IHC), enzyme-linked immunosorbent (ELISpot), and polymerase chain reaction (PCR) cannot be discarded. Because the imaging methods can only monitor the CAR-T cells in a short time, common methods for a long time monitoring are needed.

Unlike conventional drugs, CAR T-cells act as a “living drug” that can proliferate in the body. They also have significantly longer action than conventional chemotherapeutics and antibody drugs[6]. Therefore, animal models are generally recommended for evaluating cell therapies because basic information of the initial behavior, organ distribution, and targeting in vivo after cell infusion are important.

To determine the distribution of CAR-T cells after administration. we conducted *in vivo assays* using NCG mice with or without tumor cells and launched a small-scale clinical trial to study the pharmacokinetics of CD19 CAR-T in the blood of 13 B-NHL patients(NCT03528421), we found CD19 CAR-T cells can persist for a long time in mice and human.

2 Methods

2.1 Cell culture and CAR-T cell product manufacture

CD19 CAR-T cells are designed for the B-ALL and NHL by Beijing Immunochina Pharmaceuticals Co., Ltd. NALM6(ATCC) was cultured in RPMI 1640 containing FBS(10%, Wisent), L-glutamine (2 mmol/L, Gibco) and antibiotic-antimycotic (100x, Gibco). Peripheral blood mononuclear cells (PBMCs) were collected from volunteer’ (for preclinical studies) or patients’ (for clinical study) apheresis products, and $CD3^+$ T cells were purified and stimulated with CD3/CD28 Dynabeads. $CD3^+$ T cells were cultured in X-VIVO 15 medium (Lonza Group, Switzerland) supplemented with 100 U/mL of interleukin-2 (IL-2). The T cells were then transduced with CAR lentivirus within 48 hours and cultured for 9 days. Transduction efficiency and cell viability were examined on 5 to 7 days after CAR lentivirus transduction. When CAR-T cells were cultured to sufficient numbers for testing or patient infusion, cells were harvested and cryopreserved.

2.2 Biodistribution of CAR-T cells in NCG mice

Six-eight weeks immunodeficient NCG mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animal studies were approved by the Tsinghua University Animal Care and Use Committee (Beijing, China). 1×10^7 CD19 CAR-T cells in saline was intravenous injected into mice. Mice treated with saline only served as controls. Three hours, D2, D8, D15 after CAR-T infusion, the scheduled mice were intraperitoneally injected thiopentone sodium for anesthesia. After anesthesia, blood was collected from the large vein behind the abdominal cavity with a volume of about 0.5 mL(anticoagulants), and the remaining blood cells were removed by heart perfusion. Heart, lungs, liver, kidneys, spleen, brain, stomach, duodenum, uterus, ovaries, testis, epididymis, bone marrow, adipose tissue and skeletal muscle were collected for CAR-T cell detection. To establish the tumor model, NSG mice were injected with 1×10^6 NALM6 cells via the tail vein. Five days later, mice were intravenous injected with 5×10^6 CD19 CAR-T cells in saline. Mice treated with saline only served as controls. Five minutes, 30 min, 1 h, 3 h, D1, D2, D7, D14, D28, D42 and D56 after CAR-T infusion, blood(anticoagulants) was acquired the for CAR-T cell detection by qPCR and Flow cytometry. At 3 h, D2, D7, D14, D28, D42 and D56 after CAR-T infusion, six animals (3male, 3 female) were scarified every time and the organs were collected described above for CAR-T cell detection by qPCR.

In my study, all the animals were sacrificed at set time points, and no animals were euthanized. If any, the animals were euthanized by carbon dioxide asphyxiation.

2.3 qPCR methods for CAR detection

For the qPCR assay to detect CAR-T cells, DNA from different tissues was extracted using a DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions, while the concentrations of eluted DNA were quantified using UV spectrophotometry and adjusted to a suitable concentration range. Primers and probes for CAR-T cells were designed and synthesized by Biomed Biotech (Beijing, China) as listed in Table 1. The PCR experimental conditions were: 95˚C for ten minutes, followed by 40 cycles at 95˚C for 5 seconds, 55˚C for 15 seconds and 72 ˚C for 35 seconds. We used concentrations that were 1, 5, and 25 dilutions based on 10–10^6 copies/μL plasmid of CAR as a standard.

Table 1
Sequences of primers and probes for CAR-T detection

CAR-T Primers	Sequence(5'-3')	Size(bp)
WF Primer	GGCTTTCATTTTCTCCTCCTTGTA	24
WR Primer	CGGGCCACAACTCCTCATAA	20
Probe	FAM-ATCCTGGTTGCTGTCTC-MGBNFQ	17

2.4 Flow cytometry methods for CAR detection

To validate the changes of CAR T-cells in the blood after injection, we performed flow cytometry assay. Red blood cells were removed using an RBC lysing buffer (Sigma Aldrich, MO) for 5 minutes, followed by washing and re-suspension in 1x HBSS containing 1% FBS. The separated cells were used with PE-conjugated anti-CD3 and FITC-conjugated anti-CAR and placed in 4°C for 30 minutes avoiding light followed by washing and re-suspension in 1x HBSS containing 1% FBS. Data were acquired from the stained cells using BD FACS Callibur cytometry (BD Biosciences). The results were evaluated with FlowJo software.

2.5 Clinical trial

The exploratory clinical trial was launched for r/r NHL patients who showed primary resistance or recurrence after conventional chemotherapy. Patients received intravenous injection of fludarabine and cyclophosphamide for 3 consecutive days to deplete endogenous lymphocytes before CAR-T cell infusion. Response was evaluated based on the Lugano response evaluation criteria[7]. Peripheral CAR-T cell number, adverse events including cytokine release syndrome (CRS), cell-related encephalopathy syndrome (CRES), routine blood analysis, and blood biochemistry were monitored during follow-up. CTCAE 5.0 (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm) and ASTCT criteria[8] were utilized to grade the adverse events.

2.6 Statistical analysis

All data represent the means ± standard deviations (SDs) of n values, where n corresponds to the number of mice used. The two-sided Wilcoxon-Mann-Whitney test was performed to test the differences. Analyses were performed using GraphPad Prism software. A threshold of $P < 0.05$ was statistically significant for all analysis.

3 Results

3.1 Production of CD19 CAR-T cells

CAR-T cells targeting CD19 utilized CAR lentivirus carrying a FMC63-derived CD19-specific single-chain variable fragment (scFv), a 4-1BB co-stimulatory domain that can improve the expansion, persistence, and antitumor effect of CAR-T cells[9, 10], and a CD3ζ signaling domain (Fig. 1A). The T cells were purified and transduced with CAR lentivirus and then cultured for 9–13 days to form CAR-T cells **(**Fig. 1B**)**. Quality controls of CAR-T cells including viability, potency, copy number, replication-competent lentivirus (RCL), sterility, mycoplasma, and endotoxin were performed. The CAR-T cells used for all the pre-clinical and clinical studies met the defined specifications of Immunochina Pharmaceuticals.

3.2 CAR-T cells proliferated without target cells in NCG mice

Most people believed that without target cells in the mice, the CAR-T cells will disappear in a short time after infusion. To verify this point, we produced CAR-T cells targeting CD19 and transferred the cells to NCG mice through their tail vein. These mice were sacrificed at set times indicated in the Fig. 2. **A** and organs were collected for the CAR testing by qPCR. The results demonstrated that CAR-T cells still proliferated without target cells in mice. The number of CAR-T cells increased markedly in every tissue especially in spleen, peaking at 2 weeks (Fig. 2. **B and Sup. Table 1**). The AUC showed that the spleen had the most CAR copies which decreased significantly in the order of blood, lung, kidney, liver, heart, bone marrow. In the Brain, muscle and reproductive organs, low CAR copies were detected (Fig. 2. **C and Sup.**Table2).

Table 2
copies of CAR gene in blood at different time points(copies/μg DNA)

Time	Female		Male		Total	
	Average	SD	Average	SD	□□□	SD
5 min	45135.5	29994.5	22675.7	6690.3	33905.6	23002.3
30 min	5223.4	1193.7	4219.6	1909.1	4721.5	1526.5
1 h	1615.1	889.4	1746.6	1045.9	1680.8	871.3
3 h	2000.6	1853.7	1446.2	620.9	1778.8	1380.8
1d	1664.2	410.8	2999.1	962.4	2331.7	986.2
2d	1787.9	1328.3	3852.3	1586.7	2820.1	1729.5
7d	1766.7	467.2	2023.3	466.4	1869.4	428.1
14d	746.2	410.1	1037.4	69.5	891.8	293.2
28d	711.6	/	641.3	/	676.5	49.7
42d	2132.1	522.0	18925.6	10244.5	12208.2	11711.1
56d	11348.7	15338.6	4543.3	4385.6	7946.0	10013.6

3.3 CAR-T distribution in the tumor-bearing mice

To evaluate the distribution of CD19 CAR-T cells with target cells, we took advantage of the xenograft mice model with NALM6 tumor infused with CD19 CAR-T cells derived from healthy human donors. At different time points after CAR-T cell infusion, the whole blood and tissues were gathered for CAR-T cell testing (Fig. 3A).

Copies of CAR gene were detected in the peripheral blood of all animals 5 minutes after CAR T cell infusion, which showed decreasing trend and dropped to the lowest level at day 28 after treatment, then increased at day 42, and decreased again at day 56 (Fig. 3B **and** Table 2).

Three hours after administration, the CAR-T cells were mainly detected in heart, liver, lung, and the lung content was the highest. CAR copies in spleen were detected in all animals 2 days after administration, followed by a gradual overall increase to a peak at day 56. CAR-T cell detection in most of the other tissues, such as kidney, brain, stomach, duodenum, fat, muscle, colon, testis, epididymis, showed a decreasing trend after 2–14 days, and then gradually increased to the highest level at day 42. According to the results of CAR copy detection in various tissues, the number of CAR copies in most tissues increased at day 42 after administration, indicating that the activation and amplification of CAR-T cells appeared in most tissues (Fig. 3.**C and Sup.**Table 3).

Table 3
Summary of CAR-T parameters in tissues and whole blood(Average)

Type Tissue	T_{max}	Cmax	$AUC_{0-1344h}$
	h	copies/μg DNA	h*copies/μg DNA
Heart	1008	8290.2	3165207.4
Liver	1008	30327.7	14164836.0
Spleen	1344	39484.9	22287294.0
Lung	1008	39389.1	20603898.0
Kidney	1008	23099.1	9489641.0
Brain	1008	3892.4	1581518.5
Uterus	1344	9159.5	1538796.4
Testis	1008	26255.8	9344230.5
Ovary	1344	2157.9	362522.2
Epididymis	1008	37075.9	14626642.0
Stomach	1008	43912.5	15893940.0
Duodenum	1008	15141.3	5315737.1
Fat	1008	45655.4	17008538.0
muscle	1008	34794.5	11934449.0
Colon	1008	8374.4	2996989.2
Blood	0.083	33905.6	6448893.4
Bone marrow	1344	8535.2	4017144.8

The statistical data showed that CAR-T cells were most distributed in the spleen, followed by lung, fat, stomach, epididymis, liver, muscle, kidney, testis, blood, duodenum, bone marrow, heart and other tissues. The organ distribution of CAR-T cells in tumor-bearing mice was influenced by the animal model, drug administration and cell characteristics, and the results were consistent with the distribution of cell products in vivo (Fig. 3.**D and** Table 3).

3.4 CAR-T distribution and the safety in NHL patients

The clinical trial was launched for r/r NHL patients who showed primary resistance to prior chemotherapies. The study was approved by Beijing Cancer Hospital, which was carried out from May

2018 to Nov 2019. There were 13 patients with diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), marginal zone lymphoma (MZL) (Table 4**)** enrolled in this study.

Table 4
Patient characteristics and treatment

Patient No.	Diagnosis	Prior treatment	Conditioning regimen before T cell infusion	CAR-T dosage
F0104	FL/II	≥2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0106	DLBCL	≥2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0107	FL/II	2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0109	DLBCL	≥2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0110	MZL	≥2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0111	DLBCL	≥2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0118	DLBCL	≥2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0119	DLBCL	≥2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0121	DLBCL	2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0122	DLBCL	2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0123	DLBCL	2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0125	DLBCL	2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg
F0126	DLBCL	2nd line	Flu25mg/m^2 + CTX250mg/m^2	1×10^6/kg

Abbreviations: MZL, marginal zone lymphoma; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; Flu, fludarabine; Cy, cyclophosphamide; CR, complete response; PD, progressive disease

All patients underwent preconditioning regimen to deplete endogenous lymphocytes before CAR-T cell infusion. After CAR-T cell infusion, patients were followed by monitoring disease response, peripheral CAR-T cell number, and adverse events including CRS, CRES, routine blood analysis, and blood biochemistry (Fig. 4. **A**). The first response was evaluated on day 28d, the CR rate was 46%(6/13), and 2 of the CR patients maintained remission over 15 months (Fig. 4. **B**). Expansion of CAR-T cells in peripheral blood was detected in all patients, which reached the peak on day 7–21. The persistence of CAR-T cells was detected up to 510 days (Fig. 4. **C and Sup.**Table 4). The average peak concentration of CAR-T cells was about 10^8/L (Fig. 4. **D and Sup. Table 5**).

About 44% (7/13) of the patients underwent grade 1 CRS and one of them developed grade 2. All the CRS patients recovered after receiving symptomatic treatment. None of the patients experienced CRES. Other side effects were summarized in Table 5. All the adverse events were effectively controlled within two months.

Table 5
List of other adverse events

Adverse events	Adverse events degrade			
	I	II	III	IV
Blood				
Decrease of leukocyte count		3	5	3
Decrease of lymphocyte count		3		4
Decrease of neutrophil count		3	1	7
Decrease of platelet count	1	3	1	
Increase of C-reactive protein	2			
Degree III leukocyte bone marrow suppression			1	
Anemia	2	3	2	
Increase of serum ferritin	1			
Liver				
Elevated Alanine aminotransferase	1			
Elevated Aspartate aminotransferase	1			
Pain				
Pain in the jaws				1
Muscular soreness	1			
Pain in the left ilium		1		
Immune globulin				
Decrease of immunoglobulin A	3			
Decrease of immunoglobulin G	3			
Decrease of immunoglobulin M	3			
Digestive tract				
Diarrhea		2		
Gastrointestinal reaction	1			
Sick			1	
Vomit			1	
Dental ulcer	1			

Adverse events	Adverse events degrade			
	I	II	III	IV
Respiratory tract				
Cough	1			
Upper respiratory infection	1	1		
Pneumonia			3	
Cardiovascular				
Nodal tachycardia	2			
Others				
Insomnia		1		
Elevated urinary white blood cells		1		
Feeble	1			
Headache	1			
Elevated thyroid stimulating hormone	1			

4 Discussion

CAR-T cell therapy is an effective new treatment for tumors [11–13] and two CAR-T cell products have been approved for clinical use by the U.S. FDA [14]. But the distribution and location of the cells remain unclear especially after a long term persistence in the mice or human body. The influence of CAR T-cell peak in blood on the efficacy of CAR-T cell treatment also needs further investigation. Therefore, we conducted the preclinical and clinical study to investigate the distribution of CAR-T cells.

In our tumor-bearing mouse model, CAR-T cells were widely distributed in the organs well-perfused with blood, including the spleen, lung, fat, stomach, epididymis, liver, muscle, kidney, testis, duodenum, bone marrow, heart. It extensively spreaded all over the organs from 4 weeks after administration and peaked between 6–7 weeks after administration. Significant proliferation of CAR-T cells was also found in the tissues of normal NCG mice lacking target cells. One reason to explain the phenomenon is that the TCR of CAR-T cells recognized the xenogenic MHCI in mice and were stimulated to proliferate.

The process of T cell distribution is complex, such as rolling and adhesion on vascular endothelial cells, chemokine-driven extravasation, and margination to specific tissues[15]. In different species, the process and characteristics of distribution must be diverse. So we studied the CAR-T cell number in the blood of patients. Of note, in our study the dynamic changes and peak of CAR-T cells were not much associated

with the therapeutic efficacy and the adverse events which is inconsistent with the published literature[16–18]. However, this observation needs further investigation.

To summarize, we demonstrated that CAR-T cells can locate in the organs in mice which indicated that CAR-T cells may also distribute in the tissues of human. In this study, we only focused on understanding the quantitative changes of CAR-T cells in the blood of patients, the evaluation of the whole-body disposition of CAR-T cells in humans will be necessary to clarify the relationship between distribution and efficacy of CAR-T cells. To date, many new techniques to monitor the cellular locations in human body have been developed, such as positron emission tomography(PET)[19, 20], bioluminescence imaging (BLI) [21]and so on. PET imaging of herpes simplex virus thymidine kinase 1(HSV1tk+)CAR- T cells coexpressing the CAR and the reporter gene of HSV1tk + within the same cell has been tested in patients with glioma[22]. We may detect the distribution of CD19 CAR-T cells in human body using one of these methods in the furture.

Early translation of CAR-T cells in human must focus on safety and efficacy[23]. Some clinical studies about CAR-T cell therapy have indicated that severe and occasionally fatal toxicities can occur [24–26]. CRS is a major toxicity [27]. CRES[28, 29] is also emerging as a challenge in the CAR-T cell therapies. Therefore, the prediction of side effects and efficacy is a significant project worth studying, and distribution research may lead to an important breakthrough.

5 Conclusions

CAR-T cells can expand themselves with or without target cells in mice.

CAR-T cells can persistence for a long time in patients.

6 Abbreviations

CAR Chimeric Antigen Receptor

CAR-T Chimeric Antigen Receptor-modified T cells

CR Complete Remission

CRS Cytokine Release Syndrome

CRES CAR-T-related encephalopathy syndrome

DLBCL Diffuse large B cell lymphoma

FL Follicular lymphoma

MZL Marginal zone lymphoma

TCR T cell receptor

Declarations

Ethics approval and consent to participate: All animal studies were approved by the Tsinghua University Animal Care and Use Committee (Beijing, China).

Ethics approval and consent to participate: This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board at Beijing Cancer Hospital. All participants were informed of the possible risks and side effects of the therapy and provided signed informed consent.

Competing Interests: The authors declare that they have no conflict of interest.

Funding: This study was supported by Beijing Natural Science Foundation (7202026), Capital's Funds for Health Improvement and Research (2020-2Z-2157), Science Foundation of Peking University Cancer Hospital (2020-5).

Author Contributions: SY and ZJ conceived and designed the study; LX, HX, HT, QF conducted the pre-clinical experiments; YZ, WX, ZW, LN, TM, XY, PL, ZC, LW, DL, WM performed clinical examinations; LX, DY, FF, LX, DT, SY and ZJ analyzed and interpreted the data; LX, YZ , SY and ZJ wrote the manuscript; all authors read and approved the final manuscript.

Acknowledgements We are grateful for all patients who participated in our exploratory clinical studies.

Consent for publication All authors consent for publication.

Availability of data and materials: please see the supplementary files.

References

1. Quintas-Cardama A. CD19 directed CAR T cell therapy in diffuse large B-cell lymphoma. Oncotarget. 2018;9:29843–4.
2. Quintas-Cardama A. CAR T-Cell Therapy in Large B-Cell Lymphoma. N Engl J Med. 2018;378:1065.
3. **FDA**. Approves Second. CAR T-cell Therapy. Cancer Discov 2018, 8:5–6.
4. Chapelin F, Gao S, Okada H, Weber TG, Messer K, Ahrens ET. Fluorine-19 nuclear magnetic resonance of chimeric antigen receptor T cell biodistribution in murine cancer model. Sci Rep. 2017;7:17748.
5. Wen H, Qu Z, Yan Y, Pu C, Wang C, Jiang H, Hou T, Huo Y. Preclinical safety evaluation of chimeric antigen receptor-modified T cells against CD19 in NSG mice. Ann Transl Med. 2019;7:735.
6. Weist MR, Starr R, Aguilar B, Chea J, Miles JK, Poku E, Gerdts E, Yang X, Priceman SJ, Forman SJ, et al. PET of Adoptively Transferred Chimeric Antigen Receptor T Cells with (89)Zr-Oxine. J Nucl Med. 2018;59:1531–7.

7. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, Lister TA, Alliance AL, Lymphoma G, Eastern Cooperative Oncology G, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014;32:3059–68.
8. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, Maus MV, Park JH, Mead E, Pavletic S, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Transplant. 2019;25:625–38.
9. Ying Z, He T, Wang X, Zheng W, Lin N, Tu M, Xie Y, Ping L, Zhang C, Liu W, et al. Parallel Comparison of 4-1BB or CD28 Co-stimulated CD19-Targeted CAR-T Cells for B Cell Non-Hodgkin's Lymphoma. Mol Ther Oncolytics. 2019;15:60–8.
10. Ying Z, Huang XF, Xiang X, Liu Y, Kang X, Song Y, Guo X, Liu H, Ding N, Zhang T, et al. A safe and potent anti-CD19 CAR T cell therapy. Nat Med. 2019;25:947–53.
11. Zheng PP, Kros JM, Li J. Approved CAR T cell therapies: ice bucket challenges on glaring safety risks and long-term impacts. Drug Discov Today. 2018;23:1175–82.
12. Frey N. Cytokine release syndrome: Who is at risk and how to treat. Best Pract Res Clin Haematol. 2017;30:336–40.
13. Smith L, Venella K. Cytokine Release Syndrome: Inpatient Care for Side Effects of CAR T-Cell Therapy. Clin J Oncol Nurs. 2017;21:29–34.
14. Titov A, Petukhov A, Staliarova A, Motorin D, Bulatov E, Shuvalov O, Soond SM, Piacentini M, Melino G, Zaritskey A, Barlev NA. The biological basis and clinical symptoms of CAR-T therapy-associated toxicites. Cell Death Dis. 2018;9:897.
15. Khot A, Matsueda S, Thomas VA, Koya RC, Shah DK. Measurement and Quantitative Characterization of Whole-Body Pharmacokinetics of Exogenously Administered T Cells in Mice. J Pharmacol Exp Ther. 2019;368:503–13.
16. Chimeric Antigen. Receptor-Modified T Cells in Chronic Lymphoid Leukemia; Chimeric Antigen Receptor-Modified T Cells for Acute Lymphoid Leukemia; Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. N Engl J Med. 2016;374:998.
17. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368:1509–18.
18. Emami-Shahri N, Foster J, Kashani R, Gazinska P, Cook C, Sosabowski J, Maher J, Papa S. Clinically compliant spatial and temporal imaging of chimeric antigen receptor T-cells. Nat Commun. 2018;9:1081.
19. Keu KV, Witney TH, Yaghoubi S, Rosenberg J, Kurien A, Magnusson R, Williams J, Habte F, Wagner JR, Forman S, et al: **Reporter gene imaging of targeted T cell immunotherapy in recurrent glioma**. *Sci Transl Med* 2017, 9.
20. Moroz MA, Zhang H, Lee J, Moroz E, Zurita J, Shenker L, Serganova I, Blasberg R, Ponomarev V. Comparative Analysis of T Cell Imaging with Human Nuclear Reporter Genes. J Nucl Med.

2015;56:1055–60.
21. Dobrenkov K, Olszewska M, Likar Y, Shenker L, Gunset G, Cai S, Pillarsetty N, Hricak H, Sadelain M, Ponomarev V. Monitoring the efficacy of adoptively transferred prostate cancer-targeted human T lymphocytes with PET and bioluminescence imaging. J Nucl Med. 2008;49:1162–70.
22. Berger C, Flowers ME, Warren EH, Riddell SR. Analysis of transgene-specific immune responses that limit the in vivo persistence of adoptively transferred HSV-TK-modified donor T cells after allogeneic hematopoietic cell transplantation. Blood. 2006;107:2294–302.
23. Gauthier J, Turtle CJ. Insights into cytokine release syndrome and neurotoxicity after CD19-specific CAR-T cell therapy. Curr Res Transl Med. 2018;66:50–2.
24. Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: Mechanisms, manifestations and management. Blood Rev. 2019;34:45–55.
25. Neelapu SS. Managing the toxicities of CAR T-cell therapy. Hematol Oncol. 2019;37(Suppl 1):48–52.
26. Hirayama AV, Turtle CJ. Toxicities of CD19 CAR-T cell immunotherapy. Am J Hematol. 2019;94:42–9.
27. Cohen AD, Garfall AL, Stadtmauer EA, Melenhorst JJ, Lacey SF, Lancaster E, Vogl DT, Weiss BM, Dengel K, Nelson A, et al. B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma. J Clin Invest. 2019;129:2210–21.
28. Chou CK, Turtle CJ. Assessment and management of cytokine release syndrome and neurotoxicity following CD19 CAR-T cell therapy. Expert Opin Biol Ther. 2020;20:653–64.
29. Acharya UH, Dhawale T, Yun S, Jacobson CA, Chavez JC, Ramos JD, Appelbaum J, Maloney DG. Management of cytokine release syndrome and neurotoxicity in chimeric antigen receptor (CAR) T cell therapy. Expert Rev Hematol. 2019;12:195–205.

Figures

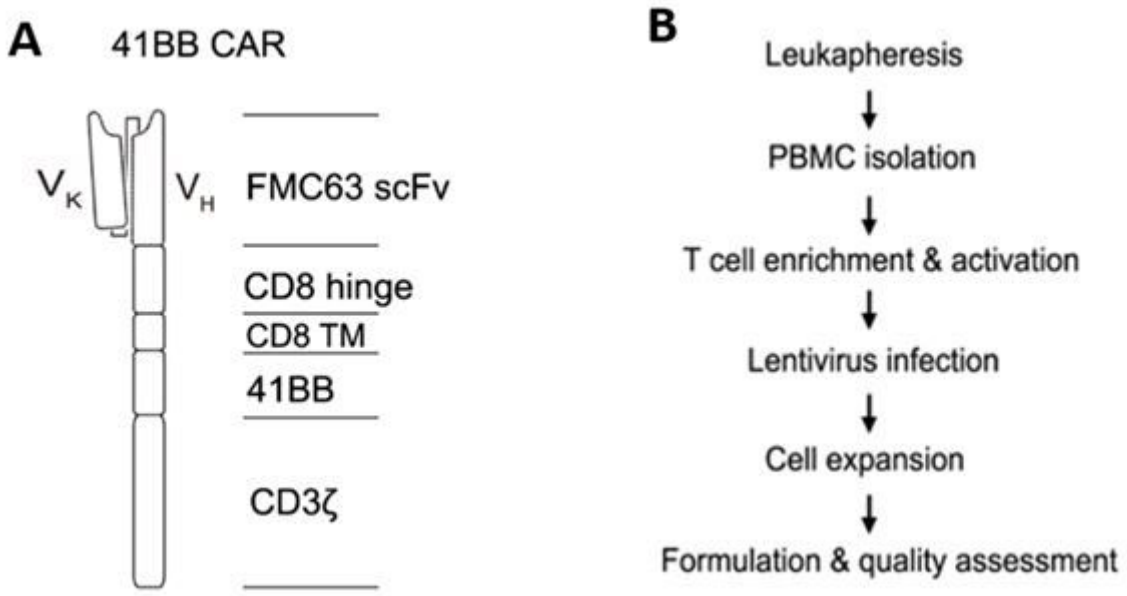

Figure 1

The basic information of CD19 CAR-T A. The construction of CAR. FMC63-derived scFv with a 4-1BB co-stimulatory domain and a CD3ζ signaling domain. B. The manufacturing time of CAR-T products. CD3+ T cells were purified from PBMC and stimulated with CD3/CD28 Dynabeads. The T cells were then transduced with CAR lentivirus within 48 hours and cultured for 9 days.

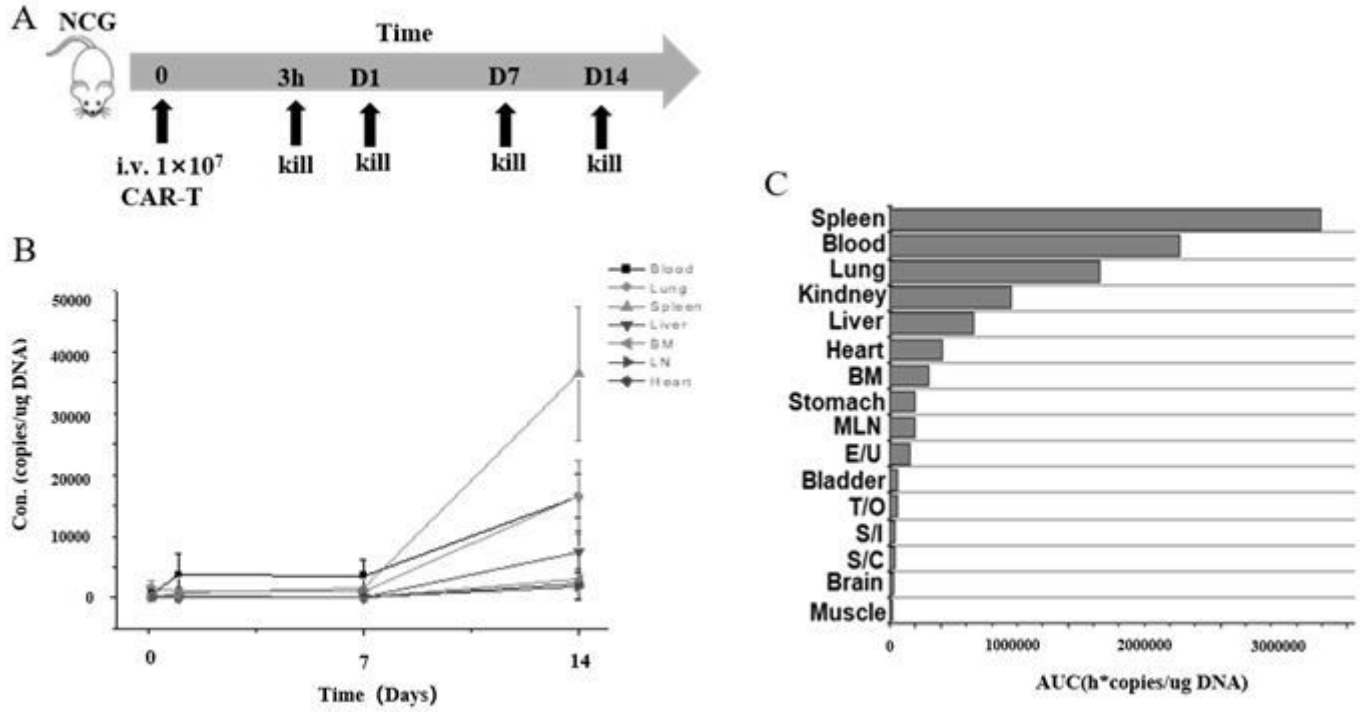

Figure 2

CAR-T distribution in the NCG mice A. Flow diagram of the experiment. Mice were sacrificed at 3h, day1,day7 and day14 after CAR-T infusion and the organs were collected to test the CAR gene copies. B. Changes of CAR-T cells in the organs over time. C. Tissue exposure of CAR-T cells in NCG mice.

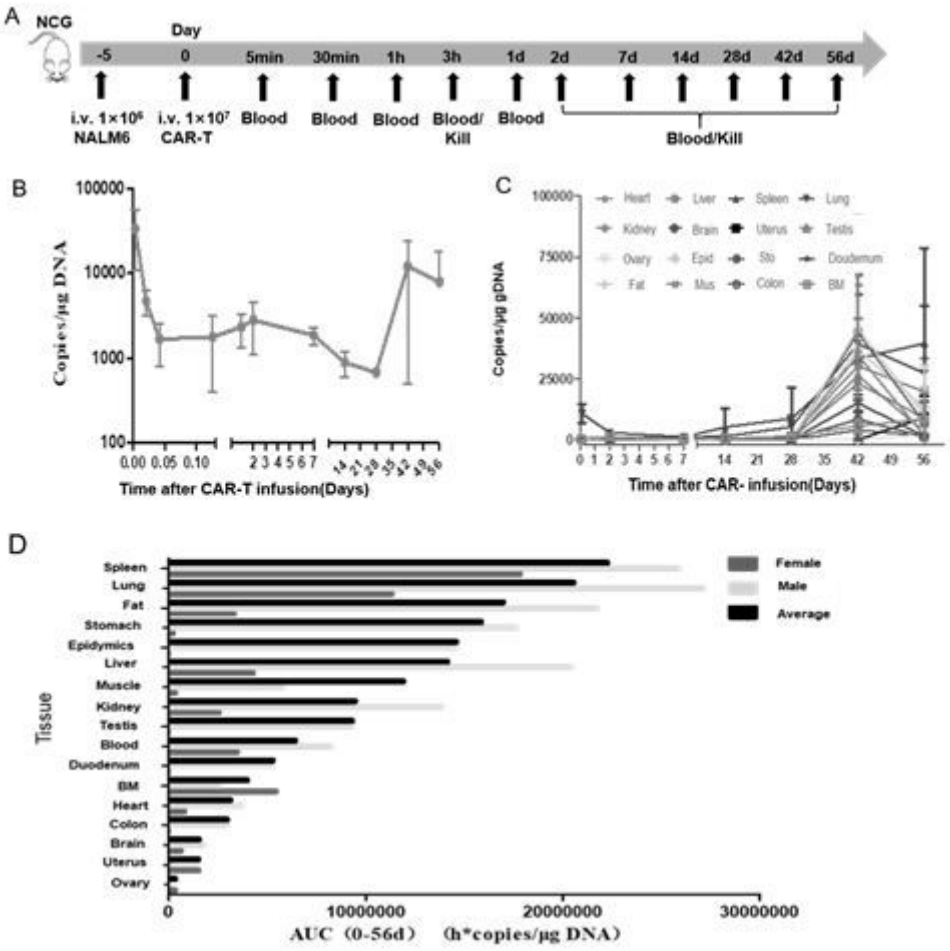

Figure 3

CAR-T distribution in the tumor-bearing mice. A. Flow diagram of the experiment. Blood an organs were taken from the tumor-bearing mice at different times(blood: 5mins, 30mins, 1h and day1; blood and organs:3h, day2, day7, day14,day28,day42 and day56) after CAR-T cell transplantation. B. Changes of CAR-T cell distribution in the blood over time. DNA from blood were extracted and detected the CAR gene copies by qPCR. C. Changes of CAR-T cell distribution in tissues over time. DNA from 16 organs were extraced and detected the CAR gene copies by qPCR. D. AUC of CAR-T cells in tissues and whole blood.

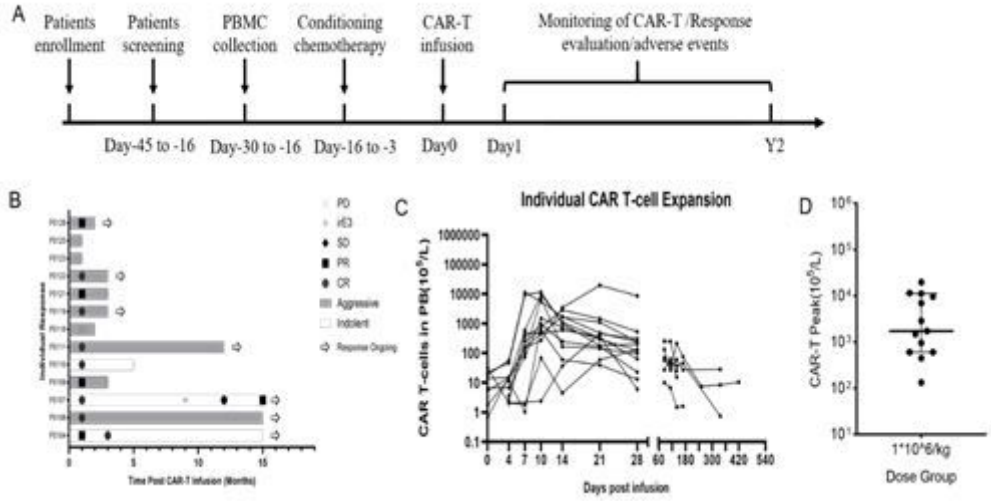

Figure 4

CAR-T distribution in NHL patients A.Flow diagram of the experiment. After CAR-T cell infusion, 13 patients were followed by monitoring disease response, peripheral CAR-T cell number, and adverse events including CRS, CRES, routine blood analysis, and blood biochemistry. B. DOR of 13 patients. The first response was evaluated on day 28d and the longest monitoring was 15 months. C. Changes of CAR T cells in peripheral blood after transfusion in 13 patients. Red blood cells were removed followed by washing and re-suspension. The separated cells were stained with PE-conjugated anti-CD3 and FITC-conjugated anti-CAR. Data were acquired from the stained cells using BD FACS Callibur cytometry. The results were evaluated with FlowJo software. D. Average CAR-T cell peak in 13 patients. The average peak concentration of CAR-T cells was about 108/L detected by FACS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DistributionmeasurementsofCD19CARTcellschecklist.doc
- supplementaryfile.docx
- ARRIVEchecklist.docx