Supplementary materials

S1. Supplementary methods

Study inclusion and exclusion criteria.

Inclusion Criteria:

1. Patients with CD19+ Refractory or Relapsed B-ALL (At least 2 prior combination chemotherapy regimens)
2. Aged between 2 to 75 years
3. Blasts in blood ≤ 30%
4. ECOG score ≤3
5. Women of childbearing potential must have a urine pregnancy test taken and proven negative prior to the treatment. All patients agree to use reliable methods of contraception during the trial period and until follow-up for the last time.
6. Voluntary participation in the clinical trials and sign the informed consent.

Exclusion Criteria:

1. Intracranial hypertension or unconsciousness
2. Respiratory failure
3. CD19 negative
4. Disseminated intravascular coagulation
5. ALT /AST>3 x normal value; Creatinine> 1.5 x normal value; Bilirubin >2.0 x normal value
6. Hematosepsis or uncontrolled active infection
7. Uncontrolled diabetes
8. Abnormal mental status
9. WHO Score >3
10. Patients who are pregnant or breastfeeding
11. Previous treatment with any gene therapy products
12. Any uncontrolled medical disorders that the researchers considered would render the patient ineligible to participate the clinical trial
13. Any situation that would increase dangerousness of subjects or disturb the outcome of the clinical study according to the researcher's evaluation.

Disease assessment

Immunophenotype analysis by flow cytometry (FCM), CD19 monoclonal antibodies (BD) were used for immunotyping. FACScanto II FCM, FACSDiva and Cell Quest pro software was used. laser 8 color flow cytometer was used to detect minimal residual disease (MRD). The Canto II flow cytometer was bought from BD bioscience company. The software was Diva 8.01 version. Most of the fluorescent antibodies were bought from BD bioscience, too. According to our routine direct stain protocol, 2X106 cells were added into the tube, and then fluorescent antibodies. Using cytoplasmatic CD79a(cCD79a) conjugated

with APC to set rough B gate, the two tubes panels were CD38 FITC/CD10 PE/CD34 PerCP/CD19 PE CY7/cCD79a APC/CD20 APC CY7/CD45 V500 and TdT FITC/CD81 PE/CD34 PerCP/cCD79a APC/CD10 PE CY7/CD45 V500. After staining for 15 minutes in room temperature, lysing and washing method were applied. One million live cells were acquired, and the sensitivity was 10^{-4}.

MRD positive criteria: 20 normal BM samples were detected as normal control, and B hematogones' patterns were observed and recorded. MRD positive cases were recognized if there were some aberrant immunophenotyping. For example, CD10bright(bri)/CD38dim, or CD45neg/CD19bri, the more patterns which different from normal (DFN) were observed, the more certain MRD results. Cytogenetic analysis was performed by the G-banding technique described in International Naming System for Human Cytogenetics (ISCN, 2016) [1].

Genetic testing methods, fusion genes, mutation genes

In our laboratory, fusion genes of the BM of the patients were detected simultaneously by multiplex nested PCR reactions. These fusion genes were as follows: AML1/ETO. CBFβ/MYH11. MLL/AFX. SIL/TAL. MLL/AF1q. MLL/AF. E2A/PBX1. NPM/ALK. PM/MLF. AML1/MDS. MLL/AF4. TEL/PDGFR. NPM/RARα. DEK/CAN. MLL/AF6. SET/CAN. MLL/AF9. TEL/ABL. BCR/ABL. MLL/AF10. dupMLL. PLZF/RARα. MLL/AF17. MLL/ENL. MLL/ELL. TEL/AML1. PML/RARα. TLS/ERG. E2A/HLF. HOX11. EVI1. ZNF198-FGFR1. NUP98-HOXA9. The sensitivity of real-time quantitative PCR is 10-5 RNA dilution. Next generation DNA sequencing technology was used to detect common gene mutations as defined by ECOG and The Cancer Genome Atlas Research Network [2, 3]. According to the characteristics of different gene mutations, some of the gene mutations were detected by DNA sequencing (detection sensitivity of about 5-20%). Other mutations were detected by capillary electrophoresis (detection sensitivity of about 2%) and DNA sequencing. For mut/wt> 20%, DNA sequencing was performed to determine the specific base changes or sequence alternations; for mut/wt <20%, due to the lower than detection sensitivity of gene sequencing, no additional sequencing was performed. We focus our sequencing effort on the mutation hotspots or the coding regions of 58 genes known to be frequently mutated in hematologic malignancies, including ALK, ASXL1, ASXL2, BCL2, BRAF, CALR, CARD11, CBL, CCND1, CD79B, CEBPA, CREBBP, CRLF2, CSF3R, CXCR4, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA2, ID3, IDH1, IDH2, IL7R, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MEF2B, MPL, MYD88, NOTCH1, NOCTH2, NPM1, NRAS, NT5C2, PAX5, PHF6, PIK3CA, PTEN, PTPN11, RHOA, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, STAT3, TCF3, TET2, TNFAIP3, TP53, U2AF1, WHSC1, WT1.

S2. Subgroups with no statistically significant differences in OS & LFS

Figure A. Kaplan-Meier plots of OS & LFS for CAR-T dose $<3x10^5$ cells/kg vs. CAR-T dose $\geq 3x10^5$ cells/kg group

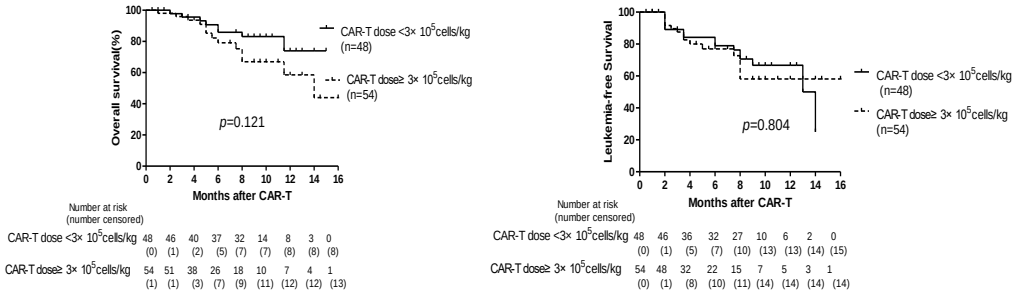


Figure B. Kaplan-Meier plots of OS & LFS for 2-14 years group vs. >14 years group

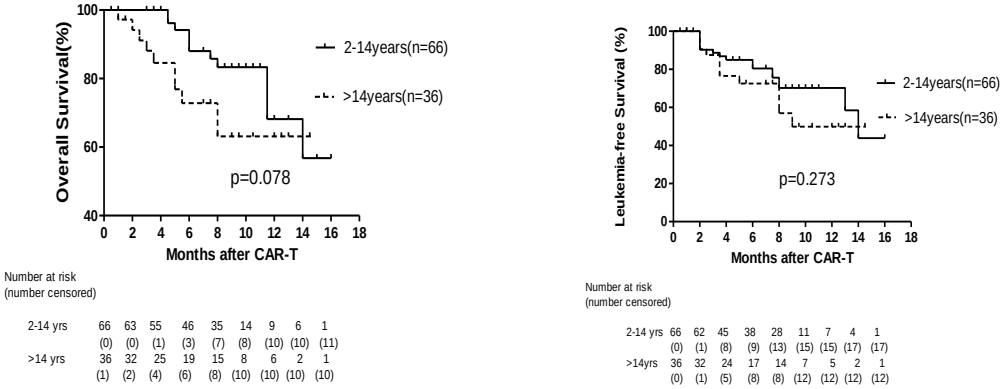


Figure C. Kaplan-Meier plots of OS & LFS of patients with EMD vs. without EMD

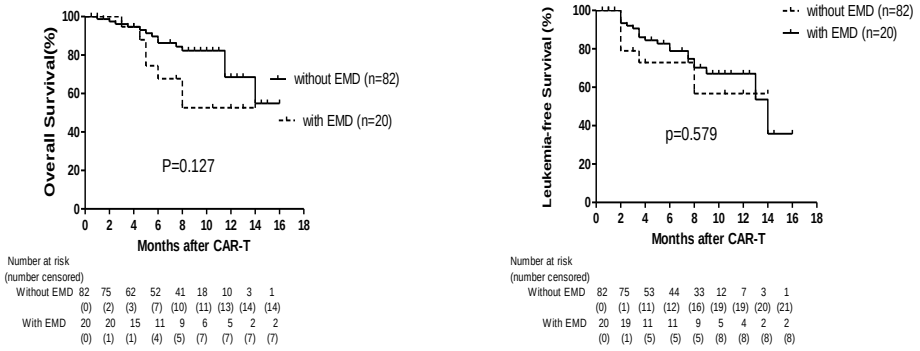


Figure D. Kaplan-Meier plots of OS & LFS for Patients with BM blasts ≤20% vs. BM blasts >20%

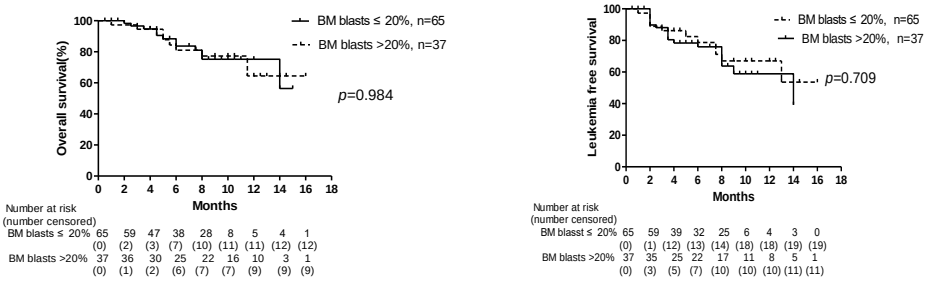


Figure E. Kaplan-Meier plots of OS & LFS for BM blast < 5% group vs.BM blast ≥5% group

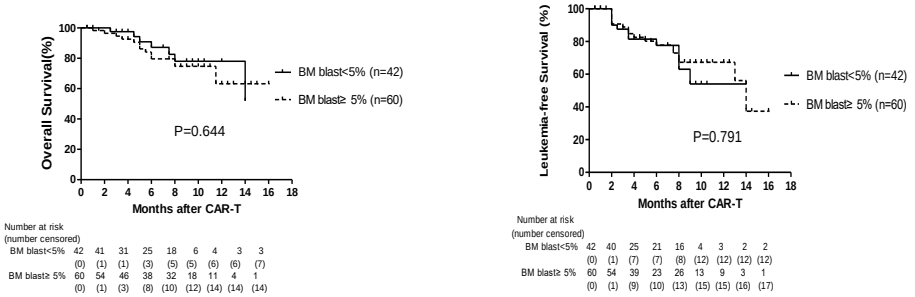
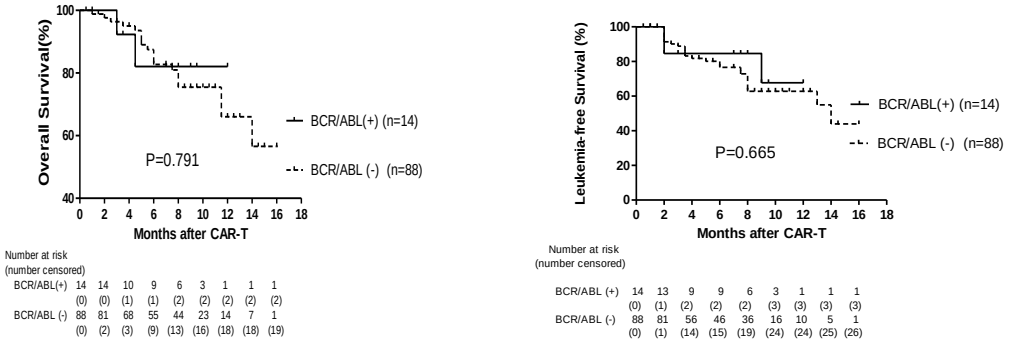
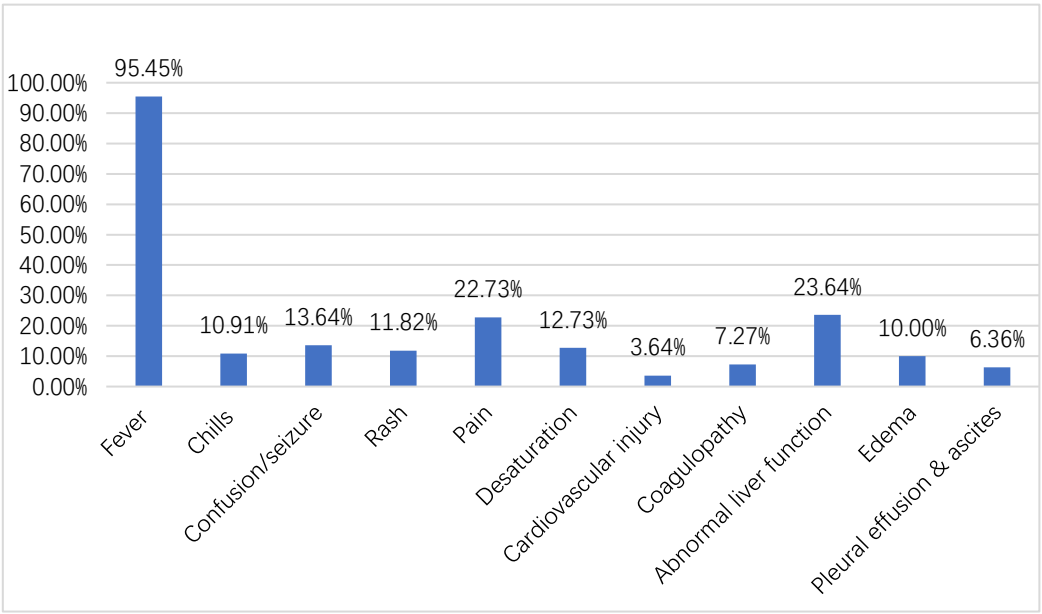


Figure F. Kaplan-Meier plots of OS & LFS of BCR/ABL (+) group vs. BCR/ABL (-)group



S3. Main adverse events after CAR-T treatment



References

1. McGowan J, Ottawa, Simons A, et al. An international system for human cytogenetic nomenclature. Cytogenetic and genome research 2016; vol.149, No.1-2.

2. Ley TJ, Miller C, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia, Cancer Genome Atlas Research Network. N Engl J Med 2013 May 30; 368(22):2059-74.

3. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med 2012 Mar 22; 366(12):1079-89.