SI Appendix

Exploratory trial of a biepitopic CAR-T–targeting B cell maturation antigen in relapsed/refractory multiple myeloma

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Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study:

1. Subjects 18~75 years of age.
2. Documented initial diagnosis of multiple myeloma according to IMWG diagnostic criteria.
3. Measurable disease at Screening as defined by any of the following:

Serum monoclonal paraprotein (M-protein) level ≥1.0 g/dL or urine M-protein level ≥200 mg/24 hours; or light chain multiple myeloma without measurable disease in the serum or the urine: Serum immunoglobulin free light chain ≥10 mg/dL and abnormal serum immunoglobulin kappa/lambda free light chain ratio.

4. Received at least 3 prior lines of treatment for multiple myeloma.

Undergone at least 1 complete cycle of treatment for each line, unless PD was documented by IMWG criteria as the best response to the regimen.

5. Received a PI and/or an IMiD.
6. Documented disease progression during, or within 12 months of, most recent anti-myeloma therapy.
7. ECOG Performance Status grade of 0 ~ 2.

8. Clinical laboratory values meeting the following criteria during the Screening Phase:

Hemoglobin ≥6.0 g/dL (without prior red blood cell [RBC] transfusion within 7 days before the laboratory test; recombinant human erythropoietin use is permitted).

Platelets $\geq 50 \times 10^9$/L (must be without transfusion support in the 7 days prior to the laboratory test).

Absolute Neutrophil Count (ANC) $\geq 2.0 \times 10^9$/L (prior growth factor support is permitted).

AST and ALT ≤2.5 × upper limit of normal (ULN).

Creatinine clearance ≥30 mL/min/1.73 m^2 based upon Modified Diet in Renal Disease formula calculation or a 24-hour urine collection.

Total bilirubin ≤1.5 × ULN; except in subjects with congenital bilirubinemia, such as Gilbert syndrome (in which case direct bilirubin ≤1.5 × ULN is required).

Corrected serum calcium ≤14.0 mg/dL (≤3.5 mmol/L) or free ionized calcium ≤6.5 mg/dl (≤1.6 mmol/L).

9. Women of childbearing potential must have a negative pregnancy test at screening and prior to the first dose of cyclophosphamide with or without fludarabine using a highly sensitive serum pregnancy test (β human chorionic gonadotropin).

10. When a woman is of childbearing potential the following are required:

Subject must agree to practice a highly effective method of contraception (failure rate of <1% per year when used consistently and correctly) and agree to remain on a highly effective method of contraception from the time of signing the informed consent form (ICF) until at least 100 days after receiving a LCAR-B38M CAR-T cell infusion.

11. Subject must sign an ICF indicating that he or she understands the purpose of and procedures required for the study and is willing to participate in the study. Consent is to be obtained prior to the initiation of any study-related tests or procedures that are not part of standard-of-care for the subject's disease.

12. Willing and able to adhere to the prohibitions and restrictions specified in this protocol.

Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study:

1. The following cardiac conditions:

New York Heart Association (NYHA) stage III or IV congestive heart failure

Myocardial infarction or coronary artery bypass graft (CABG) ≤6 months prior to enrollment

History of clinically significant ventricular arrhythmia or unexplained syncope, not believed to be vasovagal in nature or due to dehydration

History of severe non-ischemic cardiomyopathy

Impaired cardiac function (LVEF <45%) as assessed by echocardiogram or multiple-gated acquisition (MUGA) scan (performed ≤ 8 weeks of apheresis)

2. Systemic corticosteroid therapy of greater than 5 mg/day of prednisone (or equivalent dose of another corticosteroid) within 2 weeks prior to apheresis

3. Received either of the following:

An allogeneic stem cell transplant for multiple myeloma

An autologous stem cell transplant ≤12 weeks before apheresis

4. Seropositive for human immunodeficiency virus (HIV)

5. Hepatitis B infection as defined according to the American Society of Clinical Oncology (ASCO) guidelines. In the event the infection status is unclear, quantitative levels are necessary to determine the infection status.

6. Hepatitis C (anti-hepatitis C virus [HCV] antibody positive or HCV-RNA quantitation positive) or known to have a history of hepatitis C.

7. Serious underlying medical condition, such as:

Evidence of serious active viral, bacterial, or uncontrolled systemic fungal infection

Active autoimmune disease or a history of autoimmune disease within 3 years

Overt clinical evidence of dementia or altered mental status

8. Known life threatening allergies, hypersensitivity, or intolerance to LCAR-B38M

CAR-T cells or its excipients, including DMSO (refer to Investigator's Brochure)

9. Pregnant or breast-feeding, or planning to become pregnant while enrolled in this study or within 100 days after receiving study treatment.

10. Any uncontrolled diseases, other than multiple myeloma, that may lead to abnormal death.

11. Other cases excluded by the Investigators.

Preparation of LCAR-B38M CAR-T cells

Leukocytes were collected by apheresis, and then they were diluted with 0.9% NaCl solution. The diluted lymphocyte mix was layered on top of the lymphoprep medium (Fresonius Kabi, Oslo, Norway), and centrifuged at 800 g for 30 minutes at 20℃ without brakes. Lymphocyte buffy coat was then collected with a 10 ml pipette. The harvested fraction was wash twice with 0.9% NaCl. 2ml of 37℃ pre-warmed TexMACS GMP Medium (Miltenyi Biotec，Teterow，Germany) with interleukin-2 (IL-2) (Jiangsu Kingsley Pharmaceutical.Co.,Ltd, Yixing, China) was added to the cell pellet, and re-suspended softly. Cell number was determined following Trypan Blue staining, and the PBMC was ready for T cell isolation.

T cells were collected using Miltenyi Pan T cell isolation kit (Miltenyi Biotec，Teterow，Germany), following manufacturer's protocol as described and were either cultured fresh or cryopreserved and later thawed for culture. T cells were placed in TexMACS GMP Medium, supplied with IL-2, and incubated in a 37°C, 5% CO2 humidified incubator. After pre-activation with CD3/CD28 beads (Miltenyi Biotec，Teterow，Germany), the lentiviral vector containing LCAR-B38M was added and washed out on day 3 after culture initiation. Cells were expanded *ex vivo* for up to 11 days under IL-2 stimulation before harvest and preparation for infusion. All infused T cell products were required to meet predefined release criteria, including: cell viability

≥70%, $CD3^+$ cells ≥80%, endotoxin ≤3.5 EU/mL, mycoplasma negative, bacterial and fungal cultures negative, transduction efficiency by flow cytometry ≥5%.

The CAR-T cells were frozen during storage and transportation. They were put in 37℃ water bath until a suspended state is reached before infusion. Once thawed, the administration of cell delivery should be completed in 5 minutes.

Treatment administration

Approximately 4 weeks after apheresis, and after the site was notified in writing by the Legend team that manufacture of LCAR-B38M CAR-T cells had been completed, each subject would receive a conditioning regimen of cyclophosphamide 250 mg/m^2 intravenously (IV) daily and fludarabine 25 mg/m^2 IV daily for 3 days, or cyclophosphamide 300 mg/m^2 IV daily for 3 days. LCAR-B38M CAR-T cells will be administered 5 days after the start of the conditioning regimen (the first day of conditioning is Day -5 to Day -3, and the starting day of LCAR-B38M CAR-T cell infusion is Day 0). If administration of cyclophosphamide with or without fludarabine must be delayed ≥10 weeks after apheresis, the subject must undergo full rescreening prior to dosing. Cyclophosphamide ± fludarabine should be administered using administration procedures and supportive care according to the site's standard of care.

LCAR-B38M CAR-T cells were administered as summarized in the following table. The administration method includes one-time infusion and three-time infusion. Subjects should remain in the hospital for at least 2 weeks after infusion of LCAR-B38M CAR-T cells and would be discharged from the hospital per assessment of the subject's condition by the Investigator.

Dose	The dose was 0.2-2.0 $\times 10^6$ CAR-positive viable T-cells/kg. The maximum total dose of cells to be administered to any subject was 1.0 $\times$ 10^8 CAR-positive viable T-cells. The dose and administration schedule might be altered for safety purposes based on emerging data. If after apheresis and CAR-T cell preparation the quantity of LCAR-B38M CAR-T cells manufactured was not sufficient for dosing at the lower end of the dosing range, dosing for that subject may proceed, provided that a measurable quantity of LCAR-B38M CAR-positive viable T cells that pass quality testing were generated.
Route/Regimen	LCAR-B38M CAR-T cells IV infusion was to be administered under the supervision of site staff.
Dosing instructions	The actual dose for study treatment administration was based on the subject's weight (kg) at apheresis.
Schedule of administration	Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine (RJ), and Changzheng Hospital affiliated to Shanghai Second Military Medical University (CZ) used a split infusion method that a total dose of CAR-T cells was administered at day 0, day 2 and day 6 by split infusions (20%, 30% and 50% respectively). First Affiliated Hospital of Nanjing Medical University (JS) used single intravenous infusion of CAR-T cells.
Hospitalization recommendations after discharge	Subjects who experience a fever or an event of neurotoxicity after discharge from the hospital should be evaluated by the Investigator. Subjects might be re-hospitalized at the discretion of the Investigator. In case of urgency, subjects should go to the local hospital and the treating physician should contact Investigator.
Vital sign and clinical safety monitoring	On the day of infusion, blood routine, hepatic and renal functions, should be tested. Investigators examined the vital signs including temperature, blood pressure, heart rate and respiratory rate to make sure all were in normal prior to cell delivery. Vital signs and oxygen saturation should be monitored three times a day for at least two weeks post infusion.

Detection of CAR-T cells by fluorescence-activated cell sorter (FACS) and quantitative real-time polymerase chain reaction (qPCR)

Fresh PBMCs were suspended in FACS buffer (PBS containing 1% FBS, with or without 0.1% Na_3N) and stained with anti-human CD3 and FITC-labeled human BCMA protein (ACRO Biosystems). FACS data were collected using an LSRII flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and were analyzed with FlowJo software (TreeStar, Ashland, OR, USA).

For qPCR, genomic DNA was isolated from samples of whole blood obtained at serial time points before and after infusion of LCAR-B38M using ABI Taqman technology (Item 7500, Thermofisher, Singapore). A clonal cell line with known copy number of CAR was generated to produce standard curve. All samples were measured three times with a positive Ct value in 3/3 replicates with %CV less than 2%. A parallel amplification reaction was performed for apoB gene using 20ng genomic DNA to control the quality of interrogated DNA. Copies of transgene per microgram DNA were calculated from BCMA standard curve ×correction factor/amount DNA evaluated (ng) ×1000ng.

Supplementary Table 1. BCMA epitope peptide sequence

Code	Position	Amino acid residue sequence	Length
269EP001	1-10	MLQMAGQCSQ	10
269EP002	8-21	CSQNEYFDSLLHAC	14
269EP003	11-23	NEYFDSLLHACIP	13
269EP004	20-30	ACIPCQLRCSS	11
269EP005	24-42	CQLRCSSNTPPLTCQRYCN	19
269EP006	36-43	LTCQRYCNAS	10
269EP007	43-54	ASVTNSVK GTNA	12

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Supplementary Table 2. Information of CAR^+ T cells in product, conditioning therapy and method of infusion

ID	Fraction of CAR^+ T cells (%)	$CD4^+$ CAR^+ T cells (%)	$CD8^+$ CAR^+ T cells (%)	Conditioning therapy*	Method of infusion†
RJ01	58.5	38.9	61.1	FC	three-infusion
RJ02	35.0	62.5	37.5	FC	three-infusion
RJ03	35.0	64.3	35.7	FC	three-infusion
RJ04	14.5	30 59	69.4	FC	three-infusion
RJ05	42.6	17.1	82.9	FC	three-infusion
JS01	22.4	27.6	72.4	C	one-infusion
JS02	9.4	42.7	57.3	C	one-infusion
JS03	49.0	47.1	52.9	C	one-infusion
JS04	23.0	41.9	58.1	C	one-infusion
JS05	11.5	61.2	38.8	C	one-infusion
JS06	22.2	39.0	61.1	C	one-infusion
JS07	51.1	10.3	89.7	C	one-infusion
JS08	75.9	45.7	54.3	C	one-infusion
JS09	21.0	30.1	69.9	C	one-infusion
CZ01	11.5	40.3	59.7	FC	three-infusion
CZ02	22.9	25.3	74.7	FC	three-infusion
CZ03	12.3	31.4	68.7	FC	three-infusion

Note: * FC: Cyclophosphamide 250 mg/m^2 and fludarabine 25 mg/m^2 intravenously daily for 3 days; C: Cyclophosphamide 300 mg/m^2 intravenously daily for 3 days. † Two different delivery methods were used: three-infusion given at day 0, day 3 and day 6 in 8 patients; one-infusion given at day 0 in 9 cases.

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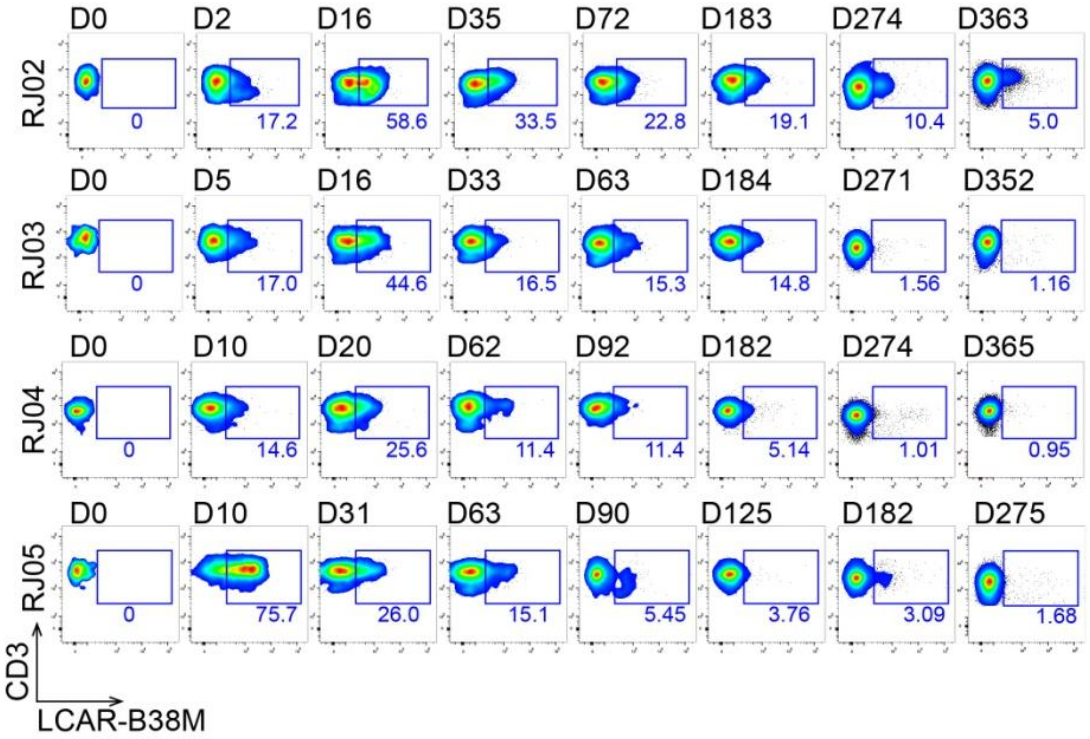
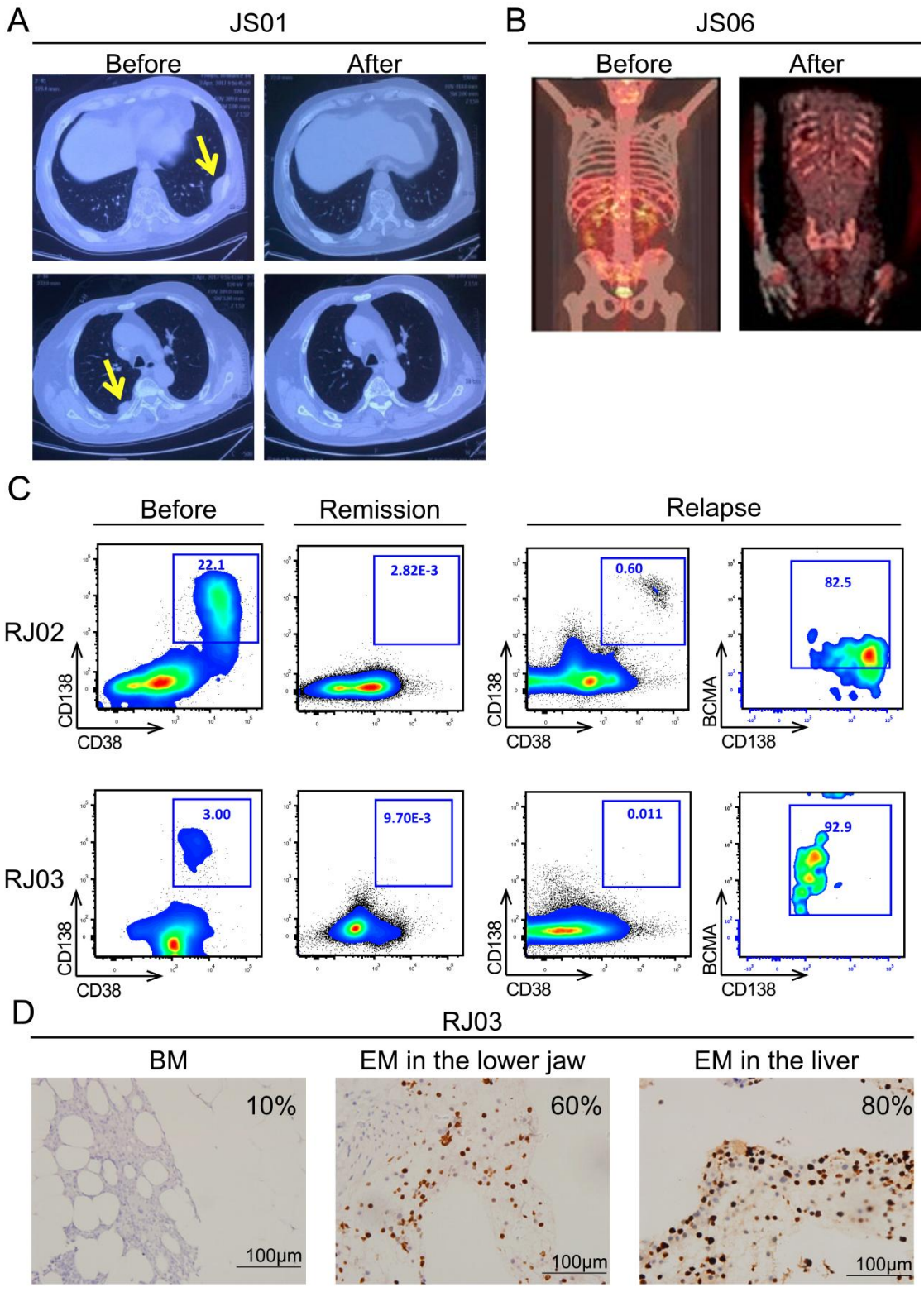
Figure S1



Supplementary Figure 1. CAR-T cells in the peripheral blood of treated patients. Flow cytometry shows the percentage of BCMA specific CAR-T cells in CD3 positive peripheral T cells of patient RJ02, RJ03, RJ04 and RJ05. The time course is indicated as days (D) after CAR-T infusion.

Figure S2

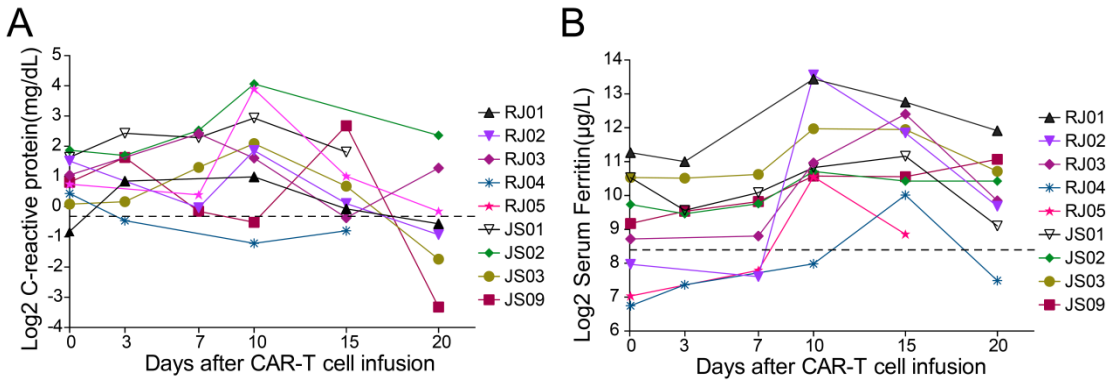


Supplementary Figure 2. The intra- or extramedullary involvements in treated patients.

(A) Patient JS01 developed extramedullary mass in pleura two years after diagnosis.

Massive plasma cells were seen in fine needle aspiration of the chest mass. One month after CAR-T therapy, CT scan of the chest revealed disappearance of pleural mass. **(B)** Patient JS06 had tumor infiltration in the pleura and peritoneum. All infiltrated lesions disappeared at 6 months post CAR-T**. (C)** Flow cytometry detection shows bone marrow (BM) plasma cells ($CD38^{+}CD138^{+}$) in patient RJ02 and RJ03 at different stages including before CAR-T therapy, remission or relapse after CAR-T treatment. The plots with $CD138^{+}BCMA^{+}$ indicate the plasma cells with expressed BCMA. **(D)** Ki-67 staining of BM and extramedullary (EM) tumor biopsies of patient RJ03 at initial CAR-T therapy.

Figure S3



Supplementary Figure 3. C-reactive protein (CRP), and ferritin levels in the serums of treated patients.

(A) The plot presents the CRP levels after infusion of LCAR-B38M. The elevated CRP was accompanied with CRS. The dashed line represents the normal upper limit of CRP. **(B)** The chart displays the ferritin levels after infusion of LCAR-B38M. The elevated ferritin was accompanied with CRS. The dashed line represents the normal upper limit of ferritin.