

Supplementary material

Materials and methods

Study procedure

This phase I clinical study (NCT05346198) was conducted in China at the First Affiliated Hospital of Zhejiang University School of Medicine. The experiment was performed according to a study protocol approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (approval number: PRO20200134). All patients agreed to participate in the study and provided written informed consent.

Eligibility criteria included an age of 18 years or older, a diagnosis of multiple myeloma, receipt of at least 3 previous lines of therapy (a proteasome inhibitor and an immunomodulatory agent), measurable disease (defined by any of the following: a monoclonal protein concentration in serum of at least 1.0 g/dL or in urine of at least 200.0 mg per 24 h; serum immunoglobulin free light chain at least 10.0 mg/dL and abnormal serum immunoglobulin kappa to lambda free light chain ratio), an Eastern Cooperative Oncology Group performance status score of 0 or 1, an expected survival time of ≥ 3 months, and adequate organ function. Additional eligibility criteria included B cell maturation antigen (BCMA) expression on $\geq 5\%$ of plasma cells and myeloma cells, confirmed by immunohistochemistry or flow cytometry. Patients were excluded if they had previously received treatment with a CAR T-cell-targeted or BCMA-targeted therapy. Other key exclusion criteria included known allergy or hypersensitivity reaction to any study treatment;

participation in another clinical trial within 4 weeks before single collection; previous autologous hematopoietic stem cell transplantation or allogeneic stem cell transplantation within 12 weeks before a single collection; previous live vaccine or attenuated vaccine therapy within 4 weeks before the BCMA CAR-T treatment; previous systemic steroid therapy (except inhalation or topical use), immunosuppressive therapy, graft vs. host therapy, or preventive central nervous system treatment within 7 days before the single collection, or as determined by the investigator to require long-term treatment during the study period; or any severe or uncontrolled illness or condition.

All patients received preconditioning chemotherapy consisting of fludarabine at 30 mg/m²/d and cyclophosphamide at 300 mg/m²/d for 3 consecutive days to deplete host lymphocytes. After a 2-day interval, all patients received a single dose of BCMA CAR-T at 2.5×10^6 , 5.0×10^6 , or 7.5×10^6 CAR-positive T cells/kg (participant body weight), according to the dose at enrollment. During the procedure, we closely monitored patients and provided essential support to prevent and treat CAR-T cell-associated toxicity (**Figure S1**).

Endpoints

The main purpose of this study was to evaluate the safety and tolerability of BCMA CAR-T and to determine the recommended dose for follow-up studies. DLT was defined as any investigational study drug-related grade ≥ 3 toxicity that occurred within the first 28 days and any grade 4 life-threatening toxicity. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for

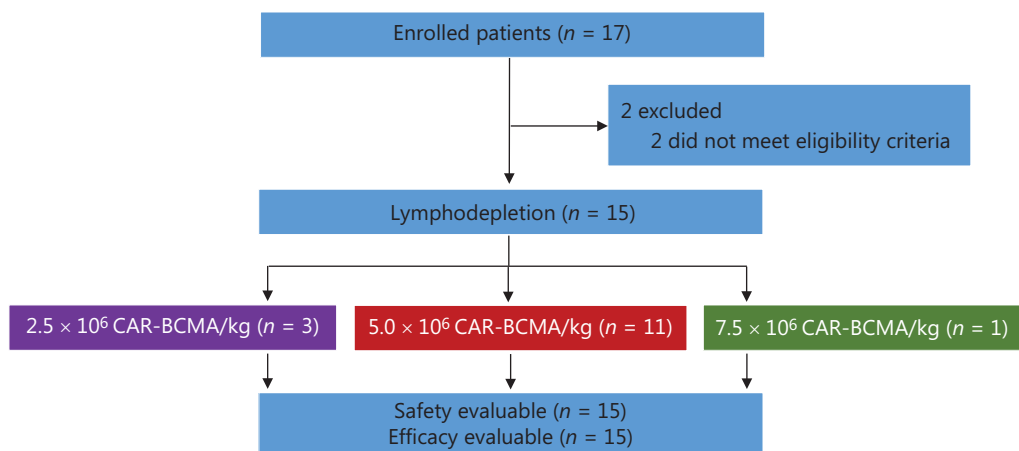


Figure S1 Study procedure. CAR-T, chimeric antigen receptor T cell; BCMA, B cell maturation antigen.

Table S1 Demographic and disease baseline characteristics of 15 patients with RRMM treated with CART-BCMA

	CART-BCMA dose (CAR positive T cells/kg)			Total (n = 15)
	2.5×10^6 (n = 3)	5.0×10^6 (n = 11)	7.5×10^6 (n = 1)	
Age (years), median (range)	63.0 (58.0–64.0)	62.0 (36.0–73.0)	64.0	63.0 (36.0–73.0)
Male, n (%)	2 (66.7)	5 (45.5)	1	8 (53.3)
Time since diagnosis (months), median (range)	52.8 (38.9–53.6)	47.6 (3.8–82.7)	56.8	52.8 (3.8–82.7)
Extramedullary plasmacytoma, n (%)	0	6 (54.5)	0	6 (40.0)
Proportion of plasma cells > 30% in bone marrow, n (%)	0	4 (36.4)	1	5 (33.3)
ECOG performance status, n (%)				
0	1 (33.3)	2 (18.2)	0	3 (20.0)
1	2 (66.7)	9 (81.8)	1	12 (80.0)
R-ISS stage, n (%)				
I	1 (33.3)	2 (18.2)	0	3 (20.0)
II	1 (33.3)	8 (72.7)	0	9 (60.0)
III	1 (33.3)	1 (9.1)	1	3 (20.0)
Cytogenetic abnormalities, n (%)				
High risk	3 (100)	6 (54.5)	0	9 (60.0)
Del (17p)	1 (33.3)	1 (9.1)	0	2 (13.3)
T (4;14)	1 (33.3)	1 (9.1)	0	2 (13.3)
T (14;16)	0	0	0	0
T (14;20)	0	0	0	0
1q21	2 (66.7)	5 (45.5)	0	7 (46.7)
Bridging therapy, n (%)	1 (33.3)	3 (23.1)	0	4 (23.5)
Previous \geq 4-line therapy, n (%)	2 (66.7)	8 (72.7)	1	11 (73.3)
Number of prior treatment lines, median (range)	5 (3–5)	5 (3–9)	4	5 (3–9)
Previous three-drug relapsed/refractory, n (%)	0	4 (36.4)	0	4 (26.7)

RRMM, relapsed or refractory multiple myeloma; CAR-T, chimeric antigen receptor T cell; BCMA, B cell maturation antigen; ECOG, Eastern Cooperative Oncology Group; R-ISS, Revised International Staging System.

Table S2 CRS-related treatment-emergent adverse events during the study

	2.5×10^6 CAR-BCMA/kg (n = 3)	5.0×10^6 CAR-BCMA/kg (n = 11)	7.5×10^6 CAR-BCMA/kg (n = 1)	Total (n = 15)
Grade \leq 2 CRS, n (%)	3 (100.0)	11 (100.0)	1	15 (100.0)
Fever	3 (100.0)	11 (100.0)	1	15 (100.0)
Anoxia	1 (33.3)	4 (36.4)	1	6 (40.0)
Hypotension	0	3 (27.3)	1	4 (26.7)
Shiver	1 (33.3)	0	0	1 (6.7)
Rash	1 (33.3)	0	0	1 (6.7)
Anorexia	0	1 (9.1)	0	1 (6.7)

CRS, cytokine release syndrome; CAR-T, chimeric antigen receptor T cell; BCMA, B cell maturation antigen.

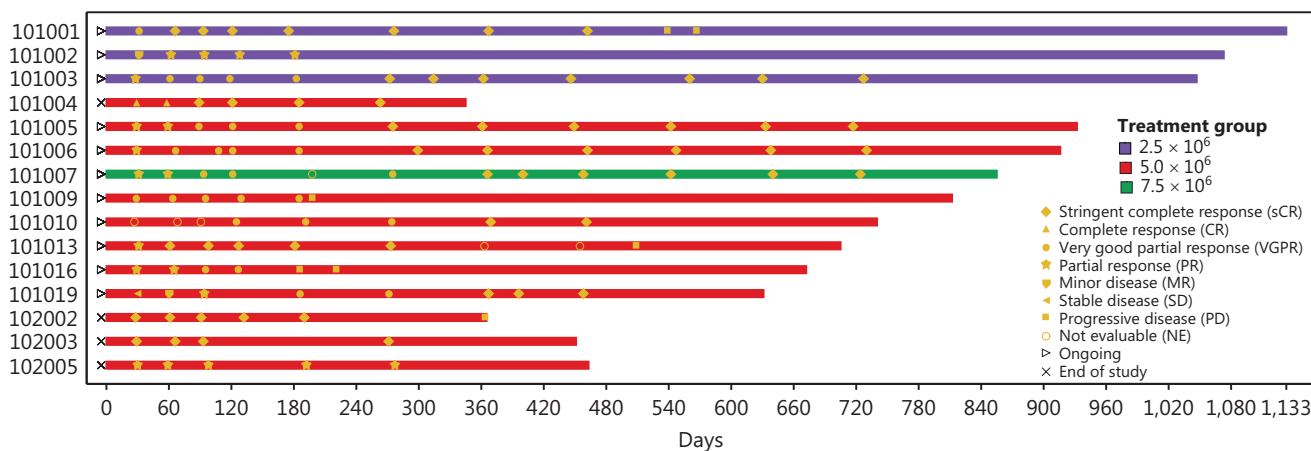


Figure S2 Swimlane plot of response duration. The length of the swimlane chart represents the number of days from the start of the infusion to the cut-off day (May 13, 2024) or end of study, whichever occurred first. Baselines with extramedullary lesions are indicated by 101009, 1010010, 101013, 101016, 102002, and 102005.

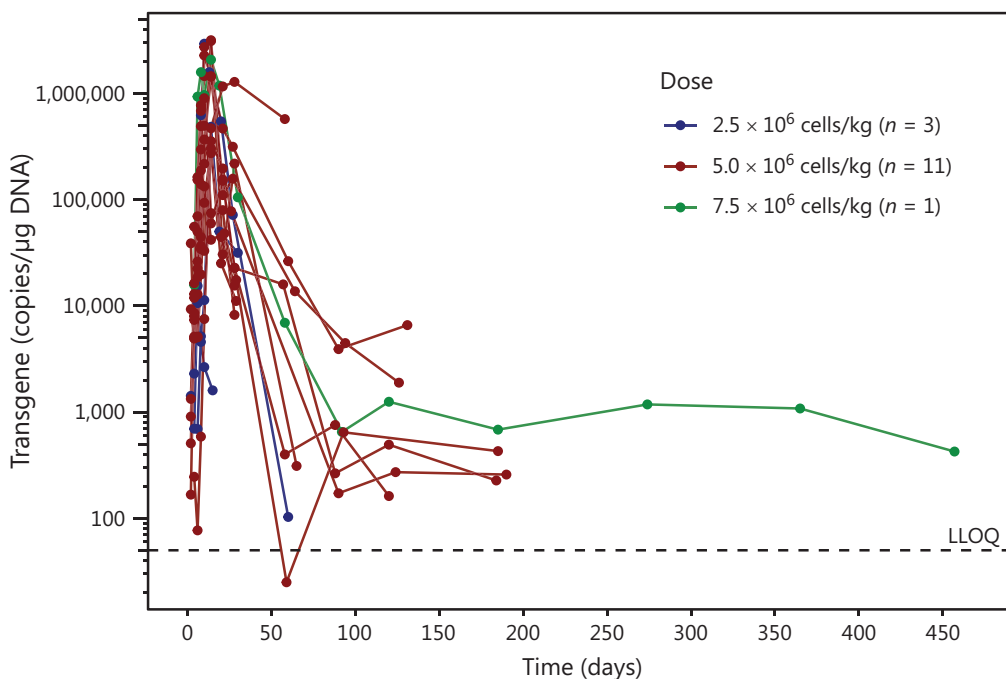


Figure S3 CAR T-cell pharmacokinetic characteristics. CAR-T, chimeric antigen receptor T cell; LLOQ, lower limit of quantification; DNA, deoxyribonucleic acid.

Adverse Events, version 5.0. CRS and ICANS were graded according to the American Society for Transplant and Cellular Therapy consensus criteria.

The secondary study purposes were to evaluate pharmacokinetic characteristics and the efficacy of BCMA CAR-T, including ORR, time to response, progression-free survival, overall survival, and duration of response.

Outcome assessments

We assessed patient responses to BCMA CAR-T according to the 2016 International Myeloma Working Group consensus criteria (20). Patient survival was confirmed at the final follow-up. We obtained the patients' subsequent therapy information from their medical records and regular disease

evaluations, including bone marrow aspiration/biopsy, serum/urine immunofixation, and positron emission tomography/computed tomography. We reviewed the patients' medical records to monitor their peripheral blood cell counts, inflammatory markers, hepatic function, and metabolic indicators. We documented the incidence of infectious diseases, physical discomfort, gastrointestinal complaints, and cutaneous lesions, on the basis of interviews and medical records.

The negative rate of minimal residual disease in bone marrow biopsy samples was detected with second-generation flow cytometry with a minimum sensitivity of 10^{-5} , and the copy number of the CAR gene in peripheral blood was detected with qPCR.

Statistical analysis

Categorical variables are expressed as numbers and percentages, whereas continuous variables are presented as medians

and ranges. The ORR is described by the number of cases and percentages, along with exact 95% confidence intervals. Time-to-event variables were estimated with the Kaplan-Meier method. For progression-free survival and duration of response, patients were censored at the date of the last disease assessment if they had no documented disease progression or death, had not initiated new anticancer therapy, had no baseline or post-baseline radiological assessments, withdrew consent, or were lost to follow-up. For overall survival, patients who were alive, withdrew consent, or were lost to follow-up were censored at the last day on which they were known to be alive. Safety data are presented as frequencies and percentages. All statistical analyses were performed in SAS Software (version 9.4, SAS Institute Inc., Cary, NC, USA), and figures were created in GraphPad Prism software (version 7, GraphPad Software Inc.; San Diego, CA, USA).