A combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, phase 2 trial

Zhiling Yan, Jiang Cao*, Hai Cheng, Jianlin Qiao, Huanxin Zhang, Ying Wang, Ming Shi, Jianping Lan, Xiaoming Fei, Lai Jin, Guangjun Jing, Wei Sang, Feng Zhu, Wei Chen, Qingyun Wu, Yao Yao, Gang Wang, Jing Zhao, Junnian Zheng†, Zhenyu Li†, Kailin Xu†*

Summary

Background **Anti-B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) T-cell therapy has been shown to have activity in patients with relapsed or refractory multiple myeloma. Reports have suggested that a small subgroup of less differentiated myeloma clones express CD19 and anti-CD19 CAR T-cell therapy has shown activity in some of these patients. We aimed to assess the activity and safety of a combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma.**

Methods **We did a single-centre, single-arm, phase 2 trial at the Affiliated Hospital of Xuzhou Medical University in China. Patients were eligible if they were aged 18–69 years, had histologically confirmed multiple myeloma, a Karnofsky Performance Score of 50 points or more, and met the International Myeloma Working Group diagnostic criteria for relapsed or refractory disease. Fludarabine (three daily doses of 30mg/m^2) and cyclophosphamide (one daily dose of 750 mg/m^2) were used to deplete lymphocytes before infusion of humanised anti-CD19 CAR T cells (1×10^6 cells per kg) and murine anti-BCMA CAR T cells (1×10^6 cells per kg). The primary outcome was the proportion of patients who achieved an overall response. Responses were assessed according to the International Myeloma Working Group criteria. This study is registered with the Chinese Clinical Trial Registration Center, number ChiCTR-OIC-17011272.**

Findings **From May 1, 2017, to Jan 20, 2019, 22 patients were enrolled and 21 received an infusion of CAR T cells and were evaluable for safety and activity analyses. At a median follow-up of 179 days (IQR 72–295), 20 (95%) of 21 patients had an overall response, including nine (43%) stringent complete responses, three (14%) complete responses, five (24%) very good partial responses, and three (14%) partial responses. The most common adverse events included cytokine release syndrome (19 [90%] of 21), including 18 patients (86%) with grade 1–2 cytokine release syndrome. The most common serious adverse events were haematological toxicities, which occurred in 20 (95%) of 21 patients. Common grade 3 or higher adverse events included neutropenia (18 [86%]), anaemia (13 [62%]), and thrombocytopenia (13 [62%]). One patient died due to cerebral hemorrhage, which was considered related to sustained thrombocytopenia. No deaths were judged to be treatment-related.**

Interpretation **Our results confirm that combined infusion of humanised anti-CD19 and anti-BCMA CAR T cells is feasible in patients with relapsed or refractory multiple myeloma, and the preliminary activity observed warrants further investigation in randomised trials. This dual CAR-T cell combinations might be a promising treatment option for relapsed or refractory multiple myeloma.**

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Introduction

Multiple myeloma is one of the most common haematological malignancies. Although the application of new drugs, such as protease inhibitors,[1] immunomodulatory drugs,[2] and daratumumab,[3] has substantially improved outcomes, prognosis is still poor for patients with relapsed or refractory disease. Studies[4–6] have confirmed the activity of chimeric antigen receptor (CAR) T-cell therapies in the treatment of relapsed or refractory B-cell tumours. Multiple myeloma is one such B cell-derived haematological malignancies, and CAR T-cell therapies targeting kappa immunoglobulin light chain,[7] CD19,[8] and B-cell maturation antigen (BCMA)[9–11] have been used to treat patients with relapsed or refractory multiple myeloma, with anti-BCMA treatment resulting in the best response.

BCMA is considered to be an ideal target for CAR T cells in the treatment of relapsed or refractory multiple myeloma,[12] because it is mainly expressed by mature B cells, normal and malignant plasma cells, and plasmacytoid dendritic cells, but not by naive B cells, memory B cells, normal haemopoietic stem cells, and

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*Joint first authors

†Joint senior authors

Blood Diseases Institute (Z Yan MD, J Cao MD, H Cheng MD, Prof J Qiao PhD, H Zhang MD, Y Wang MD, W Sang MD, F Zhu MD, W Chen MD, Q Wu PhD, Y Yao PhD, Prof Z Li MD, Prof K Xu MD) **and Cancer Institute** (M Shi PhD, G Wang PhD, Prof J Zheng PhD), **Xuzhou Medical University, Xuzhou, Jiangsu, China; Department of Hematology** (Z Yan, J Cao, H Cheng, H Zhang, Y Wang, W Sang, F Zhu, W Chen, J Zhao BS, Prof Z Li, Prof K Xu) **and Center of Clinical Oncology** (M Shi, G Wang, Prof J Zheng), **the Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China; Jiangsu Key Laboratory of Bone Marrow Stem Cells, Xuzhou, Jiangsu, China** (Z Yan, J Cao, H Cheng, Prof J Qiao, H Zhang, Y Wang, W Sang, F Zhu, W Chen, Q Wu, Y Yao, Prof Z Li, Prof K Xu); **Department of Hematology, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China** (J Lan MD, L Jin MD); **Department of Hematology, the Affiliated Hospital of Jiangsu University, Zhenjiang, Jiangsu, China** (X Fei MD); **and iCARTAB Biomedical Co Ltd, Suzhou, Jiangsu, China** (G Jing PhD)

Correspondence to: Prof Kailin Xu, Department of Hematology, the Affiliated Hospital of Xuzhou Medical University, Xuzhou 221002, Jiangsu, China, **lihmd@163.com**

Research in context

Evidence before this study

B-cell maturation antigen (BCMA) is an ideal target for chimeric antigen receptor (CAR) T-cell therapy, and promising results have been obtained in relapsed or refractory multiple myeloma. However, some patients have no response, or relapse appears soon after BCMA CAR T-cell therapy. A small component of the multiple myeloma clones express CD19 and are considered to be less-differentiated myeloma cells. Anti-CD19 CAR T cells have also been shown to be active for treating myeloma patients. We searched PubMed (Feb 4, 2019) with the terms "myeloma", "relapsed", "CD19", "BCMA", and "clinical trial" for all relevant literature published from database inception until Dec 31, 2018, without any language restrictions. We found several clinical studies on the evaluation of anti-BCMA or anti-CD19 CAR T cells in the treatment of relapsed or refractory multiple myeloma, but no published studies of a combination of humanised anti-CD19 and anti-BCMA CAR T cells for the treatment of relapsed or refractory multiple myeloma.

Added value of this study

Multiple myeloma remains an incurable disease, and different therapies are required throughout patient's life to maintain disease control. To our knowledge, this is the first study using humanised anti-CD19 CAR T cells combined with anti-BCMA CAR T cells for treatment of relapsed or refractory multiple myeloma. Our results showed that this therapy has potential as a salvage treatment when existing therapies (eg, chemotherapy, bortezomib, lenalidomide, CD38 monoclonal antibody, and transplantation) prove ineffective. Expanding the coverage of multiple myeloma cell targets and eliminating less-differentiated multiple myeloma cells might increase the activity of CAR T cells for patients with relapsed or refractory multiple myeloma.

Implications of all the available evidence

Our data showed that a combination of anti-CD19 and anti-BCMA CAR T cells is a promising treatment approach for relapsed or refractory multiple myeloma. However, several issues need to be addressed. The efficacy of the combination in comparison with anti-BCMA CAR T cells and its long-term effects need to be determined. Additionally, whether treatment efficacy could be further improved if this approach is combined with existing treatment strategies (eg, lenalidomide or immune checkpoint inhibitors) remains unknown.

other non-haemopoietic cells.[13] Studies[10,11] have reported that more than 81% of patients with relapsed or refractory multiple myeloma can achieve an objective response after infusion of anti-BCMA CAR T cells. However, the treatment is ineffective in some patients with relapsed or refractory multiple myeloma, and relapse can occur a short time after CAR T-cell therapy. Therefore, a more effective CAR T-cell regimen is required in this setting.

See Online for appendix

Expanding the coverage of multiple myeloma cell targets and eliminating less-differentiated multiple myeloma cells might increase the activity of CAR T cells for patients with treatment of relapsed or refractory multiple myeloma and improve the duration responses.[14] In murine models, a combination of anti-CS1 and anti-BCMA CAR T cells has been shown to be better at depleting myeloma cells than anti-BCMA CAR T cells alone.[15] A small component of the multiple myeloma clones are believed to express CD19, and these cells are considered to be less-differentiated multiple myeloma cells or myeloma-like stem cells.[16–18] Supporting this hypothesis, anti-CD19 CAR T-cell therapy and melphalan chemotherapy resulted in progression-free survival of more than 6 months in more than 50% of patients with relapsed or refractory multiple myeloma, indicating that anti-CD19 CAR T cells might have anti-myeloma effects.[8,19] On the basis of these preliminary data, we aimed to test the activity and safety of the combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma.

Methods

Study design and participants

We did a single-centre, single-arm, phase 2 trial at the Affiliated Hospital of Xuzhou Medical University in China. The complete protocol is included in the appendix (p 15).

Patients were eligible if they were 18–69 years of age and had histologically confirmed multiple myeloma, a Karnofsky Performance Score of 50 points or more and a life expectancy of more than 12 weeks without active infections, serious liver, kidney, heart, and other diseases, and met the International Myeloma Working Group (IMWG) diagnostic criteria for relapsed or refractory multiple myeloma, which is defined as a disease that progresses on salvage therapy or progresses within 60 days of the last treatment in patients who previously achieved at least a minimal response to treatment.[20] Female patients had to be human chorionic gonadotropin-negative, with no plans for pregnancy within 6 months of treatment. Patients were not eligible if they had received an autologous haemopoietic stem cell transplantation less than 100 days before enrolment. Patients with mental or psychological illnesses, severe allergies, or a history of severe allergies (especially those who were allergic to interleukin [IL]-2) were excluded. Detailed inclusion and exclusion criteria are provided in the appendix (pp 16, 29).

All patients provided written informed consent. The study was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University.

Procedures

All patients were confirmed as having multiple myeloma after analysis of histology, immunology, imaging, and monoclonal immunoglobulin (or kappa and lambda immunoglobulin light chain) and had been categorised into different stages according to the International Staging System.

Lymphocytes were isolated from patients using a blood cell separator and T lymphocytes were sorted using anti-CD3 immunomagnetic beads. Humanised anti-CD19 CAR T cells have been used in the treatment of relapsed or refractory acute lymphoblastic leukaemia in our centre, and the preparation process has previously been described (appendix pp 20, 33).[21] The anti-BCMA lentiviral transfer plasmid encoded the anti-BCMA single-chain variable region derived from a murine anti-human BCMA monoclonal antibody, the 4-1BB costimulatory molecule, and the CD3-ζ T-cell activation domain. CD3-positive T cells were transfected with a lentiviral vector for stable expression of CARs. Further details of the procedures are provided in the appendix (pp 24, 33).

Patients received three daily doses of fludarabine 30 mg/m² 5–3 days before CAR T cell infusion and one dose of cyclophosphamide 750 mg/m² 5 days before CAR T cell infusion. Humanised anti-CD19 CAR T cells (1×10^6 cells per kg) and anti-BCMA CAR T cells (1×10^6 cells per kg) were infused on day 0 (appendix p 6). The use of glucocorticoids to prevent allergic reactions was avoided before infusion. Owing to a risk of arrhythmias, cardiac monitoring by telemetry was advised from the time of infusion until resolution of any emergent symptoms of cytokine release syndrome (appendix p 33).

Responses were assessed according to the IMWG criteria[22] (appendix p 58). Re-evaluation of activity was done 2 weeks, 1 month, 2 months, 3 months, 6 months, and 1 year after CAR T cell infusion. After 1 year, regular follow-up was continued and adverse events were documented. The response evaluation included the number of plasma cells in the bone marrow determined by morphology, serum paraprotein and serum immunoglobulin concentration measured by electrophoresis, quantitation of 24-h urine protein, immunofixation electrophoresis, and serum free immunoglobulin light chains. Minimal residual disease was monitored at specified times after CAR T infusion (1 month, 2 months, 3 months, 6 months, 12 months, and every 6 months after 1 year) according to EuroFlow protocol[23] (appendix p 35). Further details of antibody panels for detection of minimal residual disease are in the appendix (p 61). In patients with extramedullary disease, the assessment included imaging techniques (MRI, CT, or PET-CT) and physical examination. Patients were reassessed and given salvage therapy for disease progression or relapse at any time.

There are several grading systems for evaluation of cytokine release syndrome and other toxic reactions (eg, Lee criteria, Common Terminology Criteria for Adverse Events version 4.0 version 4, 2013 National Cancer Institute Consensus, and Penn grading scale).[24–26] The adverse events were evaluated using the cytokine release syndrome evaluation criteria proposed by Lee and colleagues[24] and Common Terminology Criteria for Adverse Events version 4.0[26] (appendix p 62). Haematological toxicities were monitored once every 2 days during treatment and once a month during follow-up; immunological toxicities were monitored according to the protocol of re-evaluation of activity (2 weeks, 1 month, 2 months, 3 months, 6 months, and 1 year after CAR T cell infusion). However, complete blood cell counts and chemistry panels were done more than once per day, especially for patients at high risk of severe cytokine release syndrome or CAR-related encephalopathy syndrome or those with a high tumour burden. Treatment-related adverse events, severe adverse events, treatment-related serious adverse events were recorded. Severe adverse events that required hospitalisation, prolonged hospitalisation time, impaired work ability, endangered life, or could cause congenital malformations, whether or not it is related to the research drug, must be reported as per protocol. Clinical manifestations and vital signs associated with cytokine release syndrome, such as myalgia, fatigue, vomiting, nausea, hypoxaemia, hypotension, and neurological symptoms (eg, disorientation, limb tremor, disturbance of consciousness, and epilepsy), were recorded at any time during treatment. Peripheral blood was collected to detect IL-6, ferritin, C-reactive protein (CRP), blood cells, coagulation profiles, creatinine, liver transaminase, and bilirubin. If the patient had heart palpitations, myocardial enzymes, electrocardiogram, and troponin were measured. The assessment of cytokine release syndrome was done by three experienced clinicians and was re-evaluated if inconsistent.

To assess the amplification of CAR T cells in vivo, we designed primers for CD19-CARs and BCMA-CARs to detect the proliferation of humanised anti-CD19 and anti-BCMA CAR T cells in peripheral blood by quantitative PCR (appendix pp 56, 57). The detection of CD3+, CD4+, and CD8+ T lymphocytes and B lymphocytes in peripheral blood was done according to the re-evaluation of activity protocol during follow-up.

Outcomes

The primary outcome was the proportion of patients achieving an overall response. We determined the response according to the IMWG criteria, including stringent complete response, complete response, very good partial response, and partial response. The secondary outcome was safety, including the incidence of cytokine release syndrome, severity grading, damage to major organs (heart, liver, and kidney), and vital signs.

Statistical analysis

We assumed that the combination of humanised anti-CD19 and anti-BCMA CAR T cells for the treatment of

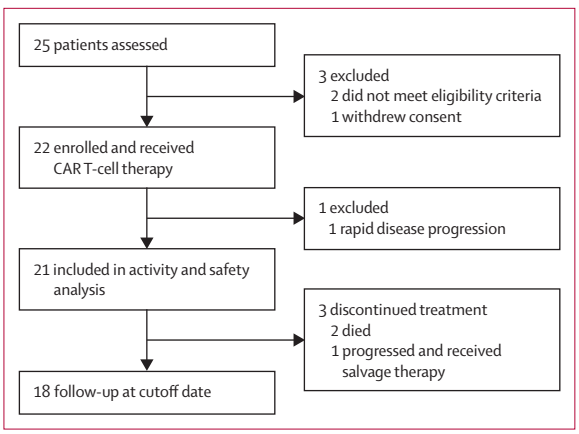

Figure 1: **Trial profile**

relapsed or refractory multiple myeloma would obtain an optimal response (proportion of patients achieving a response >80%) on the basis of published data on anti-BCMA CAR T-cell[11] or anti-CD19 CAR T-cell[8] therapy alone at the time of study design. This is an exploratory study and all the analyses are descriptive in nature. The sample size was based on clinical and practical consideration to enable exploratory evaluations of activity and preliminary safety.

Evaluable patients for safety and activity included all who received at least once dose of study protocol and had at least half a month of follow-up after CAR T-cell therapy. The best response during treatment or follow-up included stringent complete response, complete response, very good partial response, and partial response, as well as non-therapeutic-related death, relapse, or disease progression after response. Paired Student's *t* test was used to compare differences in CD3-positive cells, B lymphocytes, and monoclonal immunoglobulins before and after CAR T cell infusion. Spearman correlations were used to assess the correlation between peak frequency of CAR (CD19-CAR and BCMA-CAR) and treatment efficiency. $p<0·05$ indicated statistical significance. SPSS version 22.0 software was used for all statistical analyses.

This study is registered with the Chinese Clinical Trial Registration Center, number ChiCTR-OIC-17011272.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data and final responsibility to submit for publication.

Results

Between May 1, 2017, and Jan 20, 2019, 25 patients with multiple myeloma were screened, 22 were enrolled, and 21 received an infusion of CAR T cells and were evaluated for activity and safety (figure 1); one patient did not receive therapy owing to rapid disease progression during preparation of CAR T cells. Patients' baseline characteristics are listed in table 1. The median time from multiple myeloma diagnosis to CAR T cell infusion was 24 months (range 8–120). The patients had a median of six lines (range 4–17) of therapy before enrolment and three (14%) patients had received autologous haemopoietic stem cell transplantation (table 1). For one patient, the CAR T cells displayed expansion in vitro that did not meet the minmum requirement for infusion. However, sufficient CAR T cells were obtained after another collection of lymphocytes from this patient was done. The percentage of infusion T cells that expressed CAR-BCMA and CAR-CD19 was determined before CAR T cell infusion (appendix p 4).

At data cutoff (Jan 27, 2019), median follow-up was 179 days (IQR 72–295). Of the 21 evaluable patients, 20 (95%) had an overall response to the treatment. Nine (43%) of 21 patients achieved a stringent complete response at a median follow-up time of 268 days (IQR 91–602 days), three (14%) achieved a complete response, five (24%) achieved a very good partial response, and three (14%) achieved a partial response (figure 2; table 2). One patient (5%) had stable disease (figure 2; table 2). Of the 20 patients who responded to treatment, 17 (85%) did not relapse or progress during follow-up (17–602 days; figure 2). Patients who had a very good partial response or better had a median progression-free survival of 243 days (49–602 days). Nine (43%) of 21 patients maintained a stringent complete response or complete response for more than half a year, four (19%) maintained a stringent complete response for more than 1 year, and one (5%) patient was ongoing without relapse at 602 days (figure 2; table 2). The concentration of monoclonal immunoglobulin in the peripheral blood of patients with a response significantly decreased within 1 month after treatment compared with baseline ($p=0·0012$; figure 3).

The expression of CD19 antigen and BCMA on myeloma cells of all patients was monitored before CAR T-cell therapy. Ubiquitously expressed BCMA was detected in 20 patients who responded to treatment. Of 21 evaluable patients, 17 patients (81%) had minimal residual disease-negativity, with 16 (94%) of 17 patients obtaining negative status within 1 month after CAR T infusion. Of all the patients who had minimal residual disease-negativity, only one relapsed during follow-up (table 2).

In all 21 patients, the amplification of CD19-CARs peaked on day 7 followed by a decrease. Whereas, the copy number of BCMA-CARs peaked on day 7–14 followed by a gradual decrease (appendix p 7). A negative correlation was observed between the peak frequency of CD19-CARs ($\rho=-0·48$, $p=0·027$) or BCMA CARs ($\rho=-0·52$, $p=0·016$) and minimal residual disease-positivity. The number of plasma cells in the bone marrow was associated with the peak of BCMA-CARs ($\rho=0·62$, $p=0·0030$), but not CD19-CARs. However, there was no correlation between the peak frequencies of

	All evaluable patients, n=21
Age, years	58 (49·5–61)
Sex	
Male	10 (48%)
Female	11 (52%)
Karnofsky Performance Score	
50–60	5 (24%)
70–80	13 (62%)
90–100	3 (14%)
Bone marrow plasma cells	17% (10–32)
Monoclonal globulin	
IgG	7 (33%)
IgA	5 (24%)
IgM	1 (5%)
IgD	1 (5%)
Light chain	6 (29%)
Nonsecretory multiple myeloma	1 (5%)
High risk cytogenetics*	5 (24%)
Previous lines of therapy	6 (5–8)
International Staging System	
Stage 1	0
Stage 2	13 (62%)
Stage 3	4 (19%)
Missing	4 (19%)
Previous therapies	
Anthracyclines	12 (57%)
Cyclophosphamide	8 (38%)
Vincristine	4 (19%)
Bortezomib	18 (86%)
Thalidomide	20 (95%)
Lenalidomide	6 (29%)
Daratumumab	1 (5%)
Ixazomib	1 (5%)
Autologous stem-cell transplantation	3 (14%)
Steroid	
Yes	5 (24%)
No	16 (76%)
Tocilizumab	
Yes	1 (5%)
No	20 (95%)

Values are median (IQR) or n (%). *Fluorescence in-situ hybridisation was done at a central laboratory to detect cytogenetic mutations. High-risk cytogenetics were defined as del(17p), t(14;16), t(14;20), or t(4;14).

Table 1: **Baseline characteristics**

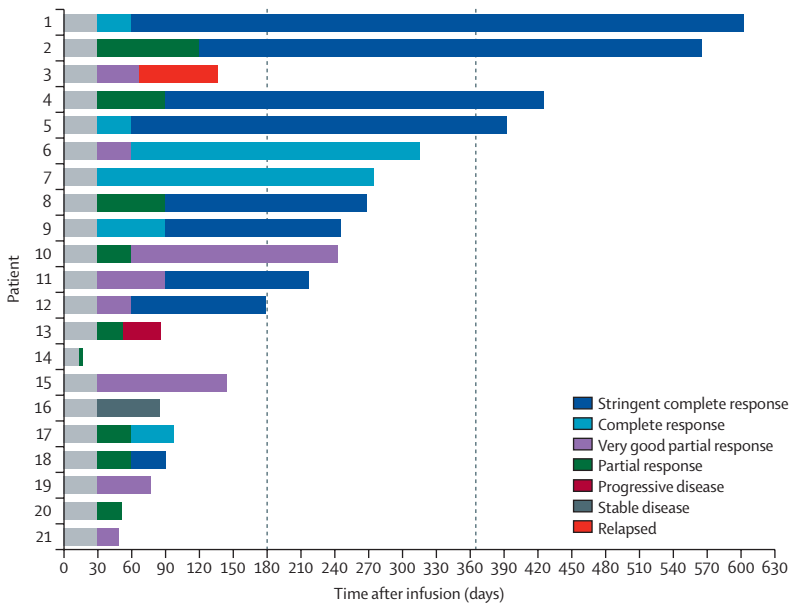

Figure 2: **Response assessment after infusion of CAR T cells**
Dotted lines mark 6 months and 12 months after infusion.

CD19-CARs or BCMA-CARs and the presence of cytokine release syndrome (appendix p 5).

The most common adverse events were cytokine release syndrome and haematological (ie, leukopenia, anaemia, and thrombocytopenia) and immunological (ie, B-cell aplasia and hypoimmunoglobulinaemia) toxicities (table 3). Cytokine release syndrome was observed in 19 (90%) of 21 patients, including 18 (86%) patients with grade 1–2 cytokine release syndrome and one (5%) with grade 3 cytokine release syndrome (table 3). Fever initiation for patients who had grade 1–2 cytokine release syndrome occurred a median of 9 days (range 1–15) after treatment and median duration was 4 days (range 1–14; appendix p 8). There was a significant difference in ferritin concentration between day 0 and day 14 (p=0·02) and a significant difference in CRP concentration between day 0 and day 14 (p=0·02) and day 14 and day 28 (p=0·04; appendix p 9). Compared with baseline concentrations, there were significant differences in ferritin, IL-6, and CRP concentrations in patients developing cytokine release syndrome (p<0·0001; appendix p 10). Two (10%) of 21 patients had CAR-related encephalopathy syndrome.

Haematological toxicities occurred in 20 (95%) of 21 patients, with 18 (86%) patients developing grade 3–4 leukopenia, 13 (62%) developing grade 3 anaemia, and 13 (62%) developing grade 3–4 thrombocytopenia (table 3). The median duration of high-grade leukopenia was 6 days (range 2–22 days), anaemia 17 days (3–37 days), and thrombocytopenia 9 days (1–37 days; appendix p 11). However, anaemia and thrombocytopenia had been present in most patients before CAR T-cell therapy. All patients had B-cell aplasia on day 7 (appendix p 12). The reconstitution of B cells was first observed 60 days after CAR T cell infusion. After 6 months, eight (80%) of ten patients showed B cell reconstitution, with five patients within the normal range. Of the 12 patients with follow-up of more than 120 days, B cell reconstitution was not seen in one (8%; appendix p 13). Hypo-immunoglobulinaemia already existed before CAR T-cell therapy and became more serious after treatment and

	Cytokine release syndrome (grade)	Neuro-toxicity	Response at 1 month	Minimal residual disease-negativity		Final outcome (days of follow-up)
				Yes or No	First confirmed time after infusion, months	
1	Yes (1)	No	Complete	Yes	2	Stringent complete response (602)
2	Yes (1)	No	Partial	Yes	1	Stringent complete response (565)
3	Yes (2)	Yes	Very good partial	Yes	1	Relapsed on day 67 died on day 136
4	Yes (1)	No	Partial	Yes	1	Stringent complete response (425)
5	Yes (3)	Yes	Complete	Yes	1	Stringent complete response (392)
6	Yes (1)	No	Very good partial	Yes	1	Complete response (315)
7	Yes (1)	No	Complete	Yes	1	Complete response (275)
8	Yes (1)	No	Partial	Yes	1	Stringent complete response (268)
9	Yes (2)	No	Complete	Yes	1	Stringent complete response (245)
10	Yes (1)	No	Partial	Yes	1	Very good partial response (243)*
11	Yes (2)	No	Very good partial	Yes	1	Stringent complete response (217)
12	Yes (1)	No	Very good partial	Yes	1	Stringent complete response (179)
13	No	No	Partial	No	..	Progressed on day 53 and received salvage therapy (86)†
14	Yes (2)	No	Death	No	..	Partial response on day 14, died of cerebral haemorrhage (17)
15	Yes (1)	No	Very good partial	Yes	1	Very good partial response (145)*
16	Yes (1)	No	Stable disease	No	..	Stable disease (85)
17	No	No	Partial	No	..	Complete response (98)
18	Yes (1)	No	Partial	Yes	1	Stringent complete response (91)
19	Yes (2)	No	Very good partial	Yes	1	Very good partial response (77)*
20	Yes (2)	No	Partial	Yes	1	Partial response (52)*
21	Yes (2)	No	Very good partial	Yes	1	Very good partial response (49)*

*The immunofixation electrophoresis is still weakly positive. †Patient lost to follow-up after 86 days.

Table 2: **Clinical responses and serious toxicities by patient**

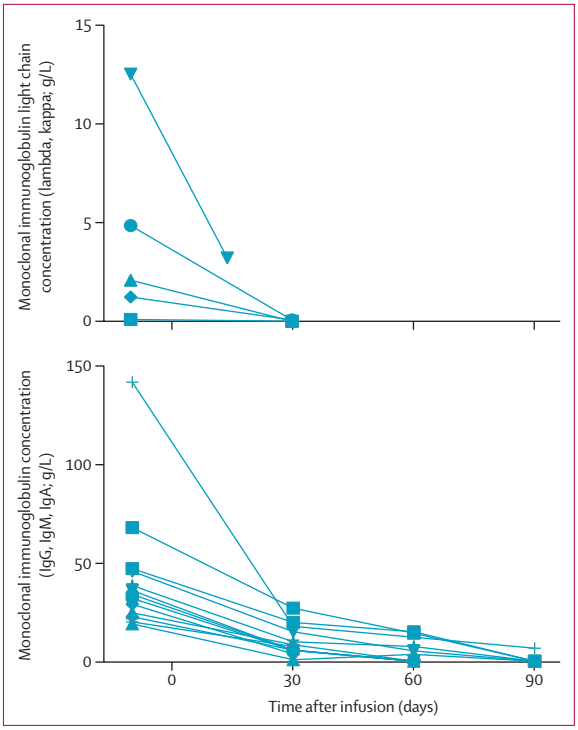

Figure 3: **Changes in monoclonal immunoglobulin concentration from baseline**
Each line represents a single patient.

lasted for a long time. IgM recovered after 3 months and IgG and IgA had not recovered to the normal concentration at data cutoff, except two patients in whom IgG recovered after 1 month and 480 days (appendix p 13). The other adverse events were mostly grade 1–2 (table 3).

Two patients died during follow-up. Patient 3 achieved a very good partial response and minimal residual disease-negative status within 1 month after CAR T cell infusion, but systemic multisite extramedullary disease was found on day 67, and myeloma cells were detected again in the bone marrow (table 2). This patient died on day 136 after giving up salvage therapy (table 2). Patient 14 had a gradually decreased platelet count due to disease progression before CAR T-cell therapy and their platelet count remained low throughout treatment. Therefore, the patient received six platelet transfusions to reduce bleeding. Cytokine release syndrome was observed 7 days after CAR T cell infusion, which was mainly characterised by fever and hypoxia without abnormal coagulation. Hypotension, hypertension, and bleeding of vital organs were not observed before this patient had a fatal intracranial haemorrhage 17 days after CAR T cell infusion (table 2). The amplification of anti-CD19 and anti BCMA CAR T cells was observed in this patient from day 7. CRP concentration peaked on day 11, IL-6 on day 11, and ferritin on day 15 (appendix p 14). No myeloma cells were detected in bone marrow 2 weeks after CAR T infusion. Serum free κ immunoglobulin light chain was reduced from 12·5g/L to 3·2 g/L, and the body temperature returned to normal after 8 days of fever. Neither of these deaths were judged to be treatment-related.

Discussion

In this study, we combined humanised anti-CD19 CAR T cells and anti-BCMA CAR T cells for the treatment of patients with relapsed or refractory multiple myeloma and obtained a partial response or better. 95% of patients achieved a response, with 43% achieving a stringent complete response and 81% achieving minimal residual disease-negativity. The exact mechanism for this effect remains unclear. Studies[8,17] have indicated that a small component of multiple myeloma clones with drug-resistant, disease-propagating properties has a B-cell

(CD19-positive) phenotype. Minimal residual disease-negative status appeared earlier than the best response in this study, possibly due to a longer metabolism time for the existing monoclonal immunoglobulin. In our research, some patients with early minimal residual disease-negative status eventually achieved a stringent complete response after a delayed period. Whether this finding indicates that patients with early minimal residual disease-negative status might have more chance of achieving a stringent complete response remains unknown and requires further observation.

Almost all patients achieved an ongoing response during follow-up. At data cutoff (Jan 27, 2019), only one patient with minimal residual disease-negative status had relapsed. Patients who had a very good partial response or better had a median progression-free survival of 243 days (49–602 days). For patients achieving a stringent complete response, the median remission time was 268 days (91–602 days). Although the follow-up is short, patients that achieve minimal residual disease-negativity and a complete response or stringent complete response might have better outcomes in the future. We found very few myeloma cells expressing CD19 antigen before CAR T-cell therapy and anti-CD19 CAR T cells might further eliminate these cells. Turtle and colleagues[27] and our previous study[21] showed that murine CAR might induce immune responses that reduce the persistence and function of CAR T cells in some patients, whereas humanised CAR might have reduced immunogenicity.

We analysed the expression of BCMA and CD19 on plasma cells of all patients before CAR T-cell therapy. BCMA was highly expressed on multiple myeloma cells in patients in whom treatment was effective. Whether low BCMA expression was a contributing factor to the poor response or whether BCMA expression before treatment can predict treatment activity requires more cases to confirm. Because this is a single-arm study, evaluation of the additive effect of anti-CD19 CAR T cells to anti-BCMA CAR T cells is difficult. Additionally, the small number of patients cautions against concluding on subgroup analyses. However, our preliminary data suggest that amplification of anti-CD19 CAR T cells might be associated with response and minimal residual disease-negative status. The specific role of anti-CD19 CAR T cells needs to be established in a controlled study.

Clinical studies[10,11,28] have suggested that the infusion dose of anti-BCMA CAR T cells is associated with the treatment activity. Dose escalation of CAR T cells in these studies[10,11,28] results in an increased response. In this study, all patients were infused with 1×10^6 cells per kg anti-BCMA and 1×10^6 cells per kg anti-CD19 CAR T cells and 20 (95%) of 21 had an overall response to the treatment. Analysis of in-vivo CAR T-cell amplification showed that CAR T cells were amplified in patients who responded to the treatment. We believe that, although the dose of the CAR T cell infusion is important, effective amplification of cells after activation in vivo is also crucial.

	Grade 1–2	Grade 3	Grade 4	Grade 5
Cytokine release syndrome	18 (86%)	1 (5%)	0	0
Haematological adverse events				
Leukopenia	2 (10%)	11 (52%)	7 (33%)	0
Anaemia	7 (33%)	13 (62%)	0	0
Thrombocytopenia	3 (14%)	9 (43%)	4 (19%)	0
Non-haematological adverse events				
Fever	16 (76%)	3 (14%)	0	0
Confusion	1 (5%)	0	0	0
Muscle weakness	19 (90%)	0	0	0
Nausea	2 (10%)	0	0	0
Vomiting	2 (10%)	0	0	0
Myalgias	2 (10%)	0	0	0
Hypotension	2 (10%)	0	0	0
Hypoxaemia	6 (29%)	1 (5%)	0	0
Aspartate aminotransferase increased	6 (29%)	1 (5%)	0	0
Alanine aminotransferase increased	4 (19%)	0	0	0
Creatinine increased	2 (10%)	0	0	0
Prolonged activated partial thromboplastin time	8 (38%)	0	0	0
Fibrinogen decreased	2 (10%)	0	0	0
Cerebral haemorrhage	0	0	0	1 (5%)
Lung infection	0	1 (5%)	0	0

All grade 3–5 events are shown and grade 1–2 events occurring in more than 10% of patients.

Table 3: **Adverse events during the treatment**

CAR T-cell therapy-related adverse events are an important consideration, because they might lead to exacerbation of conditions and treatment-related deaths. Although the incidence of cytokine release syndrome was high in our study, most cases were grade 1 or 2. In this regard, we analysed in detail the changes of cytokine release syndrome, body temperature, IL-6, CRP, and ferritin. The median time from CAR T cell infusion to fever initiation in patients who had grade 1–2 cytokine release syndrome was 9 days, which was later than the time reported by previous studies.[29] The median duration was 4 days, which was similar to previous reports.[29] The increase in IL-6, CRP, and ferritin concentrations is closely associated with the occurrence and severity of cytokine release syndrome.[25,30] Our results showed that increases in IL-6, CRP, and ferritin concentrations were lower than reported in other published literature.[25,30] Therefore, we hypothesise that the low dose of the CAR T cells infusion might be one of the reasons for the low incidence of severe cytokine release syndrome.

We observed a high incidence of haematological toxicity, but leukopenia mainly occurred after fludarabine and cyclophosphamide chemotherapy and was shorter in

duration with injection of granulocyte colony-stimulating factor. No severe infection occurred during neutropenic phase. Anaemia and thrombocytopenia were present before CAR T-cell therapy in most patients, and no clear decrease in severity occurred during CAR T-cell therapy. One patient died of cerebral haemorrhage. Because this patient did not have abnormal blood coagulation, we presume that thrombocytopenia was the main cause of the haemorrhage.

We also evaluated B-cell aplasia and hypoimmunoglobulinaemia after CAR T-cell therapy. All patients developed B-cell aplasia and hypoimmunoglobulinaemia after treatment. B cells began to recover after 2 months. However, hypoimmunoglobulinaemia lasted for a long time, especially for IgA and IgG (ongoing at data cutoff for most cases), which increased the risk of infection, including respiratory tract and digestive tract infections. However, after supplementation with intravenous immunoglobulin, only one patient developed a pulmonary infection, and they recovered after anti-infective treatment.

This study has several limitations. First, this study was uncontrolled. Second, we were unable to clarify the additive role of anti-CD19 CAR T cells to anti-BCMA CAR T cells in the treatment of relapsed or refractory multiple myeloma. Third, due to the small number of enrolled patients and the relatively short observation time, the long-term effectiveness requires further evaluation. Finally, a more optimised CAR T cell infusion dose could have been used and warrants further exploration.

In conclusion, a combination of anti-CD19 and anti-BCMA CAR T-cell therapy in patients with relapsed or refractory multiple myeloma is feasible, and the majority of patients achieved a response. However, longer follow-up is needed to assess the extend of the activity with this dual CAR-T cell therapy and randomized studies are warranted to elucidate the potential value and mechanism of the combination of anti-BCMA and anti-CD19 CAR T cells.

Contributors

ZY, JC, JuZ, ZL, and KX were responsible for study protocol design, data interpretation, and data analysis. All investigators and their research teams recruited patients and contributed to data collection. ZY, HC, JQ, ZL, and KX contributed to the first draft of the manuscript. All authors were involved at each stage of manuscript preparation and approved the final version.

Declaration of interests

GJ is an employee of iCARTAB Biomedical Co Ltd. All other authors declare no competing interests.

Data sharing

The data, except data that involves privacy or protection, will be made available when all primary and secondary endpoints have been met. Any application for data will be reviewed by the experiment manager. Access to data will be given for applicants that reasonably use the data and experimental methods. To gain access, requestors will also need to sign a data access agreement.

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