Risk Factors Associated with Durable Progression-Free Survival in Patients with Relapsed or Refractory Multiple Myeloma Treated with Anti-BCMA CAR T-cell Therapy

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ABSTRACT

Purpose: B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) T-cell therapy results in high remission rates in patients with relapsed/refractory (R/R) multiple myeloma. However, the factors associated with prognosis following CAR T-cell therapy are unknown.

Patients and Methods: Between July 1, 2018 and July 31, 2020, 61 patients with R/R multiple myeloma received anti-BCMA CAR T-cell therapy (Chictr.org number, ChiCTR1800017404). Step-wise multivariate Cox regression and competing risk analyses were conducted to identify poor prognosis–associated risk factors.

Results: Sixty patients (98.4%) experienced cytokine release syndrome (CRS), including 33, 23, and 4 cases of CRS grades 1 to 2, 3, and 4, respectively. The objective response rate (ORR) was 98.3%, and the complete remission (CR) rate was 70.3%. With a median follow-up period of 21.1 months, the 1-year overall survival (OS) and progression-free survival (PFS) rates were 78.0% and 50.2%, respectively. The median PFS was 12.7 months. Cox modeling revealed that poor PFS was associated with extramedullary disease [HR = 2.59, 95% confidence interval (95% CI) = 1.29–5.21, $P = 0.008$], light chain multiple myeloma (HR = 2.53, 95% CI = 1.03–5.97, $P = 0.035$), high-risk cytogenetics (HR = 2.80, 95% CI = 1.27–6.14, $P = 0.01$), and prior treatment with more than 3 therapeutic lines (HR = 3.14, 95% CI = 1.34–7.34, $P = 0.008$). Among the 41 CR cases, competing risk analyses demonstrated higher relapse predispositions in those with extramedullary disease (HR = 4.51, 95% CI = 1.86–10.9, $P = 0.001$), light chain multiple myeloma (HR = 4.89, 95% CI = 1.52 – 15.7, $P = 0.008$), or high-risk cytogenetics (HR = 5.09, 95% CI = 1.63–15.9, $P = 0.005$).

Conclusions: Anti-BCMA CAR T-cell therapy is safe and effective for R/R multiple myeloma. For patients with high-risk factors, improvements to extend remission and more specific individualized therapies are needed.

Introduction

Multiple myeloma is an incurable plasma cell neoplasm, typically with extramedullary involvement and bone destruction (1, 2). Although great progress has been made following the advent of proteasome inhibitors (PI), immunomodulatory imide drugs (IMiD), and mAbs, such as daratumumab, myeloma cells invariably acquire resistance, and nearly all patients ultimately experience disease progression that is refractory to available therapies (3–6). Therefore, the discovery of novel therapeutic strategies to overcome drug resistance remains a high priority.

Chimeric antigen receptor (CAR) T-cell therapy has been successful in improving treatment outcomes for patients experiencing relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL) or lymphoma by targeting cluster of differentiation 19 (CD19), which has encouraged the development of CAR T cells targeting multiple myeloma (7–11). B-cell maturation antigen (BCMA) is extensively expressed by multiple myeloma cells, normal plasma cells, and a small subset of normal B cells (12, 13). BCMA is a potential target for the treatment of multiple myeloma (14–16). Recently, multiple clinical trials have reported encouraging objective response rates (ORR) ranging from 73% to 100% and complete remission (CR) rates ranging from 33% to 76.5% for R/R multiple myeloma receiving anti-BCMA CAR T-cell therapy (16–20).

Despite encouraging results from trials investigating anti-BCMA CAR T-cell therapy for R/R multiple myeloma, some unresolved issues remain. For example, the ORR and CR rate, as well as therapy-associated complications, have varied widely among clinical trials, with a subset of patients experiencing relapse following anti-BCMA CAR T-cell treatment. The main challenge is to improve the duration of the response in these patients, and no biomarker has been recommended to date for predicting clinical outcomes following anti-BCMA

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Translational Relevance

B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory (R/R) multiple myeloma is highly effective in inducing complete remission (CR), although relapse is a frequent occurrence. However, the factors associated with long-term prognosis following CAR T-cell therapy are unknown, making it difficult to predict treatment responses. In this trial, the encouraging efficacy with the objective response rate of 98.3% and the CR rate of 70.3% was observed. The 1-year overall survival and progression-free survival rates were 78.0% and 50.2%, respectively. Furthermore, we identified extramedullary disease, light chain multiple myeloma, and certain high-risk cytogenetic factors as important independent predictors of a poor prognosis in those receiving anti-BCMA CAR T-cell therapy. For specific subsets of patients exhibiting these higher-risk characteristics, improvements in the duration of the CR are needed, and more specific individualized therapies should be developed to ensure optimal outcomes.

CAR T-cell treatment. Thus, the current study aimed to identify prognostic factors for predicting clinical outcomes following anti-BCMA CAR T-cell treatment.

Patients and Methods

Lentiviral transduction and preclinical evaluation of CAR T cells

A lentiviral vector was used to carry a second-generation BCMA-targeted CAR comprising 4-1BB costimulatory and CDζ3 signaling domains (Supplementary Fig. S1A). The antigen recognition domain of the YK-BCMA BB-002 vector was obtained from a murine hybridoma cell line raised against BCMA. CAR T-cell transduction and expansion were performed as previously described (21, 22), and CAR T-cell expression was detected via flow cytometry using biotinylated goat anti-mouse and anti-human antibodies (Jackson ImmunoResearch Laboratories, Inc.), followed by incubation with allophycocyanin (APC)-labeled streptavidin (BD Pharmingen). The *in vitro* cytotoxic capabilities of the BCMA-directed CAR T cells were assessed in HeLa cells engineered to express BCMA (HeLa-BCMA) at effector:target cell (E:T) ratios of 0.25:1, 0.5:1, 1:1, and 5:1 using a label-free iCELLigence real-time cell analyzer (RTCA) system (Agilent Biosciences, Inc.). Luminex (Thermo Fisher Scientific) assays were used to quantify the concentrations of cytokines contained in the supernatants of CAR T cells that were cocultured for 4 hours with $BCMA^+$ RPMI8226 cells. Untransduced T cells were used as the controls.

Clinical protocol design

This clinical trial was designed to assess the safety and efficacy of an infusion of autologous T cells that were modified to express the BCMA-specific CAR–4-1BB in patients with R/R multiple myeloma (Chictr.org number, ChiCTR1800017404). The study was conducted in accordance with the Declaration of Helsinki. The inclusion criteria were as follows: (i) age less than 75 years; (ii) recipient of at least three previous lines of therapy, including a PI and an IMid, or disease refractory to both drug classes; (iii) quantifiable disease, with adequate performance status and organ function; and (iv) BCMA expression on ≥30% of bone marrow plasma cells, as detected via IHC assays or flow cytometric analysis. Extramedullary disease was defined as paraskeletal soft-tissue masses, soft-tissue masses spreading outside the bone marrow, or both (17). The imaging modality (MRI, CT, or PET/CT) was used to detect extramedullary disease.

Between July 1, 2018 and July 31, 2020, 73 consecutive patients were screened. Eight patients were not eligible to participate because of severe infection ($n = 2$), disease progression ($n = 4$), or renal failure ($n = 2$). Thus, 65 patients were enrolled and underwent leukapheresis. Of these 65 patients, 4 underwent leukapheresis, but withdrew from further studies owing to severe infection ($n = 1$), disease progression ($n = 2$), or cerebral hemorrhage ($n = 1$). Finally, 61 patients were included in the trial (**Fig. 1**). The protocol was approved by the First Affiliated Hospital, School of Medicine, Zhejiang University Institutional Review Board. All patients provided written informed consent to participate in the study.

Procedure

Patients underwent leukapheresis to obtain peripheral blood mononuclear cells (PBMC) on day -14, relative to the first day of BCMA CAR T-cell infusion on day 0. Patients were subjected to a conditioning treatment for lymphodepletion (fludarabine 30 mg/m^2 on days -4 to -2, and cyclophosphamide 500 mg/m^2 on days -3 to -2), followed by an infusion of BCMA CAR T cells on day 0. Multiple myeloma response assessment was conducted according to the International Uniform Response Criteria for Multiple Myelomas (23). Cytokine release syndrome (CRS) was graded as previously described (24). Other toxicity evaluation criteria were assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE, version 5.0).

Statistical analysis

The CR rates with 95% confidence intervals (CI) were calculated using the Clopper–Pearson method. Overall survival (OS) was defined as the time from CAR T-cell infusion to death. For the PFS analysis, an event was defined as disease progression or death. Patients who did not experience an event were censored at the date of the final follow-up. Univariable and step-wise multivariable Cox regression analyses were performed to identify the risk factors associated with OS and PFS. Kaplan–Meier curves for PFS and OS were generated, and the log–rank test was used to compare differences between subgroups. The median follow-up time was estimated using reverse Kaplan–Meier curves (25). Among the patients who achieved CR, outcome events for the cumulative incidence of relapse (CIR) were defined as the time from CR to relapse. Univariable and step-wise multivariable competing risk analyses were conducted to identify the risk factors associated with relapse (26, 27). The final model selection was based on Bayesian information criteria (BIC) values (28, 29). Following the generation of the CIR plots, patients in different subgroups were compared via Fine–Gray analysis. All quoted *P* values are two-tailed, with values less than 0.05 considered to be statistically significant. All calculations were performed using R software (version 4.0.3).

Results

Preclinical evaluation of BCMA-directed CAR T-cell therapy

To evaluate the transduction efficiency of the lentiviral vector carrying a second-generation CAR against BCMA (Supplementary Fig. S1), the following preclinical evaluations were performed. Firstly, high transduction efficiency was confirmed when using the lentiviral vector in human peripheral T lymphocytes (65.2%; Supplementary Fig. S2A). Subsequently, the cytotoxicity of the BCMA-directed CAR T cells against HeLa-BCMA cells was evaluated at various E:T ratios (0.25:1, 0.5:1, 1:1, and 5:1, respectively). Compared with that of control T cells, the BCMA-directed CAR T cells demonstrated very strong

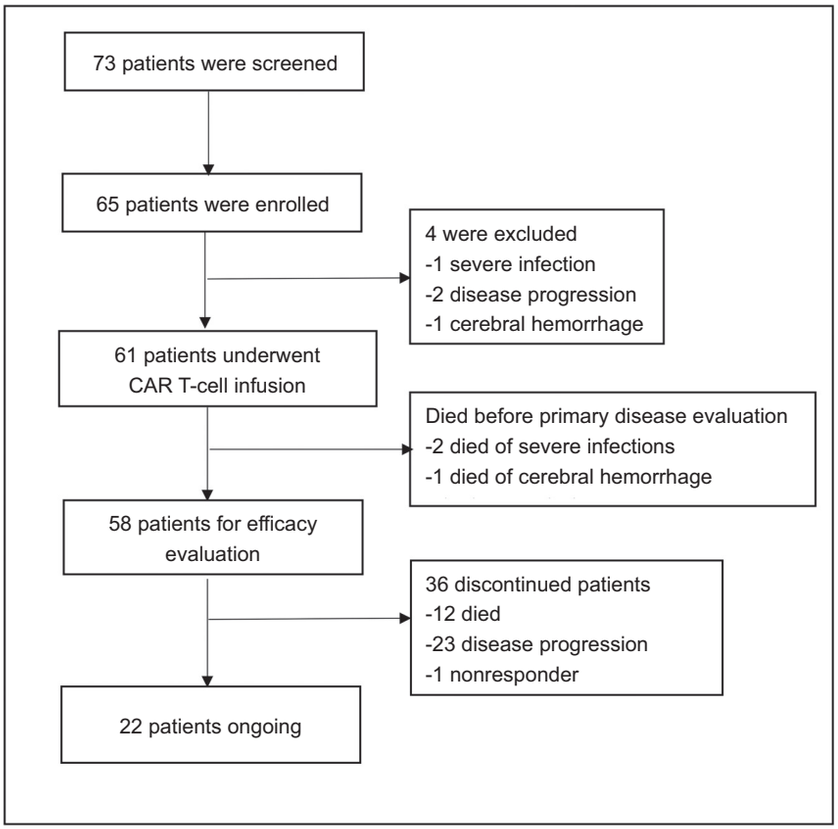

Figure 1.
CONSORT diagram.

cytotoxicity toward target cells, even at very low E:T ratios (Supplementary Fig. S2B). Finally, compared with that of control T cells, the BCMA-directed CAR T cells expressed higher levels of IFNγ, IL2, TNFα, GM-CSF, Granzyme B, and macrophage inflammatory protein 1 (MIP-1) when cocultured with RPMI8226 cells, a human $BCMA^+$ multiple myeloma cell line (Supplementary Fig. S3).

Patient baseline characteristics

A total of 61 patients were included in the trial. At the time of data cutoff (February 28, 2021), 22 patients remained ongoing (**Fig. 1**). Each patient's response and survival profiles are shown in **Fig. 2A**. The patients' baseline characteristics are summarized in **Table 1**, with each individual's details shown in Supplementary Table S1. The median number of prior lines of therapy received was 3 (range, 3–9), with 100% being refractory to both PIs and IMiDs. The median time since diagnosis was 43 months (range, 6–126 months). At least one high-risk cytogenetic abnormality was observed in 80% (49/61) of patients, with 18% (11/61) exhibiting either a 17p deletion or a TP53 mutation. Extramedullary disease afflicted 46% (28/61) of patients, 97% (59/61) had stage II or III disease, and 39% (24/61) experienced relapse following autologous hematopoietic stem cell transplantation (ASCT).

Anti-BCMA CAR T-cell characterization in CAR T-cell products

After 7 to 11 days of culture, the cells were prepared for infusion. The median transfection efficiency of the final product was 58% (range, 19%–84%). All 61 patients received CAR T-cell infusion at doses of 1.1×10^6 per kg to 6.2×10^6 per kg, with a median dose of 3.7×10^6 per kg. For all 61 patients, the median ratio of $CD8^+$ to $CD4^+$ CAR T cells in the infusion product was 0.55 (range, 0.03–2.24; Supplementary Table S2).

Adverse Events and toxicities of anti-BCMA CAR T-cell therapy

CRS was experienced by 60 of 61 patients (98.4%); of them, 33, 23, and 4 exhibited CRS of grades 1–2, 3, and 4, respectively. No grade 5 CRS occurred (Supplementary Fig. S4). CRS mostly occurred with a median of 3 days postinfusion (range, 1–10 days) and lasted for a mean of 10 days (range, 3–25 days). CRS was fully reversible in all patients and was well managed with supportive care alone ($n = 33$), supportive care *plus* the anti-IL6 receptor mAb tocilizumab ($n = 12$), supportive care *plus* corticosteroids ($n = 5$), or supportive care *plus* tocilizumab *plus* corticosteroids ($n = 10$). Reversible neurotoxicities were observed in 5 patients (patients #1, #7, #21, #35, and #51). Four patients (#34, #36, #43, and #51) received continuous renal replacement therapy for acute kidney injury. Patient 34 developed grade 4 CRS after CAR T-cell infusion. Methylprednisolone and general supportive treatment were given. Next, the patient was transferred to the intensive care unit to receive continuous renal replacement therapy and dopamine therapy because of acute renal failure, respiratory failure, and shock. One week later, the patient developed cerebral hemorrhage and died after his family refused rescue measures. The occurrence, management, and outcomes related to other specific organs are listed in Supplementary Table S3.

ORR, OS, PFS, and CIR

Within 1 month, 2 patients died of severe infections and one died of cerebral hemorrhage; thus, the malignancy responses were evaluated in 58 patients. Among the 58 evaluable patients, the ORR was 98.3% (57/58), with 70.7% (41/58) of patients experiencing CR; moreover, all experienced stringent CR (sCR). **Figure 2B** shows the rate of CR (confirmed CR or sCR) according to characteristics evaluated at baseline and during treatment. In total, 8.6% (5/58) and 19.0% (11/

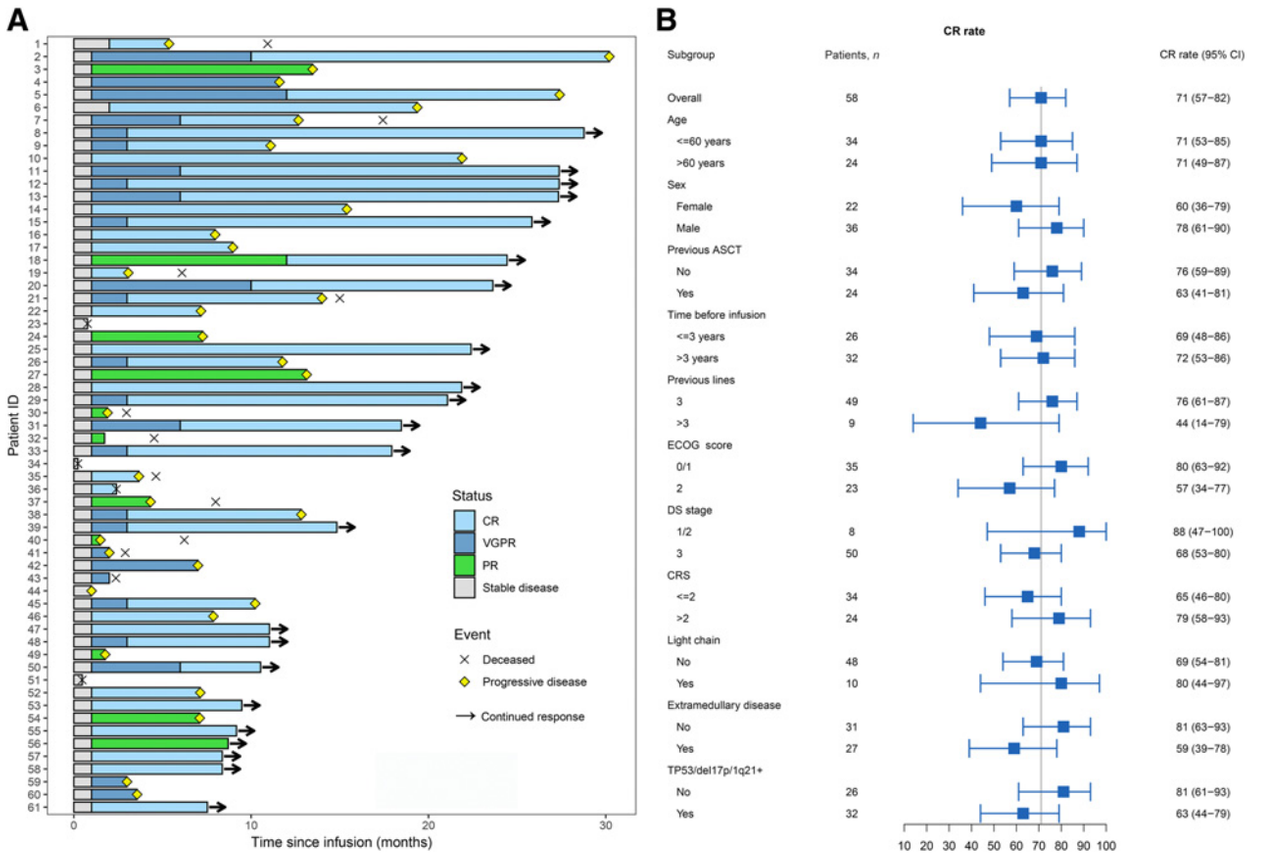

Figure 2.

Tumor response and subgroup analysis of response. **A,** Follow-up of 61 patients treated with BCMA CAR T cells. **B,** The rate of CR according to characteristics at baseline and during treatment. DS, Durie-Salmon.

58) of patients achieved a very good partial response (VGPR) or partial response (PR), respectively. With a median follow-up time following anti-BCMA CAR T-cell infusion of 21.1 months, 15 (24.6%) deaths occurred: 3 (4.8%) from severe infection during the pancytopenia period, 1 (1.6%) from cerebral hemorrhage, 1 (1.6%) from liver failure, and another 10 (12.1%) from disease progression or relapse. A total of 22 patients demonstrated ongoing responses. Representative imaging observations before and after CAR T-cell therapy confirmed the antimyeloma activity of anti-BCMA CAR T cells (Supplementary Fig. S5). The median PFS was 12.7 months (95% CI, 8.0–21.9; **Fig. 3A**). The 1-year OS (Supplementary Fig. S6A) and PFS rates were 78.0% (95% CI, 68.0–89.4) and 50.2% (95% CI, 38.7–65.2), respectively. Among the 41 patients who achieved CR, 19 (46.3%) relapsed and 1 (2.4%) died of gastrointestinal hemorrhage. The 1-year CIR rate was 38.9% (95% CI, 20.3–53.1; **Fig. 4A**).

Predictive factors for OS, PFS, and CIR

Univariable Cox regression analyses were performed to identify baseline, CAR T-cell product, and therapy-related factors associated with PFS for inclusion in the multivariable Cox regression analyses. PFS was significantly associated with sex ($P = 0.044$), extramedullary disease ($P = 0.012$), light chain multiple myeloma ($P = 0.005$), high-risk cytogenetics (TP53 mutation, deletion of 17p13 or gains/amplification of 1q21, TP53/del17p/1q21^{+}; $P = 0.002$), exposure to prior therapeutic lines ($P = 0.007$), Durie–Salmon stage ($P = 0.037$), Eastern Cooperative Oncology Group (ECOG) score ($P = 0.013$), CRS grade ($P = 0.036$), lactate dehydrogenase/upper limit of normal (LDH/ULN) values ($P = 0.012$), hemoglobin (Hb; $P = 0.008$), and the infusion dose of CAR T cells ($P = 0.02$; Supplementary Table S4).

The significant factors (with P values < 0.05) were included in the subsequent multivariable Cox regression modeling using a stepwise approach. Poor PFS was associated with extramedullary disease (HR = 2.59, 95% CI = 1.29–5.21, $P = 0.008$), light chain multiple myeloma (HR = 2.53, 95% CI = 1.03–5.97, $P = 0.035$), high-risk cytogenetics (HR = 2.80, 95% CI = 1.27–6.14, $P = 0.01$), and prior treatment with more than three lines (HR = 3.14, 95% CI = 1.34–7.34, $P = 0.008$; **Table 2**). Based on these independent factors associated with PFS identified by the multivariable Cox model, a subset of patients with multiple myeloma was identified who exhibited poor PFS following BCMA CAR T-cell therapy. Patients with extramedullary disease (1-year PFS rate: 34.4% vs. 60.2%), light chain multiple myeloma (9-month PFS rate: 18.2% vs. 68.0%), high-risk cytogenetics (1-year PFS rate: 38.5% vs. 66.3%), and those treated with more than three prior lines (1-year PFS rate: 11.1% vs. 57.5%) exhibited poor PFS (**Fig. 3B–E**). Patients with more of these independent factors had a poorer PFS. The one-year PFS rates for patients with 0, 1, 2, and 3 independent factors were 100%, 57.2%, 31.7%, and 0%, respectively (**Fig. 3F**).

To further explore the risk factors for relapse among patients who achieved CR, univariable competing risk models were generated, the results of which are shown in Supplementary Table S5. Significant

Table 1. Baseline characteristics.

	Total ($N = 61$)
Median age (range), year	59 (40–71)
Male sex, *n* (%)	39 (64)
Median time since diagnosis (range), months	43 (6–129)
ISS stage	
I	2 (3)
II	6 (10)
III	53 (87)
Types of myeloma, *n* (%)	
IgA	17 (28)
IgG	28 (46)
IgD	4 (7)
Light chain	11 (18)
Kappa	2 (3)
Lamda	9 (15)
Nonsecretory type	1 (2)
Extramedullary disease, *n* (%)	28 (46)
ECOG Performance Status score, *n* (%)	
0	9 (15)
1	27 (44)
2	25 (41)
High-risk cytogenetic profile, *n* (%)	49 (80)
TP53/del(17p)	13 (21)
t(4;14)	17 (28)
t(14;16)	3 (5)
t(14;20)	17 (28)
1q21 gain/amplification	32 (52)
Progressive disease during most recent line of therapy, *n* (%)	39 (64)
Median duration since diagnosis (range), months	43 (6–126)
Previous ASCT, *n* (%)	24 (39)
Median *n* of previous antimyeloma regimens (range)	3 (3–9)
Previous therapies, *n* (%)	
Proteasome inhibitors	61 (100)
Bortezomib	61 (100)
Isazomi	7 (11)
Carfilzomib	5 (8)
Immunomodulatory agents	61 (100)
Lenalidomide	61 (100)
Pomalidomide	13 (21)
Thalidomide	10 (16)
PIs + IMiDs	61 (100)
Selinexor	1 (2)

Abbreviations: IgA, immunoglobulin A; IgD, immunoglobulin D; IgG, immunoglobulin G; ISS, International Staging System.

factors (with P values < 0.05) were included in the step-wise multivariable competing risk models. Patients with multiple myeloma with extramedullary disease (HR = 4.51, 95% CI = 1.86–10.9, $P = 0.001$), light chain multiple myeloma (HR = 4.89, 95% CI = 1.52–15.7, $P = 0.008$), or high-risk cytogenetics (HR = 5.09, 95% CI = 1.63–15.9, $P = 0.005$) were predisposed to relapse (**Table 2**). CIR plots were generated, which showed that patients with extramedullary disease (1-year CIR rate: 68.6% vs. 16.8%, $P = 0.009$), light chain multiple myeloma (9-month CIR rate: 75.0% vs. 20.2%, $P = 0.001$), and high-risk cytogenetics (1-year CIR rate: 53.2% vs. 23.7%, $P = 0.002$) had a higher CIR rate (**Fig. 4B–D**). Patients with more independent factors had higher CIR rates. The 1-year CIR rates for patients with 0, 1 or 2, and 3 independent factors were 0%, 22.8%, and 85.7%, respectively (**Fig. 4E**).

As shown in Supplementary Table S6 and **Table 2**, the univariable and multivariable Cox regression modeling revealed that patients with light chain multiple myeloma (HR = 2.91, 95% CI = 1.01–8.41, $P = 0.048$) and those with an ECOG score of 2 (HR = 4.81, 95% CI = 1.50–15.41, $P = 0.008$) had a poor OS. The Kaplan–Meier plots for disease type and ECOG score are shown in Supplementary Fig. S6B and S6C. Compared with patients without these two independent factors, patients with an ECOG score of 2 or light chain multiple myeloma experienced poorer OS (Supplementary Fig. S6D).

Among patients with extramedullary disease, 10 were paraskeletal soft-tissue masses, 7 were soft-tissue masses spreading outside the bone marrow, and 10 had both masses. There were 5, 4, and 5 patients in these three groups of patients, respectively. The results of χ^2 test showed that there was no difference in high-risk cytogenetics among the three groups. After infusion of CAR T-cell therapy, only 1 case did not have an imaging response. As shown in Supplementary Fig. S7A and S7B, despite the results of the log–rank test showing no statistical difference among these three groups, a slightly poorer prognosis was observed in patients with soft-tissue masses spreading outside the bone marrow than those with paraskeletal soft-tissue masses.

Discussion

Despite advances in multiple myeloma treatment, almost all patients eventually relapse, with a worse prognosis expected for refractory patients (3–6). Recently, the largest clinical trial yet conducted of CAR T cells (idecabtagene vicleucel has been approved by FDA) targeting BCMA demonstrated therapeutic efficacy in heavily treated patients with R/R multiple myeloma, with an ORR of 73% and a CR rate of 33% (17). Another trial of an anti-BCMA CAR T cells (LCAR-B38M) involving 57 R/R multiple myeloma cases showed an ORR of 88% and a CR rate of 68% (30). A phase I trial (18 R/R multiple myeloma cases) of a fully human anti-BCMA CAR T cells (CT103A) showed an encouraging ORR of 100% and a CR rate of 72% (20). In our trial, the disease burden of enrolled patients appeared to be higher relative to that of other anti-BCMA CAR T-cell clinical trials. A total of 41% of patients had an ECOG score of 2, 87% of patients had ISS stage of III, 80% of patients had traditional high-risk genotypes, and 46% of patients had extramedullary lesions. In spite of the high disease burden in our trial, a very high ORR (98.3%) and sCR (70.7%) were still observed among the 61 patients with R/R multiple myeloma. Compared with other non-BCMA–directed therapy for R/R multiple myeloma, the efficacy of anti-BCMA CAR T-cell therapy was higher. Previous research reported that the ORR of melflufen plus dexamethasone in the treatment of R/R multiple myeloma was 29% to 76% (31). Single-agent selinexor showed an ORR of 4%, while combination with dexamethasone increased the ORR to 50% (32). The clinical trial of single-agent belantamab mafodotin showed an ORR of 34% (33). Despite these promising clinical outcomes of anti-BCMA CAR T-cell therapy in R/R multiple myeloma, some subsets of patients still face the risk of relapse during this potential treatment, which has become another focus of increasing attention. Thus, it is essential to identify predictive factors for the prognosis of patients with multiple myeloma receiving anti-BCMA CAR T-cell therapy.

Few studies have evaluated the risk factors affecting the prognosis of patients with multiple myeloma undergoing anti-BCMA CAR T-cell therapy. Recently, Nikhil and colleagues reported that a high infusion dose was slightly more effective (17). In this trial, the univariable analysis also demonstrated that a high infusion dose was associated with a better PFS than a lower dose, but it was not an independent predictor according to the multivariable Cox models. Univariable analyses from a trial involving 17 patients with R/R multiple myeloma suggested that those with extramedullary disease, anti-CAR T

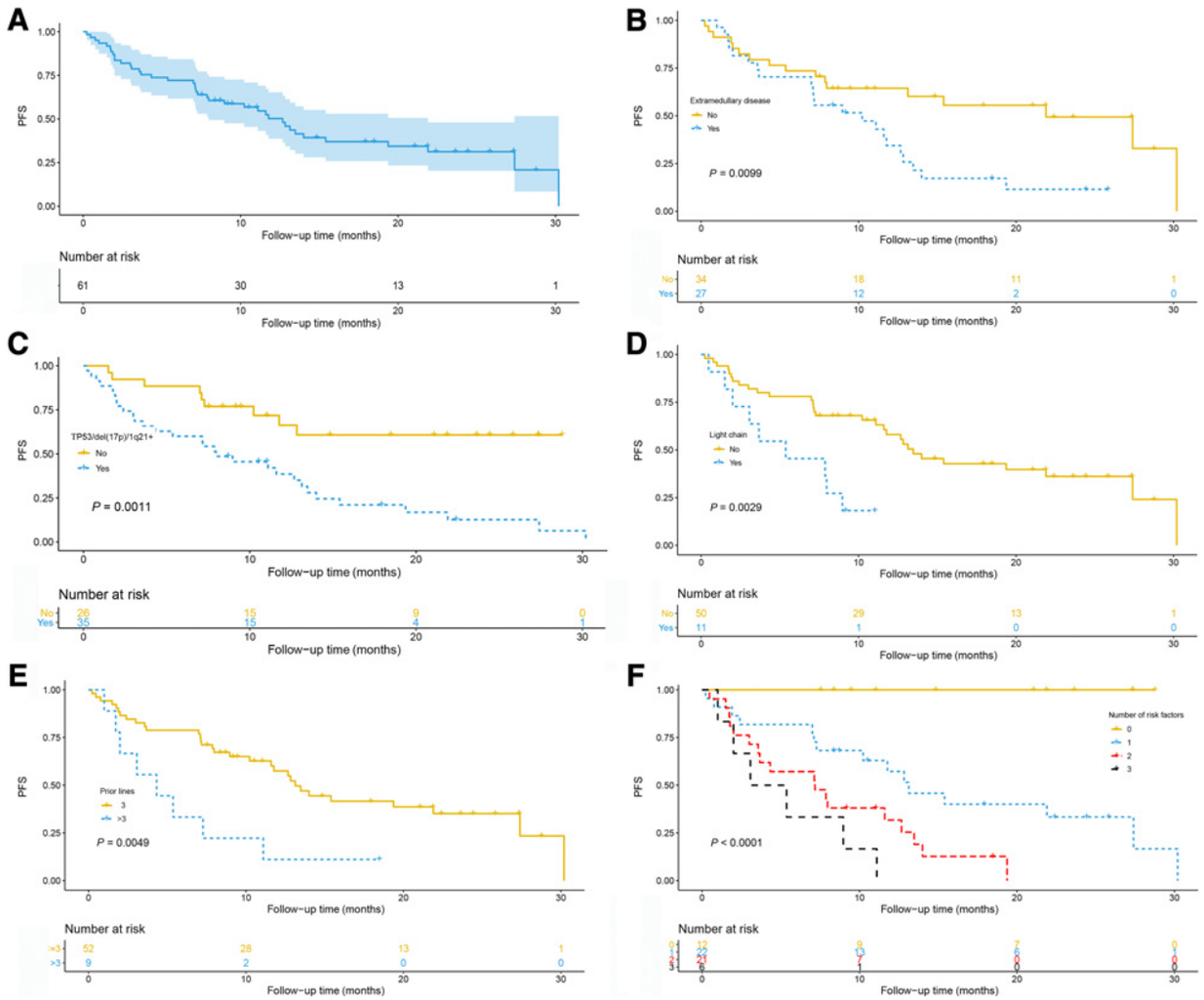

Figure 3.

PFS and subgroup analysis. **A,** The PFS in all patients. **B,** The PFS in patients with or without extramedullary disease. **C,** The PFS in patients with or without high-risk cytogenetics (TP53 mutation, del17p, or 1q21 gain or amplification). **D,** The PFS in patients with light chain or other types. **E,** The PFS in patients with light chain and other types. **F,** The PFS in patients with different number of independent factors.

antibodies, or those without prior ASCT had worse outcomes (18). Another trial involving 28 patients with R/R multiple myeloma and 2 patients with primary plasma cell leukemia (PCL) indicated that those who received previous ASCT treatment or had extramedullary disease/PCL had a poor prognosis (19). The outcomes following prior ASCT were inconsistent between the two studies; however, this analysis failed to detect an association between prior ASCT and the prognosis of patients with multiple myeloma receiving anti-BCMA CAR T-cell therapy. A significant association was also found between extramedullary disease and poor prognosis; however, it was an independent predictor of disease progression in patients who received CAR T-cell therapy and of relapse among patients who achieved CR. One possible explanation is that the microenvironment of extramedullary lesions makes it relatively difficult for CAR T cells to penetrate and exert persistent effects (34). Besides, highly heterogeneous and persistent extramedullary lesions may contain or produce clones that may be more likely to escape anti-BCMA CAR T-cell therapy (35). In addition, a slightly worse prognosis for patients with soft-tissue masses spreading outside the bone marrow was observed in this trial. A previous study indicated a similar conclusion (36). This may be because the myeloma cells from soft-tissue masses spreading outside the bone marrow usually show immature or plasmablastic morphology (36). We failed to detect significant difference in high-risk genetics among the three extramedullary disease groups. The conclusion was similar to the results of a large Spanish transplantation trial (37).

Deletion of 17p13 (the locus of the tumor-suppressor gene, TP53), t (4; 14) (p16; q32), t (14; 16) (q32; q23), t (14; 20) (q32; q12), and gains/amplification of 1q21 are the main high-risk cytogenetic and molecular abnormalities that contribute to the poor prognosis in multiple myeloma (38–42). Whether these high-risk genetic factors are significantly associated with the prognosis of patients with multiple myeloma undergoing anti-BCMA CAR T-cell therapy remains controversial. Recently, a study using single-cell RNA (scRNA) and whole-exome sequencing techniques indicated that patients with a 17p deletion should be aware of the negative recurrence of BCMA-targeted therapies (43). Among 5 patients with the negative recurrence

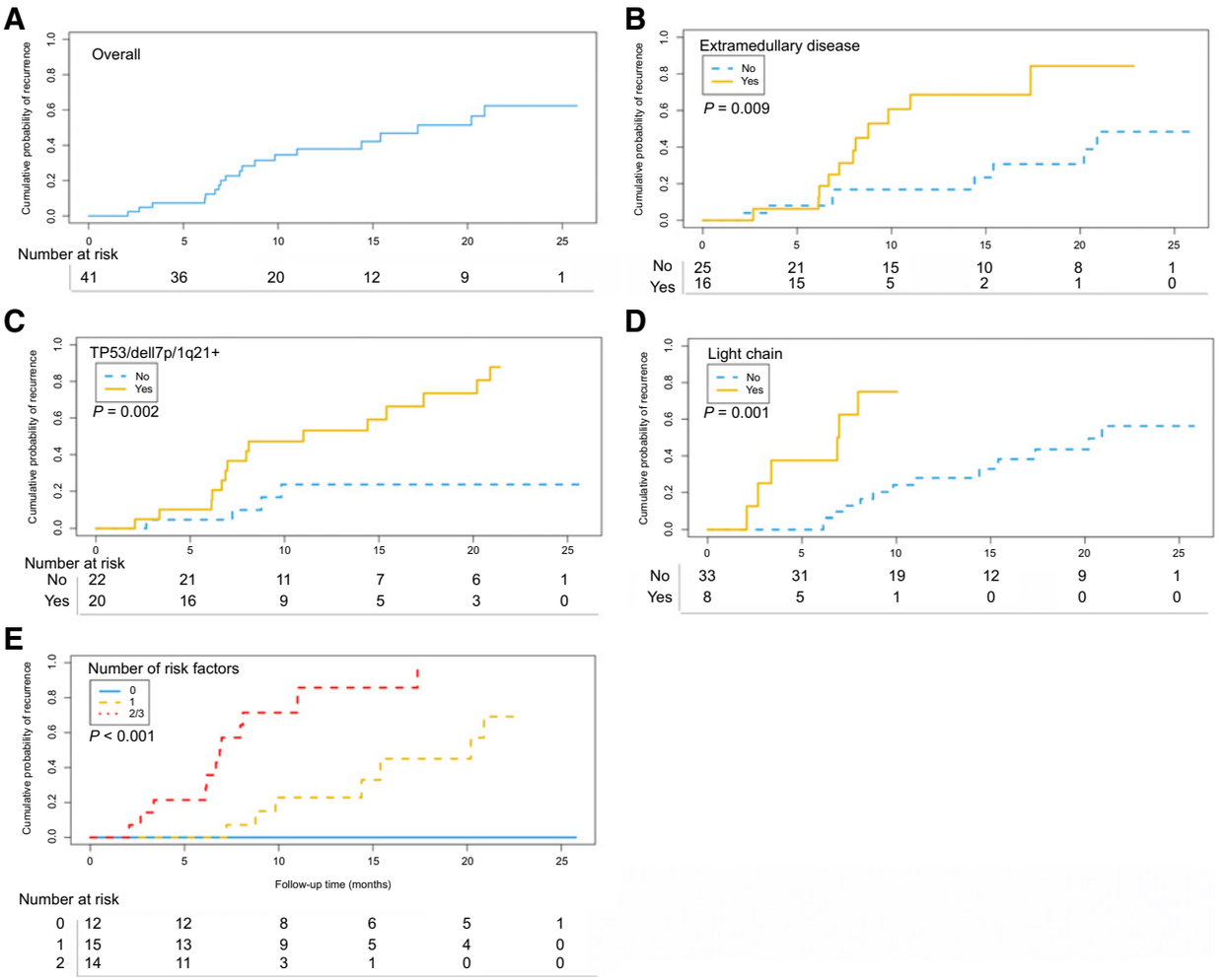

Figure 4.

CIR and subgroup analysis among 41 patients who achieved CR. **A,** The CIR in all patients. **B,** The CIR in patients with or without extramedullary disease. **C,** The CIR in patients with or without high-risk cytogenetics (TP53 mutation, del17p, or 1q21 gain or amplification). **D,** The CIR in patients with light chain or other types. **E,** The CIR in patients with different number of independent factors.

after anti-BCMA CAR T-cell therapy in our trial, 3 patients had deletion of 17p or TP53 mutation. The CIR curves in the present study led to a similar conclusion. The univariable analysis and the subsequent multivariable analysis confirmed that patients with high-risk cytogenetics experienced poor PFS and a higher CIR, and the factors were also an independent risk factor for poor clinical outcomes.

A total of 11 (18%) patients with light chain multiple myeloma were included in this trial; this proportion was similar to the 15% to 20% incidence reported in a previous study (44). Light chain multiple myeloma tumor cells only synthesize monoclonal light chains, without the corresponding heavy chains, and are characterized by the presence of prominent renal damage and poor prognosis (45). Sirohi and colleagues compared the prognosis of patients with light chain, IgA, and IgG multiple myeloma who underwent ASCT and suggested that light chain patients had a shorter OS and event-free survival; however, they failed to detect significant differences among the patients who achieved CR (46). No study has investigated whether a light chain was associated with prognosis in patients with multiple myeloma receiving CAR T-cell therapy. Interestingly, the results of the multivariable analysis showed that patients with light chain multiple myeloma were predisposed to disease progression, and among patients who achieved CR, those with a light chain were more likely to relapse. Among 11 patients with light chain multiple myeloma, 8 patients had high-risk cytogenetics. Grouped by high-risk cytogenetics for those 11 patients, we failed to detect a significant difference in prognosis. On the one hand, this may be due to the small number of light chain patients; on the other hand, it may be due to the influence of other risk factors in these patients, such as extramedullary disease and the therapy of prior lines.

Although all patients in this trial had undergone at least three lines of previous treatment and all were intolerant to bortezomib and lenalidomide, they appeared to have been exposed to fewer prior treatment lines than those in other anti-BCMA CAR T-cell trials. This may be due to the limited supply of certain drugs in China, such as carfilzomib, pomalidomide, and daratumumab. In our trial, the median number of prior lines of therapy was 3 (range, 3 to 9) and none received daratumumab therapy. In the trial of LCAR-B38M, the median number of prior lines of therapy was 3 (range, 1 to 9; ref. 30).

Table 2. Multivariable analysis for prognosis of patients with multiple myeloma with BCMA CAR T-cell therapy.

Variable	Multivariable HR (95% CI)	*P*
PFS[a]		
Extramedullary disease	2.59 (1.29–5.21)	0.008
Disease type (light chain vs. others)	2.53 (1.07–5.97)	0.035
Cytogenetic (1q21/TP53/del17p vs. others)[b]	2.80 (1.27–6.14)	0.010
Prior lines (>3 vs. 3)	3.14 (1.34–7.34)	0.008
CIR[c]		
Extramedullary disease	4.51 (1.86–10.9)	0.001
Disease type (light chain vs. others)	4.89 (1.52–15.7)	0.008
Cytogenetic (1q21/TP53/del17p vs. others)	5.09 (1.63–15.9)	0.005
OS[d]		
Disease type (light chain vs. others)	2.91 (1.01–8.41)	0.048
ECOG score (2 vs. 0/1)	4.81 (1.50–15.41)	0.008

[a]Multivariable Cox analysis for PFS of 61 patients with multiple myeloma.
[b]1q21/TP53/del17p represents deletion of 17p13, TP53 mutation, or 1q21 gain/amplification.
[c]Competing risk analysis for cumulate incidence of relapse among 41 patients who achieved CR.
[d]Multivariable Cox analysis for OS of 61 patients with multiple myeloma.

The trial of a fully human BCMA-targeting CAR T cells (CT103A) reported that the median number of prior lines of therapy was 4 (range, 3 to 11) and only 2 (13%) patients underwent daratumumab therapy (20). In the largest trial of idecabtagene vicleucel, the median number of previous antimyeloma regimens was 6 (range, 3 to 16) and 85% patients experienced daratumumab therapy (17). Zhou and colleagues reported that treatment with more than six treatment lines was a significant predictor of a VGPR or better response and suggested that earlier CAR T-cell therapy may infer additional benefits to the patients (19). Although the present study failed to detect a significant association, exposure to three prior treatment lines was an independent predictive factor for PFS. These findings support the theory that earlier CAR T-cell therapy may be more beneficial to patients.

A total of four independent risk factors for PFS were identified in this trial via stepwise multivariable analysis. The patients were classified into four subgroups according to the number of independent risk factors they exhibited, with the Kaplan–Meier plots revealing differences in PFS among the four subgroups. With a median follow-up time of 21.5 months for 12 patients without any independent risk factors, none died from disease progression, whereas those with 1, 2, or 3 independent risk factors exhibited median PFS times of 10.23, 3.57, and 2 months, respectively. Similar outcomes were observed in the 41 patients who achieved CR. None of the 12 patients without any independent risk factors experienced relapse. The 1-year CIR rates for patients with one or more independent risk factors were 22.8% and 85.7%, respectively. These results suggest that patients without any risk factors may experience a good prognosis, even without follow-up intervention, whereas those with more than one independent risk factor require early intervention to minimize the risk of disease progression.

In our trial, CRS of grade 3 or higher occurred in 27 of 61 (44%) patients and only five neurotoxicities were observed. The trial of LCAR-B38M reported 4 of 57 (7%) had grade ≥3 CRS, and 1 patient had neurotoxicity (30). However, another trial of LCAR-B38M showed 6 of 17 (35%) patients experienced severe CRS, and 1 died of a very severe CRS (18). In the largest trial of idecabtagene vicleucel, 7 (5%) cases had CRS of grade 3 or higher. Neurotoxicities occurred in 23 cases (18%) and were of grade 3 in 4 cases (3%; ref. 17). The results of CT103A showed that 4 of 18 (22%) experienced CRS of grade 3 or higher (20). Compared with these anti-BCMA trials, it appeared to be a high proportion of grade 3 CRS with our product, whereas CRS and neurotoxicity was fully reversible in all patients and was well managed.

In summary, anti-BCMA CAR T-cell therapy for patients with R/R multiple myeloma is highly effective at inducing CR, although relapse is a frequent occurrence. Extramedullary disease, light chain multiple myeloma, and high-risk cytogenetics may be important independent predictors of a poor prognosis in those receiving anti-BCMA CAR T-cell therapy. For specific subsets of patients exhibiting these higher risk characteristics, improvements in the duration of the CR are needed, and more specific individualized therapies should be developed to ensure optimal outcomes.

Authors' Disclosures

Y. Zhang reports personal fees from Shanghai YaKe Biotechnology Ltd. during the conduct of the study. S. Peng reports personal fees from Shanghai YaKe Biotechnolgy Ltd. during the conduct of the study. C.-H. Huang reports personal fees from Shanghai YaKe Biotechnology Ltd. during the conduct of the study. A.H. Chang reports personal fees from Shanghai YaKe Biotechnology Ltd. during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

M. Zhang: Conceptualization, software, formal analysis, supervision, visualization, methodology, writing–original draft, writing–review and editing. **L. Zhou:** Conceptualization, data curation, software, formal analysis, methodology, writing–original draft, writing–review and editing. **H. Zhao:** Data curation, methodology, writing–review and editing. **Y. Zhang:** Data curation, methodology, writing–original draft. **G. Wei:** Writing–review and editing. **R. Hong:** Data curation. **W. Wu:** Data curation, supervision. **H. Xu:** Data curation. **L. Wang:** Data curation. **F. Ni:** Data curation. **J. Cui:** Data curation, supervision. **S. Peng:** Data curation. **C.-H. Huang:** Data curation. **A.H. Chang:** Resources, supervision, funding acquisition. **Y. Hu:** Conceptualization, supervision, project administration, writing–review and editing. **H. Huang:** Resources, supervision, funding acquisition, project administration, writing–review and editing.

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References

1. Röllig C, Knop S, Bornhäuser M. Multiple myeloma. Lancet 2015;385: 2197–208.
2. Kumar SK, Rajkumar V, Kyle RA, van Duin M, Sonneveld P, Mateos MV, et al. Multiple myeloma. Nat Rev Dis Primers 2017;3:17046.
3. Kumar S. Treatment of newly diagnosed multiple myeloma in transplant-eligible patients. Curr Hematol Malig Rep 2011;6:104–12.
4. Kumar SK, Lee JH, Lahuerta JJ, Morgan G, Richardson PG, Crowley J, et al. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. Leukemia 2012;26:149–57.
5. Sonneveld P. Management of multiple myeloma in the relapsed/refractory patient. Hematology Am Soc Hematol Educ Program 2017;2017:508–17.
6. Nijhof IS, van de Donk N, Zweegman S, Lokhorst HM. Current and new therapeutic strategies for relapsed and refractory multiple myeloma: an update. Drugs 2018;78:19–37.
7. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015;385:517–28.
8. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014; 371:1507–17.
9. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377:2531–44.
10. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018;378:439–48.
11. Wei G, Ding L, Wang J, Hu Y, Huang H. Advances of CD19-directed chimeric antigen receptor-modified T cells in refractory/relapsed acute lymphoblastic leukemia. Exp Hematol Oncol 2017;6:10.
12. Liu D. Cancer biomarkers for targeted therapy. Biomark Res 2019;7:25.
13. Shu HB, Johnson H. B cell maturation protein is a receptor for the tumor necrosis factor family member TALL-1. Proc Natl Acad Sci USA 2000;97: 9156–61.
14. Tai YT, Anderson KC. Targeting B-cell maturation antigen in multiple myeloma. Immunotherapy 2015;7:1187–99.
15. Novak AJ, Darce JR, Arendt BK, Harder B, Henderson K, Kindsvogel W, et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. Blood 2004;103:689–94.
16. Brudno JN, Maric I, Hartman SD, Rose JJ, Wang M, Lam N, et al. T cells genetically modified to express an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. J Clin Oncol 2018;36:2267–80.
17. Munshi NC, Anderson LD Jr, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N Engl J Med 2021;384:705–16.
18. Xu J, Chen LJ, Yang SS, Sun Y, Wu W, Liu YF, et al. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. Proc Natl Acad Sci USA 2019;116:9543–51.
19. Li C, Cao W, Que Y, Wang Q, Xiao Y, Gu C, et al. A phase I study of anti-BCMA CAR T cell therapy in relapsed/refractory multiple myeloma and plasma cell leukemia. Clin Transl Med 2021;11:e346.
20. Wang D, Wang J, Hu G, Wang W, Xiao Y, Cai H, et al. A phase I study of a novel fully human BCMA-targeting CAR (CT103A) in patients with relapsed/refractory multiple myeloma. Blood 2021;137:2890–901.
21. Pan J, Yang JF, Deng BP, Zhao XJ, Zhang X, Lin YH, et al. High efficacy and safety of low-dose CD19-directed CAR-T cell therapy in 51 refractory or relapsed B acute lymphoblastic leukemia patients. Leukemia 2017;31:2587–93.
22. Pan J, Niu Q, Deng B, Liu S, Wu T, Gao Z, et al. CD22 CAR T-cell therapy in refractory or relapsed B acute lymphoblastic leukemia. Leukemia 2019;33:2854–66.
23. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International myeloma working group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;15:e538–48.
24. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood 2014;124:188–95.
25. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials 1996;17:343–6.
26. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association 1999;94:496–509.
27. Skrifvars MB, Bailey M, Moore E, Mårtensson J, French C, Presneill J, et al. A post hoc analysis of osmotherapy use in the erythropoietin in traumatic brain injury study-associations with acute kidney injury and mortality. Crit Care Med 2021; 49:e394–e403.
28. Schwarz GE. Estimating the dimension of a model. The Ann Statist 1978;6:461–4.
29. Scrucca L, Santucci A, Aversa F. Regression modeling of competing risk using R: an in depth guide for clinicians. Bone Marrow Transplant 2010;45:1388–95.
30. Zhao WH, Liu J, Wang BY, Chen YX, Cao XM, Yang Y, et al. A phase 1, open-label study of LCAR-B38M, a chimeric antigen receptor T cell therapy directed against B cell maturation antigen, in patients with relapsed or refractory multiple myeloma. J Hematol Oncol 2018;11:141.
31. Oriol A, Larocca A, Leleu X, Hajek R, Hassoun H, Rodríguez-Otero P, et al. Melflufen for relapsed and refractory multiple myeloma. Expert Opin Investig Drugs 2020;29:1069–78.
32. Chen C, Siegel D, Gutierrez M, Jacoby M, Hofmeister CC, Gabrail N, et al. Safety and efficacy of selinexor in relapsed or refractory multiple myeloma and Waldenstrom macroglobulinemia. Blood 2018;131:855–63.
33. Lonial S, Lee HC, Badros A, Trudel S, Nooka AK, Chari A, et al. Belantamab mafodotin for relapsed or refractory multiple myeloma (DREAMM-2): a two-arm, randomised, open-label, phase 2 study. Lancet Oncol 2020;21:207–21.
34. Johnson LA, Scholler J, Ohkuri T, Kosaka A, Patel PR, McGettigan SE, et al. Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma. Sci Transl Med 2015;7:275ra22.
35. Bhutani M, Foureau DM, Atrash S, Voorhees PM, Usmani Sz. Extramedullary multiple myeloma. Leukemia 2020;34:1–20.
36. Rosiñol L, Beksac M, Zamagni E, Van de Donk N, Anderson KC, Badros A, et al. Expert review on soft-tissue plasmacytomas in multiple myeloma: definition, disease assessment and treatment considerations. Br J Haematol 2021;194:496–507.
37. Rosiñol L, Oriol A, Teruel AI, Hernández D, López-Jiménez J, de la Rubia J, et al. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: a randomized phase 3 PETHEMA/GEM study. Blood 2012;120:1589–96.
38. Xiong W, Wu X, Starnes S, Johnson SK, Haessler J, Wang S, et al. An analysis of the clinical and biologic significance of TP53 loss and the identification of potential novel transcriptional targets of TP53 in multiple myeloma. Blood 2008; 112:4235–46.
39. Gertz MA, Lacy MQ, Dispenzieri A, Greipp PR, Litzow MR, Henderson KJ, et al. Clinical implications of t(11;14)(q13;q32), t(4;14)(p16.3;q32), and -17p13 in myeloma patients treated with high-dose therapy. Blood 2005;106:2837–40.
40. Hanamura I, Stewart JP, Huang Y, Zhan F, Santra M, Sawyer JR, et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. Blood 2006;108:1724–32.
41. Ross FM, Avet-Loiseau H, Ameye G, Gutiérrez NC, Liebisch P, O'Connor S, et al. Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. Haematologica 2012;97:1272–7.
42. Ross FM, Chiecchio L, Dagrada G, Protheroe RK, Stockley DM, Harrison CJ, et al. The t(14;20) is a poor prognostic factor in myeloma but is associated with long-term stable disease in monoclonal gammopathies of undetermined significance. Haematologica 2010;95:1221–5.
43. Samur MK, Fulciniti M, Aktas Samur A, Bazarbachi AH, Tai YT, Prabhala R, et al. Biallelic loss of BCMA as a resistance mechanism to CAR T cell therapy in a patient with multiple myeloma. Nat Commun 2021;12:868.
44. Kyle RA, Larson DR, Therneau TM, Dispenzieri A, Melton LJ 3rd, Benson JT, et al. Clinical course of light-chain smouldering multiple myeloma (idiopathic Bence Jones proteinuria): a retrospective cohort study. Lancet Haematol 2014;1: e28–e36.
45. Drayson M, Begum G, Basu S, Makkuni S, Dunn J, Barth N, et al. Effects of paraprotein heavy and light chain types and free light chain load on survival in myeloma: an analysis of patients receiving conventional-dose chemotherapy in Medical Research Council UK multiple myeloma trials. Blood 2006;108:2013–9.
46. Sirohi B, Powles R, Kulkarni S, Rudin C, Saso R, Lal R, et al. Comparison of new patients with Bence-Jones, IgG and IgA myeloma receiving sequential therapy: the need to regard these immunologic subtypes as separate disease entities with specific prognostic criteria. Bone marrow transplant 2001;28:29–37.