Original Research

A novel dominant-negative PD-1 armored anti-CD19 CAR T cell is safe and effective against refractory/relapsed B cell lymphoma

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ABSTRACT

Refractory/relapsed B cell lymphoma patients who received the available anti-CD19 chimeric antigen receptor (CAR) T cells may still experience a short duration of remission. Here in this study, we evaluated the safety and efficacy of a novel dominant-negative programmed cell death-1 (PD-1) armored anti-CD19 CAR T cells. A total of 9 patients (including 4 diffuse large B cell lymphomas, DLBCL, 2 transformed follicular lymphomas, TFL, and 3 follicular lymphomas, FL) received the novel CAR T cells infusion at a dose of more than 1×10^6/kg. Grade ≥ 3 cytokine release syndrome (CRS) and neurotoxicity were observed in 11.1% ($n = 1/9$) and 11.1% ($n = 1/9$) of patients, respectively. The overall response rate (ORR) was 77.8% ($n = 7/9$) and complete response (CR) rate was 55.6% ($n = 5/9$). Two patients have ongoing CR (all at 20+ months). CAR T cells expanded after infusion and continued to be detectable at 12+ months in patients with ongoing CR. This novel CD19-CAR T cell was safe and effective with durable remissions in patients with refractory/relapsed B cell lymphoma.

Introduction

Genetically engineered T cells expressing chimeric antigen receptor (CAR) targeting CD19 have shown promising clinical efficacy on multiple B cell malignancies. In multicenter trials to evaluate CAR T-cells therapy for refractory B cell lymphomas, overall remission rates (ORR) ranged from 52% to 83% and complete remission (CR) rates were 40%–58% [1,2]. Despite the considerable response rates, patients usually relapsed with a median duration of remission (DOR) of around 11.1 months [2].

Inhibitory signals that CAR T cells encountered in the tumor microenvironment are commonly reported to impair the efficacy the CAR T therapy [3]. Programmed cell death-1 (PD-1) [4] is one of the checkpoint proteins on T cells that could combine with programmed death ligand-1 (PD-L1) on tumor cells and induce T cell dysfunction and exhaustion [5]. Studies on the combination of CAR T cells and PD-L1 antibody were reported and showed an antitumor effect [6–8]. Besides co-administering with PD-L1 antibody, engineering modified CAR T cells or "armored" CAR T cells are another promising approach to enhance the efficacy of CAR T cells. Co-expression of a PD-1 dominant negative receptor or secreted PD-1-blocking single-chain variable fragments (scFv) was reported in preclin-

ical models and displayed the possibility to enhance CAR T-cell therapy [9,10].

Taken together, these studies lead to the clinical trials of modified checkpoint-blocking CAR T cells. A phase 1 clinical study (ChiCTR1900021295) of dominant-negative PD-1 armored CD19-CAR T cells involving patients with refractory aggressive B cell lymphoma was initiated. Here we reported initial results from this ongoing study.

Materials and methods

Patients

This single-arm, open-label, phase 1 study was registered in the Chinese Clinical Trial Registry (ChiCTR, Registration No.: ChiCTR1900021295) and conducted at the Department of Hematology, Yantai Yuhuangding Hospital as one of the multi-centers.

Eligibility criteria included: (1) CD19 positive histologically confirmed B cell NHL, including diffuse large B-cell lymphoma (DLBCL), transformed follicular lymphoma (TFL), or follicular lymphoma (FL); at least one measurable lesion; (2) refractory diseases (no response to sufficient first-line immune-chemotherapy; progressive disease or stable disease as best response to second or further chemotherapy; disease pro-

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gression or recurrence less than 12 months after prior ASCT) or relapsed diseases; (3) prior therapy must include an anti-CD20 monoclonal antibody and anthracycline; and (4) no evidence of CNS involvement based on magnetic resonance imaging(MRI).

Eligible patients also should meet age from 18 to 65 years; an Eastern Cooperative Oncology Group (ECOG) performance-status score of ≤2; and an absolute lymphocyte count of ≥0.1 × 10^9/L (100/ul), neutrophil count of ≥1 × 10^9/L, and platelet count of ≥75 × 10^9/L. Patients must have adequate renal, hepatic, lung, and cardiac function defined as serum creatinine clearance rate of ≥60 ml/min, serum alanine aminotransferase (ALT)/aspartate aminotransferase (AST) of ≤2.5 times the upper limit of normal, total serum bilirubin of ≤1.5 times the upper limit of normal, basic blood oxygen saturation of >92%, the cardiac ejection fraction of ≥50%, and no evidence of pericardial effusion, as determined by echocardiogram.

Key exclusion criteria included a history of malignancy other than non-melanoma skin carcinoma or carcinoma in situ, ASCT within 6 weeks of informed consent, history of allogeneic hematopoietic stem cell transplant, prior CD19-targeted therapy, prior CAR T-cell therapy, history of HIV, HBV, and HCV infection, uncontrollable viral bacterial and fungal infections, history of CNS diseases, and history of a severe allergy to aminoglycosides or any regimen involved in this study.

Written informed consents were obtained from the patients for publication of this study and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request. The Institutional Review Board/Independent Ethics Committee of Yantai Yuhuangding Hospital approved the protocol. This study was conducted following the principles of the Declaration of Helsinki.

Study design

The primary endpoints of this study were objective response rate including CR and partial remission (PR) according to the revised International Working Group (IWG) Response Criteria (Cheson 2014) [11,12]. Secondary endpoints include DOR, progression-free survival (PFS), overall survival (OS), and adverse events (AEs). The severity of AEs was graded according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. Cytokine release syndrome (CRS) was defined and graded based on DW Lee's report [13].

CAR t cell manufacturing

Eligible patients were enrolled after screening according to the criteria and underwent leukapheresis to obtain peripheral blood mononuclear cells (PBMC) for CAR T cell manufacturing on day-21.

CD4+ and CD8+ T cells were then selected from leukapheresis, stimulated by anti-CD3/CD28 beads (1:3), and cultured in T cell medium containing 12.5 ng/ml IL-2 at 5% CO2, 37 °C. Cells were then transduced with a lentiviral vector containing the anti-CD19 CAR and a dominant-negative mutated PD-1 sequence that expressed inactive PD-1. Replacement of culture medium 24 h after transduction or as required was performed to keep cell activity. About 5–6 days after transduction, cells were harvested, washed, and then suspended in the solution with DMSO, HAS, Multiple Electrolytes Injection, and Dextran 40 Glucose Injection for cryopreservation. The products were stored in the gas phase of the liquid nitrogen tank. Quality control tests on products included viability, efficiency (percentage of CAR-expressing cells), transgene copy number, cytotoxicity, endotoxin, mycoplasma, and sterility. CD19-CAR T cells were thawed for infusion at a 37 °C water bath. The dose returned was at least 1 × 10^6 CD19-CAR T cells/kg for patients.

Clinical procedures

PD-L1 expressions were detected by immunohistochemical (IHC) staining on the FFPE sections of the initial tumor tissues. The detection was performed by Shanghai Pengyuan Laboratory Medicine Insti-

tute with "PD-L1 IHC 22C3 pharmDx" (Dako, SK006). Diluted Monoclonal Mouse Anti-Human PD-L1 (3ug/mL) was used for the staining process.

Patients received lymphodepletion with fludarabine (30 mg per square meter of body surface area per day) and cyclophosphamide (500 mg per square meter per day) on days −5, −4, and −3, followed by an infusion of CAR T cells on day 0. Peripheral blood was collected on transfusion day and then every three days to the 21st day for monitoring the expansion and toxicity of CAR T cells. Clinical response was evaluated at the end of the first month and then every 3 months with PET-CT. Patients were followed until disease progression or death.

Statistical analyses

The sample size was based on clinical considerations. Descriptive statistics include means with standard deviations or medians with minimum and maximum for continuous variables and counts and percentages for categorical variables. Duration of response, progression-free survival, and associated 95% confidence intervals were estimated with the use of Kaplan–Meier methods. GraphPad Prism 8 was used for statistical analysis of the experiments, data processing, and figure generation.

Results

Patient characteristics

A total of 9 patients were enrolled in this study including 4 DLBCL, 2 TFL, and 3 FL as shown in the consort diagram (Fig 1). The median age was 51 years (range, 22 to 62). Sixty-seven percent of patients were in stage III and IV according to Ann Arbor staging system. Extranodal lesions included intestine, bone marrow, lung, breast, and skin involvements. Four patients received previous treatments of more than 3 lines. CAR T cell manufacturing was successful for all nine patients (Table 1).

PD-L1 expression was detected by IHC staining. Three cases (2DLBCL and 1 TFL) were PD-L1 positive (>20%), while six cases (2 DLBCL, 1 TFL, and 3 FL) were PD-L1 negative (range 0–10%) (Fig 2A).

Safety

All the AEs in the first 30 days were reported and graded. All patients experienced CAR T cells related AEs of any grade with the most common AEs including fever (100%), neutropenia (100%), and fatigue (100%). AEs of Grade≥3 were reported in eight of nine patients. The most common hematological AEs were neutropenia (100%), anemia (66.7%), and thrombocytopenia (55.6%) that were unrelated to the lymphodepletion regimens (Table 2).

CRS occurred in all nine patients treated with CD19-CAR T cells. A total of eight (88.9%) patients had grade 1 CRS. The most common CRS-related symptoms were pyrexia (100%), tachycardia (100%), hypotension (22.2%), and hypoxia (22.2%). Only one patient (patient 8) experienced grade 3 CRS with pyrexia, hypotension, hypoxia, headache, and notable elevated IL6 concentration in serum. This patient received Tocilizumab on days 3–4 and blood filtration on day 4 for management of CRS. The severity of CRS seemed unrelated to the CD19-CAR T-cell expansion since patient 8 experienced grade 3 CRS but the expansion of CD19-CAR T cells was not higher compared with other patients. Patients 1, 5, and 6 who exhibited obvious peaks of CD19-CAR T-cell counts only had CRS of grade 1 (Table 3).

Patients 8 who displayed grade 3 CRS also experienced neurotoxicity of grade 1 with symptoms of encephalopathy, tremor, delirium, and restlessness, while other patients didn't exhibit any symptoms of neurotoxicity.

CRS and neurotoxicity occurred early. The median time to develop CRS and neurotoxicity was 5 days (range: 0–9 days; n = 8) and 3 days (patient 8), respectively, with a median duration of 3 days (range: 1–6
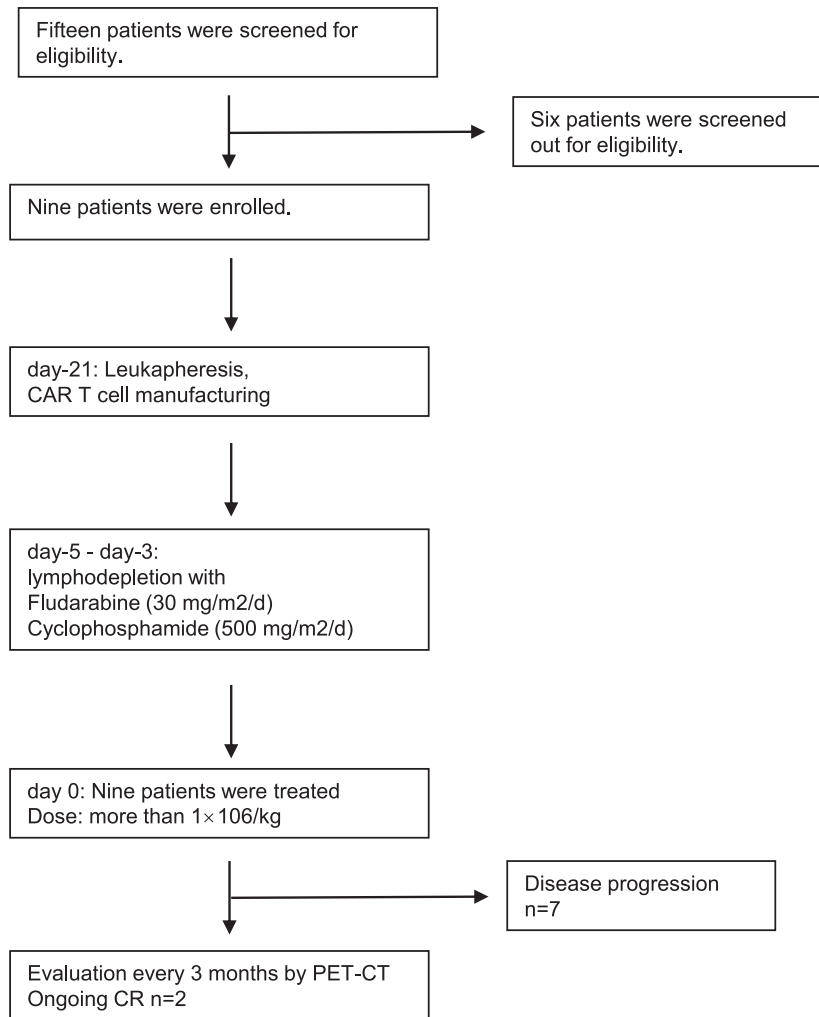
Consort diagram

Fig. 1. Consort diagram.

Fifteen patients were screened for eligibility.

Six patients were screened out for eligibility.

Nine patients were enrolled.

day-21: Leukapheresis, CAR T cell manufacturing

day-5 - day-3: lymphodepletion with Fludarabine (30 mg/m2/d) Cyclophosphamide (500 mg/m2/d)

day 0: Nine patients were treated Dose: more than 1×106/kg

Disease progression n=7

Evaluation every 3 months by PET-CT Ongoing CR n=2

days; $n = 8$) and 1 day (patient 8), respectively. In most of the patients, CRS was self-limiting and reversible.

Efficacy

All patients received the positron emission tomography-computed tomography (PET-CT) scan on the 30th day after CD19-CAR T-cell infusions. Seven (77.8%) of nine patients achieved an objective response with two (22.2%) patients having CR and five (55.6%) patients having PR (Fig 2B). In the five patients with PR at 1 month, two patients achieved CR at 3 months and one patient was evaluated as CR at 6 months. In refractory DLBCL patients, one (25.0%) patient achieved CR and two (50.0%) patients achieved PR. In refractory TFL and FL patients, three (60.0%) patients got CR and one (20.0%) patient PR.

The median duration of follow-up till now was 20 months (range: 13–24 months). Two patients (one TFL and one DLBCL) were in ongoing CR at 24 and 23.5 months. Progressive disease was reported in patients 2, 4, 5, and 9 at 1–3 months, respectively. Patients 6, 7, and 8 with FL may relapse with diffuse high metabolic lesions (SUVmax 6.9–8.0) less than 1.0 cm in diameter by PET-CT at 9–11 months, rejected receiving re-biopsy and subsequently quitted (Fig 2C).

In Fig 2D, patient 1 diagnosed as refractory TFL with 3 lines of previous treatments (R-CHOP, R-GDP, and MA) was evaluated as PR on day 30 and CR on day 90 showed by the representative PET-CT scans. Patient 1 kept in CR when evaluated at 24 months after infusion. Patient

3 defined as refractory DLBCL was previously treated with R-CHOP, R-GDP, ESHAP, MA, and HD-MTX. He achieved PR on day 30 and CR on day 90 after CAR T-cell infusion showed by the representative PET-CT scans (Fig 2D). The response has been ongoing at 23+ months. Patient 8 was diagnosed as refractory FL with a bulky disease in the abdomen. He achieved PR at 1 month and CR at 3 months after infusion (Fig 2D).

CAR T-cell expansion and cytokine secretion

As shown in Fig 3A, the peak of CD19-CAR T-cell expansions detected by flow cytometry occurred between 5 and 23 days after infusions with a median of 8 days. The counts of CD19-CAR T cells at peaks ranged from 0.78 to 84.63 × 10^4/ml. The largest count of CD19-CAR T cells was observed in patient 5 on day 12. Cell counts tested by flow cytometry showed consistent with CAR copy numbers tested by PCR (Fig 3A). CD19-CAR T cells were still detectable via qPCR and flow cytometry in the latest follow-up in the peripheral blood of the two patients with ongoing CR (Suppl Table 1).

The final CD19-CAR T-cell products were a mixture of CD4+ and CD8+ T cells. We monitored the CD8/CD4 ratios during CAR T-cell expansion in vivo. The ratios of CD8/CD4 increased after CAR T-cell infusion and the peak (2.26–16.98, median: 5.2) came on day 3–8, which was earlier than that in the CAR T cells counts (5–23, median: 8) and then went down as CD4+ CAR-T cells expanded later.
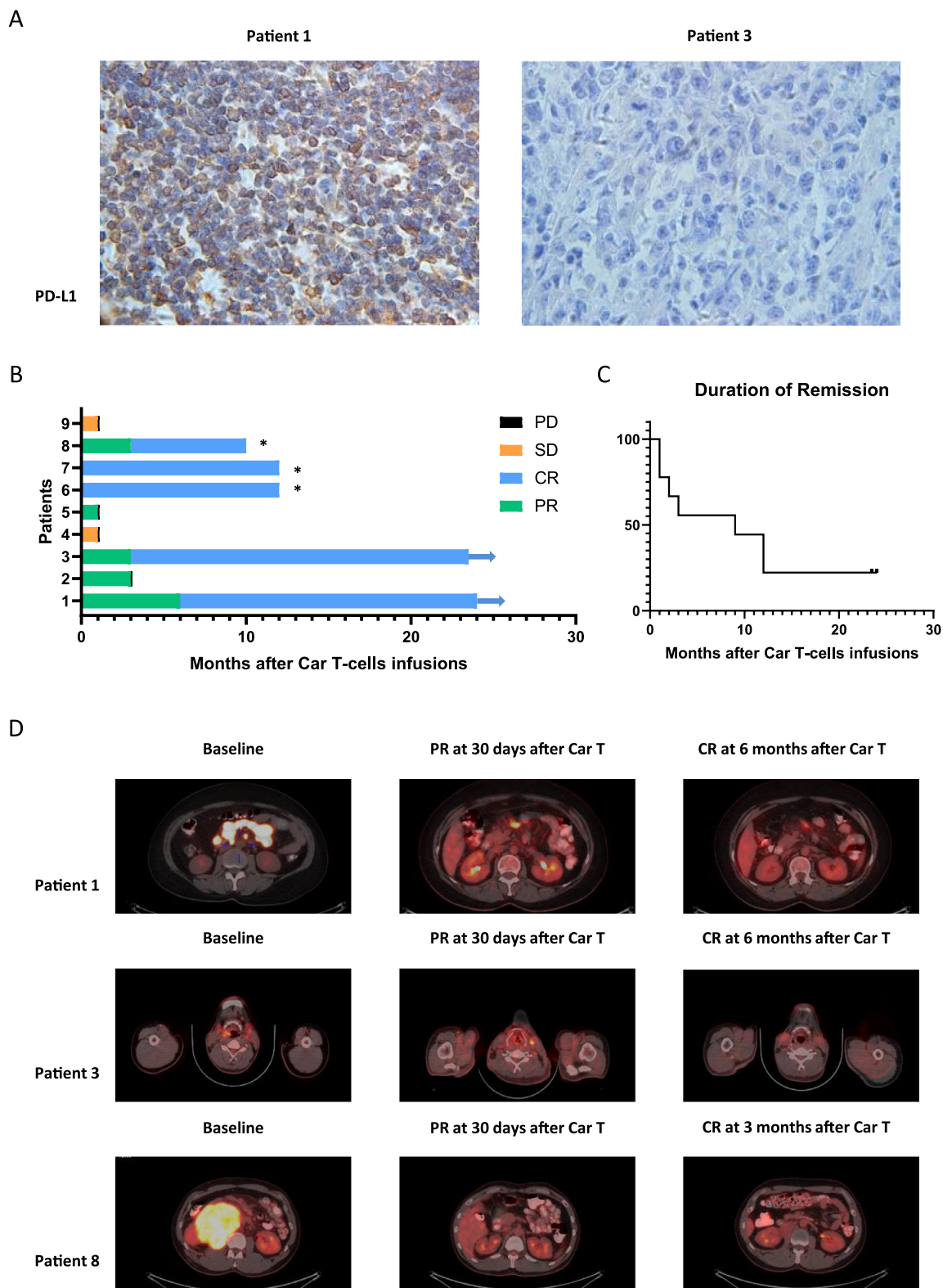
Fig. 2. Clinical efficacy after CAR T-cell infusion
(A) PD-L1 expression in FFPE sections of tumor tissues from patients 1 and 3
(B) Follow-up in nine patients (* Withdraw from the clinical trial)
(C) Duration of response post-infusion with CAR T cells
(D) Representative images of PET-CT scan at baseline and post-infusion in three individuals.

Table 1
Baseline characteristics.

Characteristic Patients	Patients 1	2	3	4	5	6	7	8	9
Diagnosis	TFL	DLBCL (GCB)	DLBCL (GCB)	TFL	DLBCL (GCB)	FL	FL	FL	DLBCL (non-GCB)
Age(years)	52	62	22	42	53	51	34	37	62
Sex	female	male	male	male	female	female	female	male	male
ECOG	2	1	0	1	1	0	0	0	1
Disease stage	IV	IV	II	III	IV	IV	IV	I bulk	II bulk
Prior therapies	RCHOP*5RGDP*2 MA*1	RCHOP*2R-CHOPE R-GDP CHOE	RCHOP*4R-GDP*5 ESHAP MA HD-MTX	RCHOP*6	CHOP*5 DT56Gy/28F/2Gy	RCHOP*6	RCHOP *8 R *6	RCHOP*4	RCHOP*4 RCHOPE *2 R-GDP*1 RDHAP *1
Prior lines of therapy	3	4	5	1	2	1	1	1	4
R/R status	refractory ≥second line of therapy	refractory≥second line of therapy	refractory ≥second line of therapy	relapsed after first line therapy	refractory ≥second line of therapy	relapsed after first line therapy	relapsed after first line therapy	relapsed after first line therapy	refractory≥ second line of therapy
R/R LDH(U/ml)	907	730	1382	353	250	160	281	189	438
Extra nodal involvement	bone marrow	small intestine	no	no	lung, breast	bone marrow	skin, bone marrow	no	no
PD-L1 expression	80%	5%	0	1%	60%	1%	10%	1%	25%

TFL, transformed follicular lymphoma; DLBCL, diffuse large B cell lymphoma; GCB, germinal center B-cell-like lymphoma; FL, follicular lymphoma; non-GCB, non-germinal center B-cell-like lymphoma; ECOG PS, eastern cooperative oncology group performance status; MA, high dose-MTX+ intermediate dose cytarabine; R, rituximab; HD-MTX, high dose MTX; R/R, relapsed and refractory; LDH, lactate dehydrogenase.

Table 2
Grade 3 or higher AEs.

Event	any, n (%)	grade 3, n (%)	grade 4, n (%)	grade 5, n (%)
febrile neutropenia	5(55.6)	5(55.6)	0(0)	0(0)
encephalopathy	1(11.1)	0(0)	0(0)	0(0)
neutropenia	9(100)	3(33.3)	5(55.6)	0(0)
anemia	6(66.7)	4(44.4)	0(0)	0(0)
hypoxia	2(22.2)	1(11.1)	0(0)	0(0)
somnolence	0(0)	0(0)	0(0)	0(0)
thrombocytopenia	5(55.6)	1(11.1)	2(22.2)	0(0)
acute kidney injury	0(0)	0(0)	0(0)	0(0)
agitation	0(0)	0(0)	0(0)	0(0)
ascites	0(0)	0(0)	0(0)	0(0)
ALT increased	0(0)	0(0)	0(0)	0(0)
cardiac failure	2(22.2)	0(0)	0(0)	0(0)
delirium	1(11.1)	0(0)	0(0)	0(0)
fatigue	9(100)	1(11.1)	0(0)	0(0)
hemorrage intracranial	0(0)	0(0)	0(0)	0(0)
hypocalcemia	4(44.4)	0(0)	0(0)	0(0)
hyponatremia	4(44.4)	0(0)	0(0)	0(0)
hypophosphatemia	5(55.6)	3(33.3)	0(0)	0(0)
hypotension	2(22.2)	1(11.1)	0(0)	0(0)
metabolic acidosis	0(0)	0(0)	0(0)	0(0)
oral herpes	0(0)	0(0)	0(0)	0(0)
pseudomonal sepsis	0(0)	0(0)	0(0)	0(0)
pyrexia	9(100)	2(22.2)	0(0)	0(0)
restlessness	1(11.1)	0(0)	0(0)	0(0)
tremor	1(11.1)	0(0)	0(0)	0(0)
urinary tract infection	0(0)	0(0)	0(0)	0(0)

AEs, adverse events; ALT, alanine aminotransferase.

A series of cytokines were monitored during this study. With the expansion of CD19-CAR T cells, a range of cytokines and inflammatory factors were induced, elevated, and then cleared that regulated T cell proliferation, activation, and function. The peak values of markers including IL2, IL4, IL6, IL10, TNF-α, and IFN-γ occurred within 7 days after infusions and typically went down to baseline in the first month. IL6 was considered as one of the major cytokines that contributed to CRS [14]. In these patients, patient 8 showed a peak value of 2002.33pg/ml on day 1 of post-infusion with grade 3 CRS, and other patients with

grade≤ 2 CRS showed lower IL-6 level (peak values range from 6.96 to 454.55pg/ml). Other elevated cytokines such as IL2, IL4, IL10, TNF-α, and IFN-γ were also consistently noted in these patients (Fig 3B).

Discussion

CAR T-cell therapy is a novel and hot frontier in cancer immunotherapy. Although over 1000 clinical trials are registered and promising efficacy occurs on series of malignant diseases, as we know, this is the
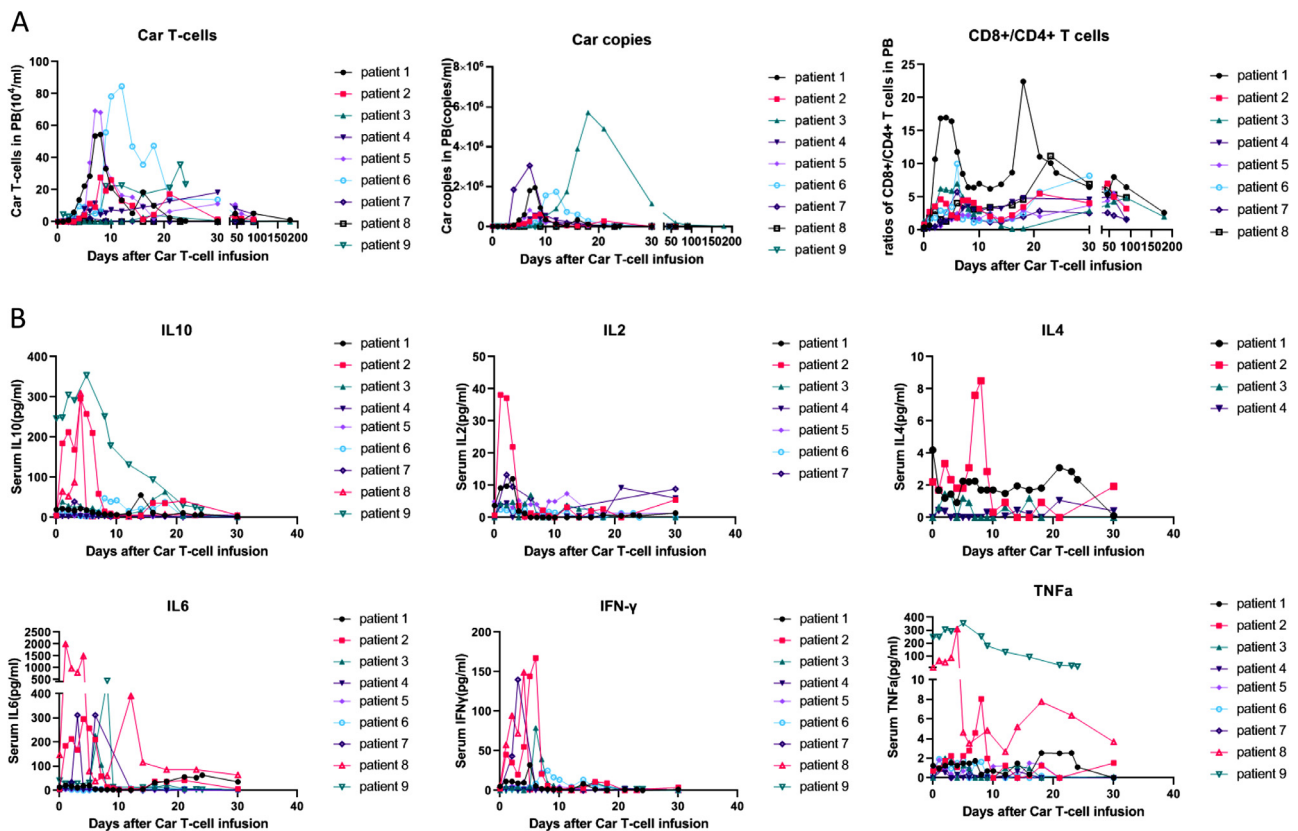
Fig. 3. Kinetics of CAR T cell in PB and serum biomarkers

(A) Car T-cell counts and ratios of CD8+ *T* cells to CD4+ *T* cells in PB detected by flow cytometry and CAR copies in PB detected by PCR. CAR T cells expanded after infusion and the peak occurred on 5 to 23 days after infusions in all patients. Cell counts detected by flow cytometry showed consistent with CAR copy numbers determined by qPCR. CAR T cells persisted in the latest follow-up in the peripheral blood of the two patients with ongoing CR. The ratios of CD8+ *T* cells to CD4+ *T* cells increased after CAR T-cell infusion and the peak occurred on day 3–8, and then went down as CD4+ CAR T cells expanded later.

(B) Analysis on serum level of critical biomarkers related to CAR T-cell function and CRS. Cytokines and inflammatory factors were induced and elevated quickly after infusion and generally resolved within the first month. In patients 8, a significantly elevated IL6 level was detected with grade3 CRS.

Table 3
Cytokine release syndrome and neurotoxicity.

Event	any, n (%)	grade 3, n (%)	grade 4, n (%)
CRS, any	9(100)	1(11.1)	0(0)
pyrexia	9(100)	2(22.2)	0(0)
hypotension	2(22.2)	0(0)	0(0)
tachycardia	9(100)	0(0)	0(0)
acute kidney injury	0(0)	0(0)	0(0)
cardiac failure	2(22.2)	0(0)	0(0)
headache	1(11.1)	0(0)	0(0)
hypoxia	2(22.2)	1(11.1)	0(0)
metabolic acidosis	0(0)	0(0)	0(0)
neurotoxicity, any	1(11.1)	0(0)	0(0)
encephalopathy	1(11.1)	0(0)	0(0)
tremor	1(11.1)	0(0)	0(0)
somnolence	0(0)	0(0)	0(0)
agitation	0(0)	0(0)	0(0)
aphasia	0(0)	0(0)	0(0)
delirium	1(11.1)	0(0)	0(0)
dizziness	0(0)	0(0)	0(0)
dyskinesia	0(0)	0(0)	0(0)
hallucination	0(0)	0(0)	0(0)
restlessness	1(11.1)	0(0)	0(0)

first reported clinical study on PD-1 modified CD19-CAR T cells. Here in this study, CD19-CAR T cells expanded sufficiently in the first two weeks post-infusion and then decreased notably in the first month. Different from only one peak of CAR T-cell expansion in most of the patients shown in other studies [15–18], the second peak of CD19-CAR T cells was observed in almost all the patients between day 15 and day 30 in this clinical trial. In CR patients, CD19-CAR T cells were detectable by flow cytometry and PCR in the long-term follow-up. The expression of dominant-negative PD-1 was considered to contribute to the enhanced expansion and persistence of CD19-CAR T cells [9].

The efficacy of the modified CD19-CAR T cell is notable. Although only 2 patients achieved CR in the first month, 3 patients with PR evaluated in the first month continued to respond to therapy and achieved CR in 3 months. Eventually in this study, the ORR is 77.8% and CR is 55.6%. The DOR ranged from 1 to 24 months. The ORR was lower than that in refractory DLBCL patients ($n = 3$, ORR 100%) who received the equal novel CD19-CAR T cell reported by Chen et al. in another center of this trial [19]. The response rate and the duration indicated that these modified CD19-CAR T cells may be a promising approach to treating refractory B cell lymphoma.

This modification on CAR T cells does not induce extra toxicity. Severe CRS (grade 3–4) was only observed on one patient and reversed after Tocilizumab and steroid injection. Neurotoxicity of grade 1 occurred on one patient with mild symptoms. Elevated IL6, IFN-ɣ, and TNF-α levels were observed with severe CRS on patient 8 indicating that these cytokines might contribute to the onset of CRS. The elevated cytokines and inflammatory factors could drop to baseline in the first month.

We enrolled transformed follicular lymphoma, follicular lymphoma, and diffuse large B cell lymphoma in this trial. Compared to DLBCL, FL and TFL showed similar response rates (80% in FL and TFL, while 75% in DLBCL), which may be caused by the bias of enrollment that FL patients experienced fewer previous lines of treatment. But notably,

patient 1 with refractory TFL that experienced 3 lines of previous treatment keeps in ongoing CR in 23+ months. Three FL patients in this study experienced progression of disease within 24 months (POD 24) which predicted the poor outcomes [20,21]. Since clinical trials should be considered as the primary treatment options for FL patients experiencing POD 24 [22], we enrolled these three early-relapsing FL patients in this study. Compared to the previous clinical studies on the second-line treatment of refractory FL, the ORR and DOR of this novel CD19-CAR T cells therapy are competitive to other second-line regimens including Obinutuzumab, lenalidomide, ibrutinib, and PI3K inhibitor [23–26]. However, even all of them achieved CR after infusion, DOR ranged from 9 to 11 months confirmed by PET-CT.

Second generation CAR T cells including co-stimulatory domains are now most widely used in clinical trials [27]. The co-stimulation domains of CD28 or 4–1-BB are expressed with CAR and TCR-associated activation domains to enhance the TCR signal, cell activation, and expansion [28,29]. CAR T-cell therapy for refractory B cell lymphomas has shown effective response rates in either single center or multi-center clinical trials [1,2,16,30,31]. The ORR was usually higher than 50% and CR rates were 40%–60%. Despite the considerable response rates, two-third of patients relapsed with a median DOR of around 11 months.

One mechanism for disease relapse or resistance to CAR T cells is that T cell function and efficacy are inhibited in the immune-suppressive microenvironment via the PD-1/PD-L1 axis [32]. Preclinical studies demonstrated that several approaches to decreasing PD-1 expression were helpful to enhance CAR T-cell efficacy including the application of PD-1/PD-L1 antibody [6,33,34], co-expression of a PD-1 dominant negative receptor [9], secreted PD-1-blocking scFv [10], or ablation of PDCD1 with Crispr/Cas 9 technique [35].

CD19-CAR T cells used in this study were modified successfully to co-express dominant-negative PD-1 on cell surface thus inhibiting the binding of normal PD-1 on CAR T cells with PD-L1 on the surface of tumor cells and subsequently down-regulate T cell exhaustion that may happen in PD-L1 positive tumors. Before infusion, PD-L1 expression was detected in all nine patients. DLBCL patients exhibited higher PD-L1 expression compared to FL patients which were consistent with the findings from previous studies [36,37]. In the two CR ongoing patients, case 3 is PD-L1 negative DLBCL while case 1 is PD-L1 positive transformed FL. These data indicated that dominant-negative PD-1 expression may protect CAR T cells from exhaustion induced by the PD-1/PD-L1 axis and the efficacy of this PD-1 modified CD19-CAR T cell could conquer the inhibition of PD-1/PD-L1 axis on CAR T cell expansion. Thus, the novel CD19-CAR T cell showed the same efficacy on PD-L1 positive tumor with that on PD-L1 negative tumor as we expected. According to the level of PD-L1 expression, we may select those patients who can have a better chance of responsiveness to this novel treatment method compared to other therapies.

This study has three main limitations. This is a single-arm and open-label study without sample size calculation. The sample size was only based on clinical considerations. Since the small population of enrollment, we only have one single arm with open-label without random comparison.

In conclusion, the result of this phase 1 study, the first study of dominant-negative PD-1 armored anti-CD19 CAR T cells on refractory B cell lymphoma, demonstrated that the modified CD19-CAR T cells were safe and tolerable without extra toxicity. It also indicated that the efficacy of this new CD19-CAR T cell was promising for the management of TFL and early-relapsed FL. More importantly, this novel CD19-CAR T cell showed the same efficacy on PD-L1 positive tumor with that on PD-L1 negative tumor as we expected. It proved the possibility that CAR T-cell function could be improved by interrupting the PD-1/PD-L1 axis which was validated preliminarily in this clinical study.

Declaration of Competing Interest

None.

Author contribution

Xiaoqian Liu: Conceptualization, Methodology, Software Graphpad Prism8.0: formal analysis, Writing- Original draft preparation, Writing - Review & Editing.

Yuanfeng Zhang: Investigation, Resources, Writing - Review & Editing

Kaimin Li: Investigation, Resources

Yinghui Liu: Project administration

Junqing Xu: Investigation, Resources

Junjie Ma: Investigation, Resources

Licai An: Investigation, Resources

Hui Wang: Investigation, Resources

Xiaoxia Chu: Supervision, Conceptualization, Writing - Review & Editing

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2021.101085.

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