Clinical Cancer Research

Autologous T Cells Expressing CD30 Chimeric Antigen Receptors for Relapsed or Refractory Hodgkin Lymphoma: An Open-Label Phase I Trial

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Abstract

Purpose: Relapsed or refractory Hodgkin lymphoma is a challenge for medical oncologists because of poor overall survival. We aimed to assess the feasibility, safety, and efficacy of CD30-targeting CAR T cells in patients with progressive relapsed or refractory Hodgkin lymphoma.

Experimental Design: Patients with relapsed or refractory Hodgkin lymphoma received a conditioning chemotherapy followed by the CART-30 cell infusion. The level of CAR transgenes in peripheral blood and biopsied tumor tissues was measured periodically according to an assigned protocol by quantitative PCR (qPCR).

Results: Eighteen patients were enrolled; most of whom had a heavy treatment history or multiple tumor lesions and received a mean of 1.56×10^7 CAR-positive T cell per kg (SD, 0.25; range, 1.1–2.1) in total during infusion. CART-30 cell infusion was tolerated, with grade $\geq$3 toxicities occurring only in two of 18 patients. Of 18 patients, seven achieved partial remission and six achieved stable disease. An inconsistent response of lymphoma was observed: lymph nodes presented a better response than extranodal lesions and the response of lung lesions seemed to be relatively poor. Lymphocyte recovery accompanied by an increase of circulating CAR T cells (peaking between 3 and 9 days after infusion) is a probable indictor of clinical response. Analysis of biopsied tissues by qPCR and immunohistochemistry revealed the trafficking of CAR T cells into the targeted sites and reduction of the expression of CD30 in tumors.

Conclusions: CART-30 cell therapy was safe, feasible, and efficient in relapsed or refractory lymphoma and guarantees a large-scale patient recruitment. *Clin Cancer Res; 23(5); 1156–66.* *©2016 AACR.*

Introduction

Hodgkin lymphoma is characterized by a paucity of malignant Hodgkin and Reed-Sternberg (HRS) cells and an abundance of inflammatory/immune cells, including T- and B-reactive lymphocytes, macrophages, granulocytes, and fibroblast-like cells (1). About 80% of patients with Hodgkin lymphoma are substantially improved or likely to be cured by the first-line therapy using multidrug chemotherapy and localized radiotherapy. However, for patients with Hodgkin lymphoma who are refractory to or relapse after the first-line treatment or autologous stem cell transplantation (ASCT), the prognosis is rather poor (2, 3). So it is imperative to develop novel approaches to improve the prognosis for patients with relapsed or refractory Hodgkin lymphoma.

CD30, a member of the TNF superfamily, is selectively overexpressed in HRS cells and exhibits very low expression in normal tissues, rendering this antigen a promising target for novel treatment strategy (4). SGN35, an anti-CD30 antibody conjugated to the antitubulin agent monomethyl auristatin E (MMAE), has been used in the treatment of relapsed or refractory Hodgkin lymphoma and demonstrated good tolerance and promising antitumor activity (5), although the long-term disease control capacity of this drug has yet to be fully appreciated.

Chimeric antigen receptors (CAR) combine an extracellular antigen-binding domain of an antibody (scFv) with a transmembrane domain, linked to one or more intracellular T-cell signaling domains (6, 7). In recent years, CAR-modified T cells targeting CD19 or CD20 have shown encouraging antitumor activity in relapsed or refractory lymphocytic leukemia (8, 9) and B-cell lymphomas (10). There is no public report on CART-30 cell therapy in the world to date. In this phase I trial, we define its feasibility, safety, and efficacy in subjects with progressive relapsed or refractory Hodgkin lymphoma following the administration of CD30-targeting CAR T cells.

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Translational Relevance

In a phase I clinical trial, we report the safety, feasibility, and clinical efficacy of CD30-targeting CAR T cells in patients with progressive relapsed or refractory Hodgkin lymphoma. Our data show that the treatment is well tolerated without severe toxicity. The CART-30 cell infusion yields a 39% objective response for those patients with relapsed or refractory Hodgkin lymphoma, even though who failed with autologous stem cell transplantation or brentuximab treatment. In the future, on the basis of the efficacy of CART-30 cell infusion, combination or consolidation treatment with other anticancer therapies not only improved clinical response and long-term disease control for patients with primary refractory or relapsed Hodgkin lymphoma but also could be administrated in early-disease patients to reduce the long-term toxicity of chemoradiotherapy. What's more, CART-30 cell infusion will be further developed for therapy of other $CD30^+$ lymphoma.

Patients and Methods

Patients

Between December 1, 2014 and July 31, 2015, 18 patients were enrolled. This trial is registered with ClinicalTrials.gov, number NCT02259556. To be eligible for enrollment in this study, patients had to be 8 to 75 years old with $CD30^+$ relapsed or refractory lymphoma confirmed by immunohistochemical (IHC) evidence, have an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less, have $\geq$1 cm of measurable lesion, previous treatment with at least 2 systemic chemotherapy regimens which must be finished at least 4 weeks, and no ASCT or brentuximab vedotin within 12 weeks. Primary exclusion criteria were severe organs dysfunction, a history of or active systemic autoimmune/immunodeficiency disease, and a treatment history of immunosuppressive agents or glucocorticoids within a month. All patients provided written informed consent in accordance with the Declaration of Helsinki before enrolling in the study. The study protocol and the consent forms were approved by the Institutional Review Board at the Chinese PLA General Hospital.

Constructs and lentivirus package

The single-chain fragment variable (scFv) sequence specific for the CD30 antigen was derived from AJ878606.1. The CAR.30-CD137ζ vector harboring anti-CD30 scFv and human CD137 and CD3ζ signaling domains were generated (Fig. 1A). The cassette was cloned into a lentiviral backbone. A pseudotyped, clinical-grade lentiviral vector was produced according to current good manufacturing practices with a 3-plasmid production approach. The GFP-harboring vector CAR.30-CD137ζ-GFP was also constructed to verify transduction efficiency.

Generation and expansion of CAR T cells

CAR T cells were generated as previously described (9, 10). Lentivirus-mediated CAR transduction was performed twice on days 2 and 3 of cell culture, respectively. Composition and purity were assessed by fluorescence-activated cell sorting (FACS) and were harvested beginning on days 10 to 12.

Treatment plan

All enrolled patients' overall disease burden was assessed via imaging with positron emission tomography (PET)/computed tomography (CT). Patients received a conditioning regimen that included 3 forms: (i) FC (fludarabine, 3 daily doses of 25 mg/m^2 + cyclophosphamide, at a total dose of 30 mg/kg) to deplete endogenous leukocytes (11, 12); (ii) GMC-like chemotherapy (gemcitabine 1 g + mustargen 10 mg + cyclophosphamide, at a total dose of 30 mg/kg) to inhibit the disease progression in a short time and eliminate endogenous leukocytes; and (iii) PC (nab-paclitaxel 150 mg/m^2 + cyclophosphamide 30 mg/kg) to deplete the stromal compartment and eliminate endogenous leukocytes; the individual absolute dose was administrated at the discretion of a physician according to treatment history and bone marrow tolerance (Supplementary Table S4). A conditioning regimen was followed 2 to 3 days later by the infusion of CART-30 cells, the administration of which was a starting dose of 3.2×10^5 CAR T cells per kg and then infused by 5-fold increments over 3 to 5 consecutive days (the intended dose is 1×10^7 to 3×10^7 CAR T cells per kg stated according to previous clinical trials (9, 10). Clinical response was assessed by radiographic imaging 4 weeks after cell infusion. Patients were eligible to receive a second CART-30 cell infusion after disease progression if they obtained clinical benefit, defined as a complete remission (CR), partial remission (PR), or stable disease (SD) $\geq$3 months from the first cycle of treatment. Peripheral blood samples and pathologic biopsies were obtained at predetermined time points to evaluate toxicity and the CART-30 cells' persistence, expansion, and homing. Toxicity was monitored according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, ver. 4.0. Treatment response to Hodgkin lymphoma was defined according to standard international criteria (13). Follow-up continued until April 30, 2016.

Laboratory measurements

Real-time PCR was used to quantify the level of the CAR gene according to the protocol described previously (9, 10). A 153-bp fragment containing portions of the CD8a chain and adjacent CD137 chain were amplified using the forward primer 5′-GGTCCTTCTCCTGTCACTGGTT-3′ and reverse primer 5′-TCTTCTTCTTCTGGAAATCGGCAG-3′. A standard curve was prepared for absolute quantitation of CAR transgene copies by making serial dilutions of the plasmid that encoded the CAR. A 7-point standard curve was generated consisting of 100 to 10^8 copies/mL CAR plasmid spiked into 100-ng nontransduced control gDNA. Amplification of β-actin was used for normalization of DNA quantities.

Cytokines and chemokines, including C-reactive protein (CRP), IFNγ, TNFα, VEGF, IL2, IL4, IL6, IL8, IL10, IL12p70, IL12P40/IL23, and granzyme B, were measured by immunoassay. The analyte concentrations were determined using a standard curve prepared with each assay. The IHC, immunophenotyping, and cytotoxicity assays of CART-30 cells are described in Supplementary Data.

Outcomes and statistical analysis

As an open-label, phase I study, the primary objectives were to define the safety and feasibility. Secondary objectives included defining antitumor activity of CART-30 cells, the progression-free survival, expansion and persistence of CART-30 cells and determining the relative subsets of CART-30 cells.

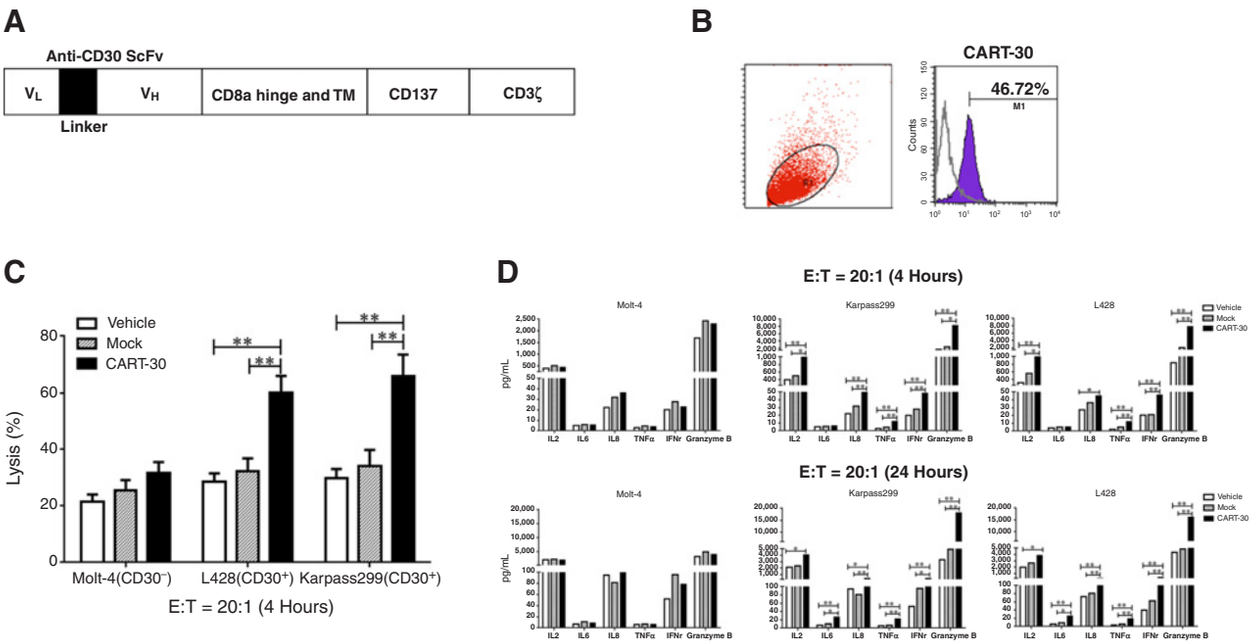

Figure 1.
Expansion and cytotoxicity of CART-30 cells. **A,** Schematic representation of the CAR.30-CD137ζ chimeric T-cell receptor cDNA plasmid, not to scale. **B,** Expression of CART-30 was assessed by FACS analysis. **C,** Cytotoxic activity of vehicle, mock, and CART-30 cells, using the following target cells: Molt-4 cell line (acute lymphoblastic leukemia, $CD30^-$), L428 ($CD30^+$) cell line (Hodgkin lymphoma cell lines), and Karpas299 ($CD30^+$) cell line (ALCL cell lines). Cytotoxic activity was evaluated in a 4-hour CFSE staining assay, and the results are shown at E:T ratios of 20:1. The data are represented as the mean of triplicate values from each patient, and error bars represent SEM. *, $P < 0.05$; **, $P < 0.01$. **D,** Expression of cytokines by CART-30 cells. Effector cells: vehicle, mock, and CART-30 cells. Target cells: Molt-4 cell line, L428 cell line, and Karpas299 cell line. Effector cells were adjusted to a final concentration of 2×10^6 cells/mL with medium, with no factor or serum. Effector cells were mixed with target cells at E:T ratio of 20:1 in mixed culture for 4 (up) or 24 hours (down). The supernatants were then collected and analyzed for the secretion. For cytotoxicity assay, each experiment was performed in triplicate and was repeated at least 3 times. The results are expressed as mean ± SD. *, $P < 0.05$; **, $P < 0.01$.

There was no significant selection bias from the clinical trial design to follow-up of patients. The results are shown as the mean ± SD of triplicate measurements (wells). Data were plotted using GraphPad Prism version 5.0. Progression-free survival was determined by the Kaplan–Meier method. Two-way ANOVA was used to determine the significance of the differences between means in all experiments. $P < 0.05$ was considered to be statistically significant.

Results

Patients

Eighteen patients were enrolled into this trial. Table 1 shows the clinical and disease-specific characteristics of the patients. The median age was 33 years (range, 13–77 years) and 72% were males. The 18 patients included 1 with primary cutaneous anaplastic large cell lymphoma (ALCL) and 17 with Hodgkin lymphoma of 3 different subtypes, most of which were nodular sclerosis. Fourteen of 18 patients had primary refractory disease (no achievement of CR with 4 cycles of first-line chemoradiotherapy); the remaining 4 patients had disease that was relapsed as well as refractory to the most recent prior therapy. The disease statuses of all 18 patients were progression before CART-30 cell infusion. All of them had heavy pretreatment histories that exceeded 10 cycles of cytotoxic chemotherapy regimens were 13, moreover, included radiotherapy ($n = 10$, 56%), ASCT ($n = 9$, 50%), and brentuximab vedotin ($n = 5$, 28%). Multiple tumor lesions included extensive abnormal lymph node regions (range, 0–7) and extranodal disease involving bone, lung, liver, pleura, mammary glands, kidney, and soft tissues found in 83% of the patients at the time of enrollment into the CART-30 cell protocol (Table 1; Supplementary Table S3).

Generation, characterization, and *in vitro* cytotoxicity of CART-30 cells

CART-30 cells were initially generated from the peripheral blood mononuclear cells (PBMC) of 50 to 80 mL of the peripheral blood of each patient. After 10 to 12 days of culture, the cells were ready for infusion. A mean of 95.9% ± 8.8% of the infused cells were $CD3^+$ cells principally composed of the $CD8^+$ subset (average, 76%), and a mean of 48% (range, 36%–68%) of the cells expressed CAR (Fig. 1B and Supplementary Table S1). The final number of infused cells and the corresponding immunophenotypic data for each patient are summarized in Supplementary Table S1. As illustrated in Fig. 1C, to evaluate the killing effects of CART-30 cells on $CD30^+$ Hodgkin lymphoma cell lines in *vitro*, carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled CART-30 cells were cocultured with Molt-4 (acute lymphoblastic leukemia, $CD30^-$), L428 (Hodgkin lymphoma cell line, $CD30^+$), and Karpas299 (ALCL cell line, $CD30^+$) cells at effector:target

(E:T) ratio of 20:1. After 4-hour coculture, cells were stained with phycoerythrin (PE)-labeled Annexin V and 7-aminoactinomycin D (AAD) and then cells without CFSE staining were subjected to survival analyses by FACS, CART-30 cells possessed prominent cytotoxicity activity against $CD30^+$ Hodgkin lymphoma cells and ALCL cells, but not $CD30^-$ Molt-4 cell line. To evaluate the potential antitumor activity of CART-30, we measured the expression levels of IL2, IL6, IL8, TNFα, IFNγ, and granzyme B in NT, mock T, and CART-30 cells after the killing of $CD30^+$ and $CD30^-$ tumor cells. The levels of all these cytokine secretions by CART-30 cells were significantly higher than NT and mock T cells after killing $CD30^+$ tumor cells but had no obvious variation in after killing $CD30^-$ tumor cells (Fig. 1D).

Adverse events

Patients received a mean of 1.56×10^7 CAR-positive T cells per kg (SD, 0.25; range, 1.1×10^7 to 2.1×10^7) in total during the infusion (Table 2). Thirteen patients received 1 cycle of CAR T-cell infusion and 5 received 2 cycles. Nearly all of them experienced cytopenias, including neutropenia, thrombocytopenia, or anemia,

Table 1. Characteristics of patients ($n = 18$)

					Disease burden at baseline[b]		Prior treatment			
Patient No.	Age, y	Sex	Diagnosis/ Stage	Disease status[a]	Lymph node regions	Extranodal sites	Chemotherapy regimens[c]	RT	ASCT	Others
1	31	M	NSHL/IV	Primary refractory	5	Lung, muscle, bone	ABVD × 6, COEP × 1, BEACOPP × 3, GDP × 3, MOAP × 4	N	Y	Brentuximab vedotin × 6, cytokine-induced killer cells × 3
2	55	F	LDHL/IV	Primary refractory	0	Lung, liver, bone	ABVD × 6, COPP × 2, GDEP × 6, MOAP × 9	N	Y	N
3	36	M	MCHL/IV	Primary refractory	7	Muscle, bone, kidney	BEACOPP × 6, IGVD × 2, MINE-BeanI × 2, MOAP × 1, MICP × 1	Y	N	Brentuximab vedotin × 6
4	27	M	MCHL/III	Relapsed	4	Bone	ABVD × 6, ICE × 3,	Y	Y	N
5	39	M	NSHL/III	Primary refractory	3	Bone	ABVD × 6, ICE × 2, MOAP × 4, ICE × 1	Y	N	N
6	21	M	MCHL/IV	Primary refractory	4	Lung	ABVD × 3, BEACOPP × 4, DHAP × 2, ICE × 1, MOAP × 8	Y	Y	N
7	41	M	NSHL/IV	Primary refractory	4	Bone	ABVD × 6, DICE × 3	N	Y	N
8	26	M	NSHL/IV	Primary refractory	4	Bone	ABVD × 7, BEACOPP × 4, GVD × 1, IVC × 2, GP × 5	N	N	Thalidomide
9	19	F	NSHL/IV	Primary refractory	0	Lung	ABVD × 5, GDP × 1, DICE × 1, MOAP × 3	N	N	N
10	13	F	NSHL/II	Primary refractory	1	None	ABVDE × 4, ABVD × 2	N	N	N
11	34	M	NSHL/IV	Primary refractory	2	Thoracic wall, parotid gland, bone	ABVD × 4, GDP × 1, MOAP × 2	Y	N	N
12	44	M	NSHL/IV	Primary refractory	2	Lung, muscle	BEACOPP × 4, NVB+IFO+DDP +DXM × 4, EPOCH × 2, ABVD × 5, DICE × 2	Y	Y	Enzastaurin DC + cytokine-induced killer cells
13	39	M	NSHL/IV	Relapsed	6	Bone, intravertebral mass, muscle, hip soft tissue	ABVD × 9, CHOP × 2, NVB+DDP × 2, BECOP × 10	Y	Y	N
14	25	F	NSHL/IV	Primary refractory	3	Lung, thoracic wall	ABVD × 4, CHOP × 2, ICE × 4, EPOCH × 1	N	Y	Brentuximab vedotin × 4
15	77	M	C-ALCL	Relapsed	0	Skin	CHOP × 7, CHOP + PEG-L-ASP × 3	Y	N	Brentuximab vedotin × 4 surgery × 3
16	19	M	NSHL/IV	Primary refractory	4	Mediastinal mass	ABVD × 6, GDHAP × 1, GEM+NVB+DDP+DEX × 3, DHAP × 1, MOAP × 1	Y	N	N
17	26	M	NSHL/III	Relapsed	1	None	ABVD × 6, GDP × 2, CHOP × 1	Y	Y	Brentuximab vedotin × 3
18	31	F	NSHL/IV	Primary refractory	4	None	ABVD × 2, GDP × 2, ICE × 2, DHAP × 2, MOAP × 6	N	N	N

Abbreviations: ASCT, autologous stem cell transplantation; C-ALCL, cutaneous ALCL; LDHL, lymphocyte depleted Hodgkin lymphoma; MCHL, mixed cellularity Hodgkin lymphoma; NSHL, nodular sclerosis Hodgkin lymphoma; RT, radiation therapy.

[a]Primary refractory: no achievement of CR with 4 cycles of first-line chemoradiotherapy.

[b]The results obtained according to PET/CT.

[c]Detailed prior chemotherapy regimens for all patients are listed in Supplementary Data.

Table 2. Patients' response and toxicity

Patient no.	Disease status at study entry	Conditioning regimen before T-cell infusion	No. of CAR-positive T cells infused ($\times 10^7$/kg)	Outcome				
				Response		Change in target lesions[a] %		
				Type	Duration, Mo	Lymph Nodes	Extranodal sites	Grade $\geq$ 3 toxicities[b]
1	PD	FC	1.5	SD	2	+10.4	+8.3 (Lung)	None
2	PD	FC	1.3	PR	2	NM	−65 (Liver); +36.9 (Lung)	None
3	PD	FC	1.4	PD		+45.7	+41.6 (Kidney)	None
4	PD	FC	2.1	PR	3.5	−71.4	NM	None
		GEMC	2.1	PR	5	−69.6	NM	None
5	PD	FC	1.7	SD	4.5	−17.7	NM	None
		GEMC	1.9	SD	6	−33.2	NM	None
6	PD	EAMC	1.6	PD		+49.5	+21.5 (Lung)	None
7	PD	MAMC	1.2	PR	6	−71.9	NM	None
		GMC	1.7	PR	6.5[+c]	−78.9		None
8	PD	GEMC	1.8	SD	3	−37.0	NM	None
9	PD	GEMC	1.4	SD	4.5	NM	−36.5 (Lung)	None
		PC	1.1	PR	9[+]	NM	−52.1 (Lung)	None
10	PD	GMMC	1.5	PR	3	−56.4	NM	None
11	PD	GEMC	1.4	PD		+23.0	NM	None
12	PD	GEMC	1.2	SD	12	−12.1	−25.0	None
13	PD	GEMC	1.3	PR	6	−54.0	−35.8 (Intravertebral mass)	Left ventricular systolic dysfunction
14	PD	GEMC	2.0	PD		−18.2	+76.1 (Lung)	None
15	PD	None	1.5	PR	3	NM	−57.6 (Skin)	ALT increased, AST increased, γ-GGT increased
		GMC	1.3	PR	5[+]	NM	−92.3 (Skin)	
16	PD	PC	2.0	PD		+40.7	+62.0 (Mediastinal mass)	None
17	PD	PC	1.2	SD	8.5[+]	−40.8	NM	None
18	PD	PC	1.6	SD	3	−9.2	NM	None

NOTE: Treatment response was defined according to revised response criteria for lymphoma: CR, disappearance of all evidence of disease; PR, regression of measurable disease ($\geq$50% decrease) and no new sites; SD, failure to attain CR/PR or PD; PD, any new lesion or increase by $\geq$50% of previously involved sites from nadir. Abbreviations: +, the percentage increase; −, the percentage decrease; EAMC, etoposide + cytarabine + mustargen + cyclophosphamide; FC, fludarabine + cyclophosphamide; GEMC, gemcitabine + epirubicin + mustargen + cyclophosphamide; GMC, gemcitabine + mustargen + cyclophosphamide; GMMC, gemcitabine + mitoxantrone + mustargen + cyclophosphamide; MAMC, mitoxantrone + cytarabine + mustargen + cyclophosphamide; NM, not measurable; PC, nab-paclitaxel + cyclophosphamide.

[a]Tumor burden was measured as the sum of the products of at most 6 measurable target lesions.

[b]Nearly all patients had cytopenias, including neutropenia, thrombocytopenias, or anemia resulting from chemotherapy, and febrile syndrome occurred 0.5 to 2 hours after cell infusion and self-recovered overnight; these are not listed.

[c]Indicates ongoing response.

after the conditioning regimen. During the period of CART-30 cell infusion, all the patients underwent grade 1/2 febrile syndrome (shiver and fever) and could self-recovered overnight. Delayed toxicities mostly occurred 2 to 4 weeks after cell infusion; no treatment-related deaths happened during the study. The possible treatment-related adverse events were nausea and vomiting (27.8%), urticarial-like rash (11.1%), breathlessness (5.6%), psychiatric abnormalities (5.6%), joint swelling (5.6%), dizziness (5.6%), and pneumonitis (5.6%). Chemical laboratory abnormalities were included the increase of alanine aminotransferase (ALT; 5.6%), aspartate aminotransferase (AST; 5.6%), γ-glutamyltransferase (γ-GGT; 5.6%), and triglyceride (16.7%; Supplementary Table S5). Grade 3 and 4 toxicities experienced by patients were abnormalities of aminotransferase (patient 15) and left ventricular systolic function (patient 13; Table 2), the most likely cause of which was attributed to drug toxicities of the conditioning regimen and a previous megadose of Adriamycin, respectively. Among the enrolled patients, patient 5 experienced instantaneous psychiatric abnormalities of mild anxiety and delirium along with fever syndrome in the first cycle of the CART-30 cells' infusion process and recovered rapidly. He also developed joint swelling and urticaria intermittently for 5 days 2 weeks after treatment, which resolved without drug intervention (Supplementary Fig. S3). At 2 weeks after the second cycle of CART-30 cell infusion, patient 5 developed dizziness for 5 days again and remission with the intervention of Phenergan. However, he did not develop a significantly high elevation in plasma cytokines around the time of peak toxicities. The level of patients' plasma cytokines, including IL2, IL4, IL6, IL8, IL10, IL12p70, IL12/IL23p40, IFNγ, TNFα, VEGF, and granzyme B, as well as CRP, were analyzed at serial time points before and after the CART-30 cell infusion. There was a significant increase ($P = 0.031$) in the level of TNFα and IL12p70 at 1 week after the CAR T-cell infusion (Fig. 2A); however, no significant difference among the patients with different clinical response was observed for TNFα and IL12p70 (data not shown). Besides, no dramatic change in other cytokines was detected (Fig. 2A and Supplementary Fig. S1).

Detection and persistence of CART-30 cells *in vivo*

To detect the persistence of CART-30 cells *in vivo*, we measured CAR genes of peripheral blood and biopsied tissues by qPCR at designed time points after the CAR T-cell infusion. As

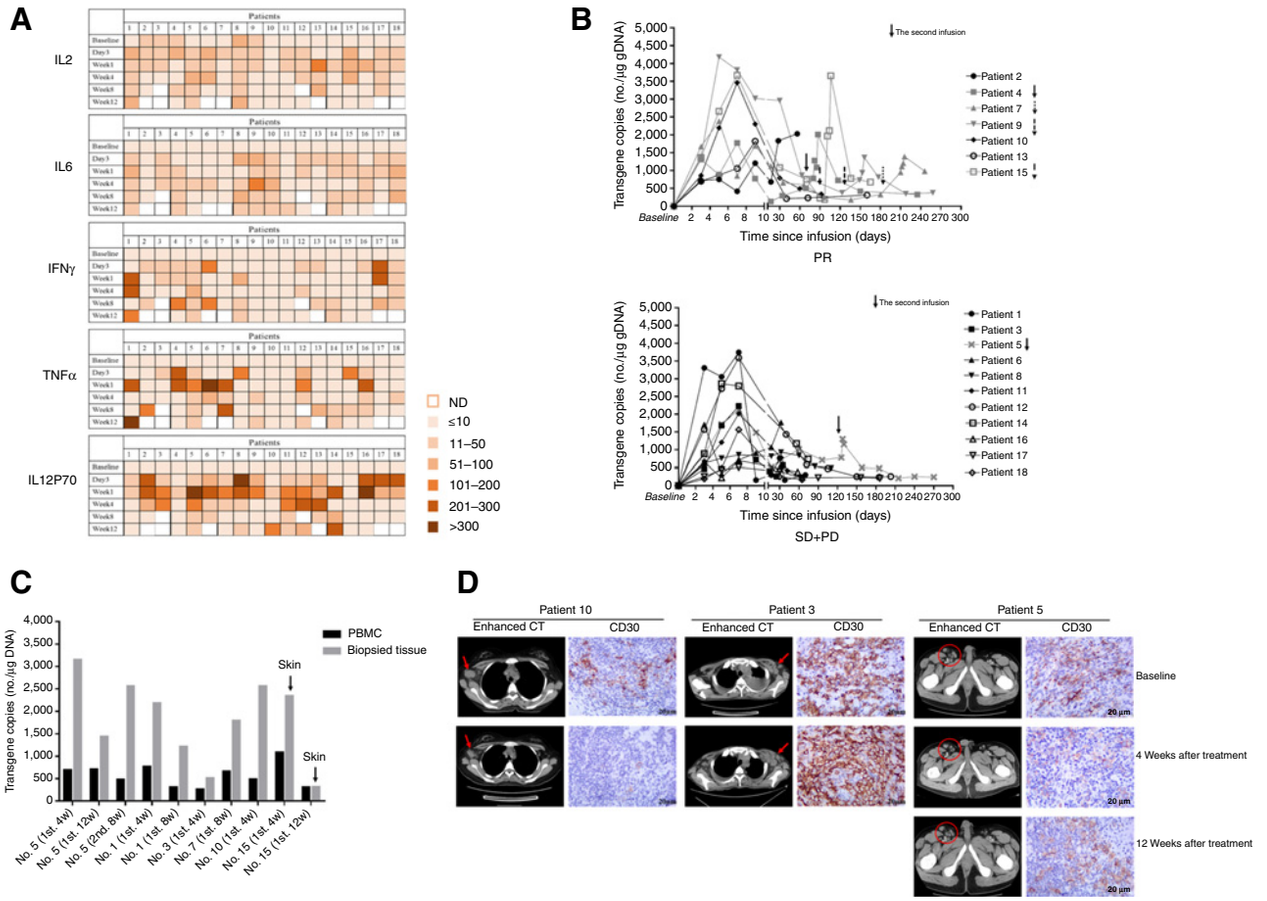

Figure 2.

Changes in the patients' serum cytokine levels within 3 months and *in vivo* persistence of CART-30 cells after infusion. **A,** Cytokines came from serum of each patient's peripheral blood, which was collected before and at serial time points after cell infusion, and were measured by FACS. The shade of brown represents the fold value of cytokines. Results for IL2, IL6, IL12P70, IFNγ, and TNFα are shown. **B,** gDNA was measured by quantitative real-time PCR harvested from each patients' PBMCs collected before and at serial time points after CART-30 cell infusion using primers specific for the transgene, the samples of which were divided into 2 groups according to clinical response. Black lines represent patient receiving one cycle cell infusion, and gray lines indicate patient with 2 cycles of cell infusion. Black arrowhead indicates the initial point of the second infusion. **C,** Detection of CAR T cells by quantitative real-time PCR assay was performed in biopsy tissues at different time points. Biopsy tissues are lymph node mass and one skin sample, obtained by ultrasound-guided fine-needle aspiration. The data are represented as the mean values (±SD) of at least 2 assays per time point, with each sample assessed in triplicate. 1st indicates the first cycle of infusion; 2nd indicates the second cycle of infusion. **D,** IHC for CD30 expression in tumor biopsy. The number of $CD30^+$ tumor cells was counted with the use of manual counting: all slices were observed by 3 experimenters independently under light microscope. Each person was selected by 10 representative high vision (10 × 40 times). $CD30^+$ tumor cells of axillary lymph node masses in patient 10 showed significant reduction ($P = 0.018$) as well as the shrinkage of size after CAR T-cell infusion was detected; there were no obvious changes of $CD30^+$ tumor cells and axillary lymph node masses in patient 3 after treatment; patient 5 showed reduction of $CD30^+$ tumor cells ($P = 0.031$) detected 4 weeks after treatment, but augmentation 12 weeks after treatment; no obvious change was observed in the size of inguinal lymph node masses.

shown in Fig. 2B, the copy numbers of CAR transgenes in peripheral blood reached their peak between 3 and 9 days, in which time a significant increase in TNFα and IL12p70 was detected (Fig. 2A) and gradually dropped to a negligible level 4 to 8 weeks after infusion in most patients. However, in the meantime, higher numbers of CAR transgene copies were detected in biopsy tissues (Fig. 2C), indicating that CART-30 cells could traffic to tumor sites. We also detected $CD3^+$ T cells by IHC and presented high expression of CD3 in biopsied tissues both before and after treatment, which conformed the feature of microenvironment of Hodgkin lymphoma (data not shown). Next, we detected the change of $CD30^+$ tumor cells to identify the targeting of CAR T cells. Five patients underwent ultrasound-guided fine-needle aspiration on their lymph node masses to receive formalin-fixed slides, as labeled in Fig. 2C. The IHC staining of patients 1 and 7 showed large amount of fibrous tissues; in the other 3 patients after treatment (Fig. 2D): (i) a decrease of $CD30^+$ tumor cells occurred ($P < 0.05$), as did the shrinkage of lymph node masses in patient 10 accompanied by a higher number of CAR transgene copies detected; (ii) there were no obvious changes of $CD30^+$ tumor cells and lymph node masses in patient 3, perhaps because of the poor infiltration of CAR T cells; and (iii) after 4 weeks of treatment, there were a statistically significant low number of $CD30^+$ tumor cells compared with baseline ($P < 0.05$) in patient 5 accompanied by a high number of CAR transgene copies detected; but

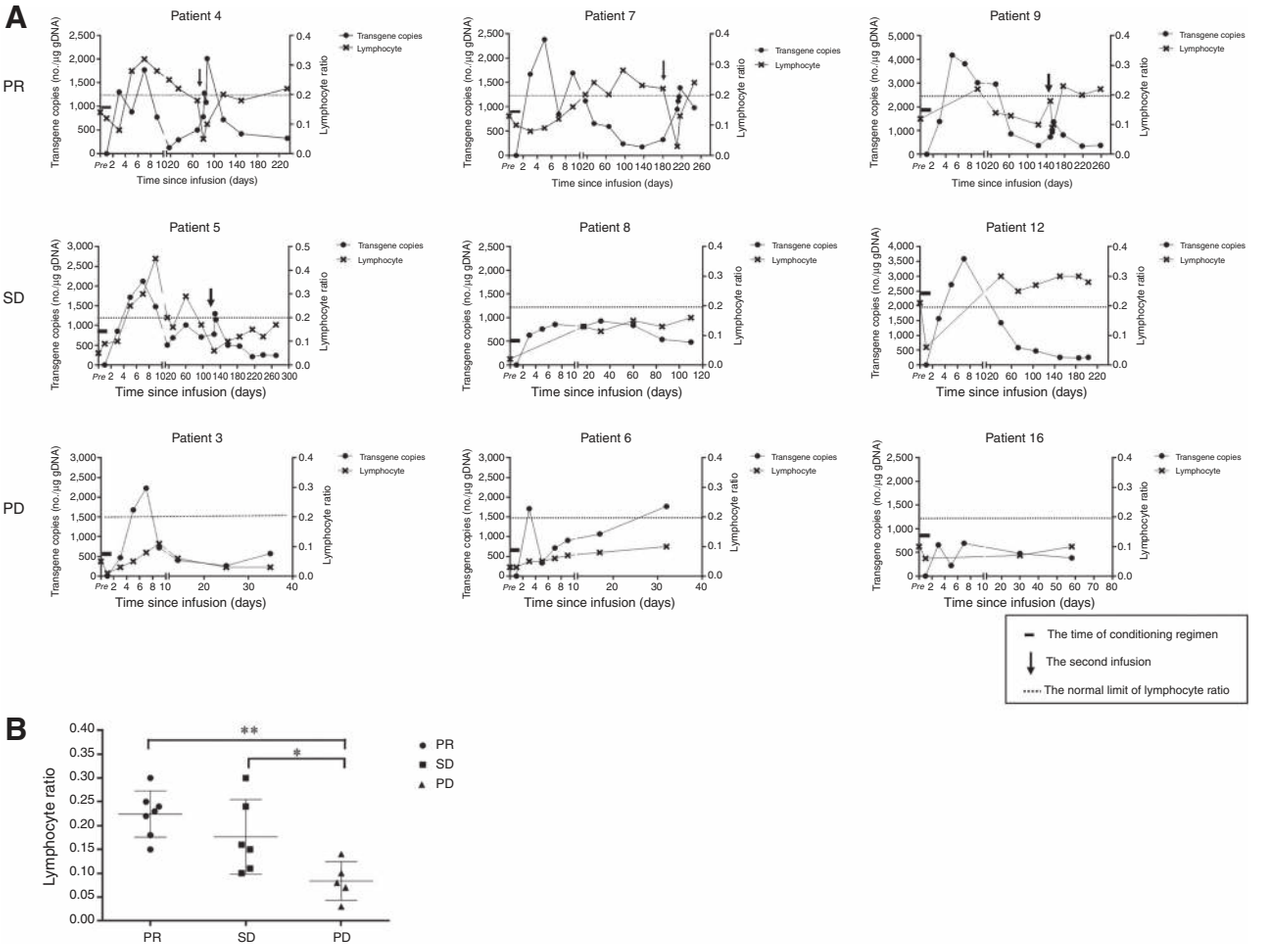

Figure 3.

Circulating lymphocyte recovery is a probable indictor of response. **A,** Lymphocyte ratios were obtained along with the copy numbers at serial time points in the process of protocol and were divided into 3 groups (CR/PR; SD; PD) according to clinical response. Black original point indicates the transgene copies and black cross represents the ratio of lymphocyte. Dash line indicates the normal limit of lymphocyte. *pre* represents the time before conditioning regimen. **B,** Number of lymphocyte ratios in the peripheral blood at 4 weeks after CAR T-cell infusion is shown. The mean and SD are depicted for the patient groups stratified on the basis of clinical response. *, $P = 0.0409$; **, $P = 0.0004$, 1-way *t* test between these 2 groups.

$CD30^+$ tumor cells increased again 12 weeks after treatment with the reduction of CAR transgene copies. Nevertheless, no obvious change was measured in the size of lymph node masses before and after treatment.

Lymphocytes recovery associated with CART-30 treatment

There is a general lower level of lymphocytes in the peripheral blood of patients with relapsed or refractory Hodgkin lymphoma. In our results, Fig. 3A shows that lymphocyte ratios dramatically increased along with the number of CAR transgene copies and was maintained for a long time until disease progress in patients obtained PR and SD, especially PR; however, lower level of lymphocyte ratios continued to exist in patients with progressive disease (PD) after treatment (PR vs. PD, $P = 0.0004$; SD vs. PD, $P = 0.0409$; PR vs. SD, $P = 0.2064$), suggesting that lymphocyte recovery should be induced by CART-30 cell infusion and could be a potential biologic parameter correlated to clinical response (Fig. 3B; Supplementary Fig. S2).

Antitumor activity

Of the 18 evaluable patients, 7 achieved PRs and 6 had SDs after the infusion of CART-30 cells. The objective response was 39% (Table 2, Supplementary Fig. S6). All patients were observed for at least 2 months for response assessment; the median PFS was 6 months (range, 3–14 months; Supplementary Fig. S4) and 4 patients continued to have a response at the time of this writing. Of the 5 patients receiving 2 cycles of CART-30 cell infusion, 3 maintained PR and 1 maintained SD; another was assessed as having SD after the first treatment and achieved PR after the second treatment (Fig. 4A). The decrease of tumor burden was more significant ($P = 0.045$) after the second CAR T-cell infusion compared with the first (Fig. 4B). In 5 patients in whom previous brentuximab treatment failed, 1 had a PR and 2 had a SD; moreover, among 9 patients who had disease recurrence after ASCT, 4 had a PR and 3 had a SD. Figure 4B shows the maximum change of measurable lymph nodes and extranodal burdens from baseline, respectively, indicating that lymph nodes presented a

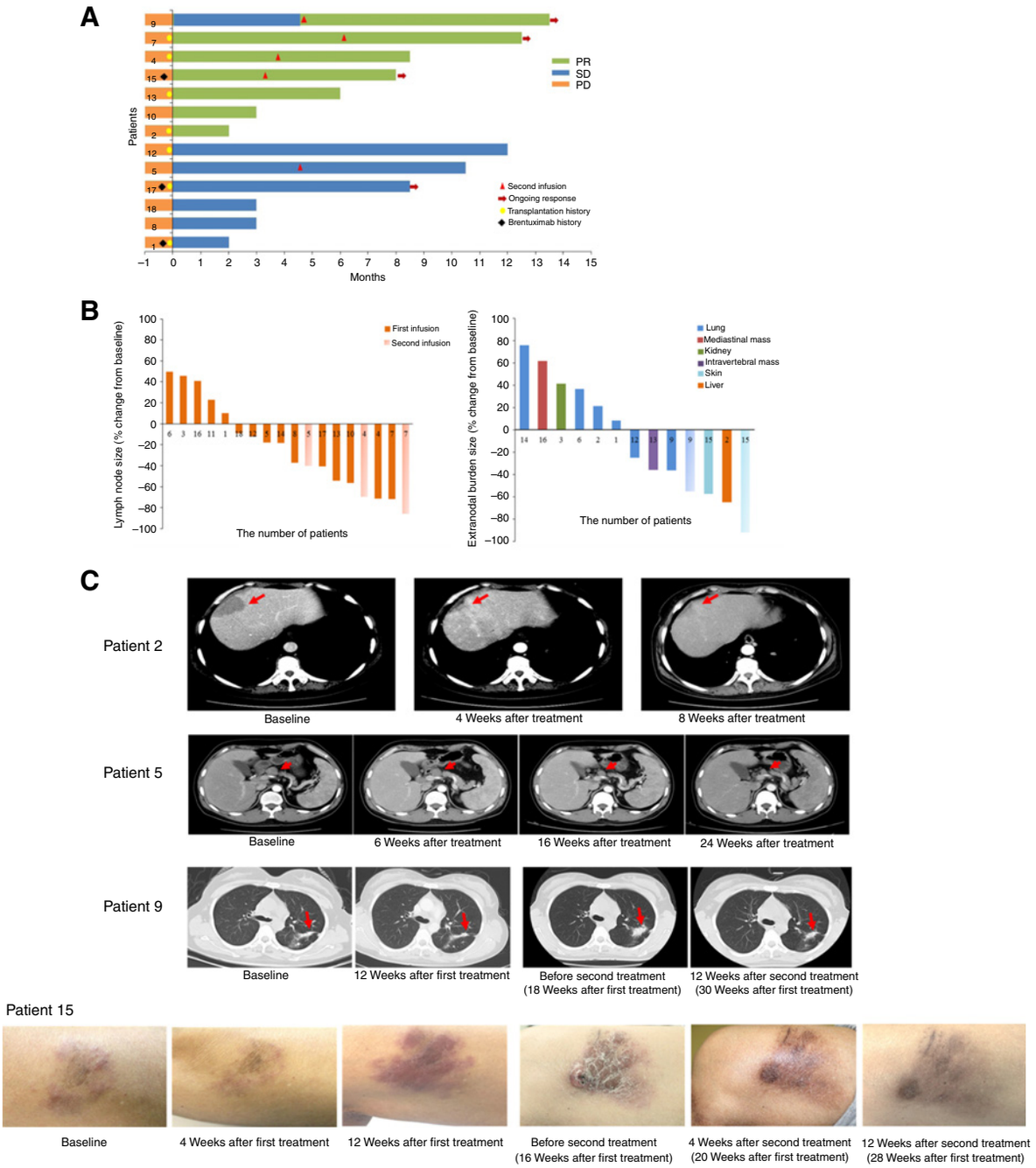

Figure 4.

Response characteristics and changes in tumor burden of patients after CART-30 cell protocol therapy. **A,** Clinical response and duration for 13 enrolled patients obtained disease control after CAR T-cell infusion and all patients were observed at least 2 months after CART-30 cell infusion. The color and length of each bar indicate the response to the treatment with CART-30 cell and the duration of response, respectively. Five patients received 2 cycles of treatment (the red triangle indicates the initial response time of second cycle of infusion), and 4 patients have a continued response at last follow-up (indicated by an arrowhead). Yellow original point and black diamond represent the treatment history of transplantation and brentuximab, respectively. **B,** Maximum reduction in target lesion size after CART-30 cell infusion. Left, the percentage reduction in lymph node mass from baseline; right, the percentage reduction in extranodal lesion from baseline. Blue: lung, red: mediastinal mass, green: kidney, purple: intravertebral mass, aquamarine: skin, orange: liver. The *x*-axis shows the number of patients, and light- colored bar indicates the result of second infusion. **C,** Representative tumor response images for patients after CART-30 cell infusion, including: (i) contrast-enhanced CT scans show liver lesions reduced significantly in patient 2; (ii) contrast-enhanced CT scans demonstrate a delayed shrinkage of abnormal abdominal lymph node until the 16th week of the second cycle of CART-30 cell treatment in patient 5; (iii) contrast-enhanced CT images show that better response of lung lesions shrinkage after the second cycle of CART-30 cell treatment in patient 9; (iv) patient 15, the only one enrolled patient diagnosed with ALCL, achieved partial reduction of masses after the first treatment without conditioning treatment. The masses obtained significant shrinkage (reduced from 4.68 to 0.36 cm^2 by ultrasonography) and showed hyperpigmentation after the second treatment.

better response than extranodal lesions; on the other hand, the response of lung lesions was likely to be relatively poor (Supplementary Table S6).

Besides, the tumor changes of different patients also presented diversified characteristics (Fig. 4C): (i) Patient 2, with lymphocyte-depleted Hodgkin Lymphoma (LDHL), was treated with 23 cycles of different treatment regimens, as well as ASCT; the result was progressive lymphoma. After treatment with the CART-30 cell infusion, there was a large and sustained diminution in most of her multiple liver lesions. (2) Patient 5 had primary refractory nodular sclerosis Hodgkin Lymphoma (NSHL) who progressed after 13 cycles of chemotherapy and radiotherapy. He achieved disease stability for 4.5 months after the first CART-30 cell infusion. To our surprise, after 16 weeks of his second treatment, a delayed response of tumor shrinkage was observed and was ongoing after 24 weeks, indicating the delayed response of immunotherapy. (iii) Patient 9 was also diagnosed with primary refractory NSHL. She was treated with 10 prior regimens and presented resistance to chemotherapy. A significant shrinkage of lung lesion was obtained and sustained after the second treatment, suggesting that a better clinical response may be obtained from multiple infusions. (iv) Only one enrolled patient diagnosed with ALCL achieved a 3-month PR after the first CAR T0cell infusion without a conditioning regimen, demonstrating the effective of CART-30 cells, and after the second treatment, the mass of skin almost disappear although some hyperpigmentation remained.

Discussion

In this trial, we evaluated the safety, feasibility, and antitumor response of CART-30 cells in patients with relapsed or refractory Hodgkin lymphoma. All 18 patients before enrolled onto this protocol received heavy treatment and had a considerable burden of lymphoma (Table 1). All of these patients are at "high risk" (14) for relapse and should receive new approaches to improve their poor prognosis (15).

Our data showed that the infusion of between 1.1×10^7/kg and 2.1×10^7/kg of CART-30 cells was well tolerated. Only 2 patients experienced grade 3 and 4 toxicities most likely because of previous chemotherapy. The most probable related adverse event was an anaphylaxis event manifesting as urticarial-like rash and arthroncus 2 weeks after the cells infusion. Although the reason and mechanism of the local anaphylaxis we observed are unclear as of now, Maus and colleagues first reported clinical anaphylaxis resulting from CAR-modified T cells and suggested that anaphylaxis was most likely triggered by an IgE antibody specific for the murine-based antibody sequences present in the CAR-modified T-cell product (16). As the expression of CD30 in normal tissues identified a rare population of large lymphoid cells in sections of lymph node, tonsil, thymus, and endometrial cells with decidual changes (4, 17, 18), it may be one interpretation of CART-30 cell therapy without significant on-target, off-tumor toxicities.

Compared with the frequency of CAR T cells in the blood (Fig. 2B), the number of CAR T cells infiltrating lymphoma masses could be one more important indicator of effectiveness in treating lymphoma by CAR T-cell therapy (19, 20). Interestingly, we observed that lymphoma masses of one patient (patient 5) infiltrated high number of CAR T cells and reduced the $CD30^+$ tumor cells, but the size of lymphoma masses was no regression probably correlated with tissue fibrosis. On the other hand, the reason for the slight expression of CD30 after 4 weeks of treatment in the presence of high CAR transgene copies is likely that the existence of different immunosuppressive pathways can hinder the full potential of CAR T-cell therapy in the microenvironment of Hodgkin lymphoma (21, 22). Because lymphocytopenia (a lymphocyte count $< 0.6 \times 10^9$/L or <8% of the white cell count or both) is 1 of the 7 adverse factors with similar independent prognostic effects composing the International Prognostic Score (IPS) in 1998 (23), thus the lymphocyte recovery along with CAR T-cell infusion is likely to be another important indicator of clinical benefit for CART-30 cell therapy.

It has been reported that conditioning chemotherapy is an indispensable regimen that can enhance the engraftment of transferred T cells and improve the objective response of patients with tumor (24–26). In this study, 3 different conditioning regimens (FC, GMC-like, and PC) were administered before CART-30 cell infusion. The aim of PC is to deplete the stromal compartment of microenvironment of Hodgkin lymphoma (27), which was characterized as containing a collagen-rich extracellular matrix (ECM), some mesenchymal stem cells, and a large number of fibroblasts (28, 29). This special microenvironment contributes to not only enhancing tumor cell proliferation but also increasing tumor interstitial fluid pressure, which resulted in blocking perfusion of the anticancer therapies to the tumor cells (30, 31). Nab-paclitaxel was proved to be able to improve drug perfusion in a primary human xenograft model for pancreatic cancer by degrading the ECM in a previous report (32). So nab-paclitaxel was used for degrading the ECM to facilitate the perfusion of CART-30 cells in our trials. Unexpectedly, our results showed that there was no significant statistical difference among the 3 conditioning regimens (Supplementary Table S7), which would be affected by disease status, tumor burden, and few cases of patients. However, current data may provide a preliminary guideline for patients to choose conditioning regimens: FC is suitable for patients with small lymphoma masses; GMC-like is a priority selection for large tumor burdens; and PC should be considered for extranodal lesions. Nevertheless, the final formulation of conditioning regimens needs to be validated by more sample data.

The patient (patient 15) who achieved a 3-month PR after CART-30 cell infusion without conditioning regimen demonstrated the antitumor activity of CART-30 cell therapy. It should be noted that CART-30 cell treatment alone is also beneficial to the patients who failed with ASCT or brentuximab treatment (Fig. 4A). Besides, there are 2 unique characteristics manifested by the activity of infused CART-30 cells. First, better response was noted in lymph nodes than in extranodal lesions and the response of lung lesions seemed to be relatively poor. Although no clear evidence explains the poor response of lung lesions in patients receiving cell infusions as of now, the lung lesion shrinkage of patient 9, who underwent 2 cycles of CART-30 cell treatment with different conditioning regimens (the first treatment with GEMC and the second with PC) was much greater after the second cell infusion than the first, suggesting that the microenvironment of Hodgkin lymphoma in the lung was likely to contain more ECM components hindering the efficient trafficking of cells. So, every opportunity to study the microenvironment of lymphoma masses should be taken. Second, multiple cycles of cell infusions might generate better clinical responses. According to the protocol, patients who obtained clinical benefit from CART-30 cell infusion could receive the second treatment. Although no radiologic complete response was observed after the second treatment, the

shrinkage degree of measurable target lymphoma masses was more significant ($P < 0.05$) and PFS was longer than the first infusion (Fig. 4A and B), indicating that multiple-cycle CART-30 cell therapy protocol will be designed in the next work.

In summary, our study shows that infusion of CART-30 cells is well tolerated without severe toxicity and can traffic to tumor sites accompanied by immune reconstitution; at the same time, it yields a high clinical benefit to some extent for those patients with relapsed or refractory Hodgkin lymphoma. Future clinical trial protocols need to consider the further optimization of conditioning regimens, the trial of multiple-cycle infusions of CAR T cells, and intervention of the CART-30 cell protocol in the early-disease stage. Besides, identifying the possible biomarkers or parameters associated with an efficient clinical response in this disease will be indispensable to determine appropriate patients for the CART-30 cell protocol. On the basis of the efficacy of CART-30 infusion alone, combination or consolidation treatment with other anticancer therapies not only improved long-term disease control for patients with primary refractory and relapsed Hodgkin lymphoma, but also could be administrated in early-disease patients to reduce the long-term toxicity of chemoradiotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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