Original Article

Overall survival benefits provided by lenalidomide maintenance after chimeric antigen receptor T cell therapy in patients with refractory/relapsed diffuse large B-cell lymphoma

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Contributions: (I) Conception and design: Z Jin, D Wu; (II) Administrative support: None; (III) Provision of study materials or patients: N Ping, C Qu, M Li, L Yan, L Yu, L Kang; (IV) Collection and assembly of data: N Ping, C Qu, M Li, X Chen, Q Wu, D Kong, F Xia, H Yao; (V) Data analysis and interpretation: N Ping, C Qu, M Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Background: Chimeric antigen receptor T cell (CAR-T) therapy has achieved remarkable effects in refractory/relapsed (R/R) diffuse large B-cell lymphoma (DLBCL). However, many patients receiving CAR-T therapy still eventually die of disease recurrence or progression due to target antigen loss or exhaustion of CAR-T cells. Therefore, maintaining the efficacy of CAR-T has become a particular research focus. As lenalidomide can regulate T cell function, we conducted a study to evaluate the efficacy of lenalidomide maintenance after CAR-T therapy in R/R DLBCL patients.

Methods: Seven R/R DLBCL patients who received lenalidomide maintenance after CAR-T therapy and nine DLBCL patients that underwent CAR-T treatment alone were included. The clinical data of all subjects were collected to evaluate the efficacy of lenalidomide maintenance. In order to understand the possible mechanisms of lenalidomide in CAR-T therapy, CAR-T copies of peripheral blood were regularly detected by quantitative real-time polymerase chain reaction, and an *in vitro* test was also conducted.

Results: Overall survival (OS) was significantly prolonged in the lenalidomide maintenance group. Furthermore, one case responding to CAR-T therapy initially but suffering a relapse shortly achieved complete remission again after lenalidomide exposure, with an increase in the number of CAR-T copies detected. The *in vitro* test showed that lenalidomide could delay the exhaustion of CAR-T cells.

Conclusions: Lenalidomide maintenance after CAR-T therapy is a safe and effective choice for R/R DLBCL patients. We confirmed that lenalidomide maintenance can improve patients' OS, and the delayed exhaustion of CAR-T cells may contribute to this OS benefit.

Keywords: Refractory/relapsed (R/R); diffuse large B-cell lymphoma (DLBCL); chimeric antigen receptor T cell (CAR-T); lenalidomide

Submitted Dec 07, 2021. Accepted for publication Mar 01, 2022.
doi: 10.21037/atm-22-20
View this article at: https://dx.doi.org/10.21037/atm-22-20

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a common aggressive malignancy that accounts for 30% of non-Hodgkin lymphomas (NHL) (1). Most DLBCL patients are sensitive to standard immune-chemotherapy regimens containing rituximab and regimens containing anthracyclines (2). Nevertheless, approximately 40% of patients develop resistance to these standard therapies or eventually relapse (3). Furthermore, only half of these refractory/relapsed (R/R) DLBCL patients respond to second-line high-dose polychemotherapy and eventually proceed to autologous stem cell transplantation (ASCT) (4,5), and the prognosis of the other half of R/R DLBCL patients is considerably worse (5,6).

It is noteworthy that in the past few years, chimeric antigen receptor T cell (CAR-T) therapy has achieved an encouraging curative effect in patients with R/R B-cell lymphoma or B-cell acute lymphoid leukemia (ALL) (7,8). Regrettably, almost all CAR-T-related clinical trials have the same problem; that is, many CAR-T therapy cannot result in durable cures due to target antigen loss or poor CAR-T persistence (9). As a result, preserving the efficacy of CAR-T therapy and prolonging the overall survival (OS) of patients has become the focus of current research.

Lenalidomide is an effective and safe oral immunomodulatory drug, and acts mainly through direct anti-tumor, as well as immune and tumor microenvironment regulation (10), and it is reported to be active in various tumors either as a single-agent or in combination with other drugs (10-12). CAR-T cells are genetically modified human lymphocytes; some previous studies have reported that lenalidomide enhances the function of CAR-T cells against multiple myelomas in both *in vivo* and *in vitro* experiments, as well as lymphoma (6,13,14). Consequently, we conducted a study to evaluate the efficacy of lenalidomide maintenance after CAR-T therapy in R/R DLBCL patients. We also performed an *in vitro* test, and the preliminary data suggested that application of lenalidomide dramatically relieved the exhaustion of anti-CD19 CAR-T cells. We present the following article in accordance with the TREND reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-20/rc).

Methods

Patients and study design

The criterion for inclusion in this study was adult R/R DLBCL patients who received maintenance lenalidomide after CAR-T therapy. From January 2017 to October 2019, all DLBCL patients who received CAR-T therapy were screened to determine their eligibility, and a total of seven patients met the above criterion. For comparison, nine R/R DLBCL patients who received CAR-T treatment alone were included. The clinical data of all enrolled subjects were collected to evaluate the efficacy of lenalidomide maintenance after CAR-T therapy.

All patients enrolled in this study were confirmed by immunohistochemical staining according to the 2008 World Health Organization (WHO) guidelines. The study was approved by the ethics committee of the First Affiliated Hospital of Soochow University (FAHSU) (No. 2017053) and informed consent was taken from all the patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

CAR-T products preparation

We collected T lymphocytes from the patients' or donor's peripheral blood through a blood cell separator and degradable anti-CD3 magnetic microbeads. Immediately after the collection of target-cells, anti-CD3 monoclonal antibody (OKT3) (Miltenyi) and anti-CD28 monoclonal antibody (Miltenyi) were co-cultured with them to stimulate cell proliferation for 24 h. Next, lentivirus was added directly to the medium to transduce recombinant lentiviral vectors comprising the anti-CD19/CD20/CD22 single-chain variable fragment (scFv), the cytoplasmic portion of the 4-1BB costimulatory moiety, and the CD3z T-cell activation domain. After 48 h, the transfected cells were expanded *in vitro* with X-vivo 15 medium containing IL-7 and IL-15 cytokines until their number met the pre-set value. All cell cultures were performed at 37 ℃ and 5% CO_2 humidified condition.

Ultimately, the corresponding CAR-T products were harvested after passing various quality tests including transduction efficiency, purity, cell viability, $CD4^+$ cell ratio, $CD8^+$ cell ratio, killing ability *in vitro*, cytokine release capacity, quantity of endotoxin, mycoplasma, bacteria, fungi, and so on. All CAR-T products used in our study were provided by the Unicar-Therapy Bio-Medicine Technology Co. (Shanghai, China).

CAR-T therapy

All eligible patients were given a FC regimen

(cyclophosphamide 300 mg/m^2 × 3 d and fludarabine 30 mg/m^2 × 3 d) for lymphodepletion chemotherapy followed by CAR-T cells infusion. The total doses of each CAR-T product administrated in both groups ranged from 1×10^7/kg to 2×10^7/kg of body weight. To alleviate serious cytokine release syndrome (CRS), each patient's CAR-T products were deliberately divided into three parts (proportions of 10%, 30%, 60%), and were infused by escalation within 3 days. The infusion of CAR-T products was successfully performed in all cases without any occurrence of CAR-T-related death.

Lenalidomide therapy

Seven DLBCL patients received lenalidomide maintenance after CAR-T therapy, of which six were administrated lenalidomide for 21 days from the 15th day after the infusion of CAR-T cells in a 28 days cycle. One case responded to CAR-T therapy initially but suffered a relapse 2 months later was exposed to lenalidomide. It is important to consider the manageability of treatment-emergent adverse events (TEAEs) of lenalidomide maintenance, the most common grade 3–4 TEAEs were neutropaenia and cutaneous reactions, as a result, we required all patients to perform blood-related tests consisting of hemogram every 2 week, as well as liver and renal function. Although lenalidomide was started at a dose of 25 mg/d, this was reduced to 10mg/d when the patients' neutrophil counts were less than 2×10^9/L or platelet counts were than 50×10^9/L, and suspended when patients' neutrophil counts fell below 0.5×10^9/L or platelet counts below 25×10^9/L during lenalidomide treatment. Appropriate symptomatic treatment measures would also be given if necessary until the indicators improved. The treatment of lenalidomide in the CAR-T therapy (C+Len) cohort was continued until disease progression or relapse.

Response evaluation and follow up

Positron emission tomography and computed tomography (PET/CT) or contrast-enhanced CT scan was used for disease evaluation at the 1-, 3-, and 6-month stages after CAR-T cells infusion, followed by assessment every 6 months thereafter. We detected CAR-T cell copies in peripheral blood by quantitative real-time polymerase chain reaction (qPCR) as frequently as routine imaging surveillance. Patients with any new suspicious tumor-related lesions were evaluated at any time during follow-up. All efficacy evaluations were based on The Lugano Classification (2014).

In vitro test

Anti-CD19 CAR-T cells from healthy donors were cultured in AIM-V media (Thermo Fisher Scientific) supplemented with 10% feta bovine serum (Gibco; Thermo Fisher Scientific), 100 IU/mL IL-2 (PeproTech), 5 ng/mL IL-7 (PeproTech), and 5 ng/mL IL-15 (PeproTech) *in vitro* test. To examine the effective function of lenalidomide, we cultured the above CAR-T cells in the presence or absence of 10 μmol/L lenalidomide. To draw the cell-growth curve, the CAR-T cells were labeled with protein L-FITC (ACRO Biosystems, Beijing, China) and analyzed using an Attune NxT flow cytometer (Thermo Fisher). The surface markers (CD4 and CD8) of the CAR-T cells above treated with different concentrations of lenalidomide were also detected using the Attune NxT flow cytometer (Thermo Fisher) at different time points. The experiments were performed three times.

Statistical analysis

The patients' characteristics were analyzed by χ^2 or Fisher's exact tests. Survival analyses were performed using the Gehan-Breslow-Wilcoxon test. Calculations were performed using the SPSS software package (version 17.0) or Graphpad Prism 5.0, and $P<0.05$ was considered significant.

Results

Clinical features of patients

From January 2017 to October 2019, a total of 16 cases were enrolled in our study, comprising 10 males and 6 females, with a median age of 59 years (range, 39–68 years). The frequency of non-GCB subtype (n=15, 94%) was significantly higher than that of GCB in all 16 DLBCL patients. The majority of patients had stage III or IV disease prior to CAR-T cell administration (n=15, 94%). Also, the entire cohort was R/R DLBCL, and had previously received therapy comprising rituximab. In this study, refractory disease was defined as not achieving at least a partial response (PR) after chemotherapy (>4 cycles of the first-line therapy or >2 cycles of later lines of therapies) or relapse ≤12 months post-ASCT, and relapsed disease was defined as the recurrence of any new lesion after complete remission (CR) (15).

Table 1 Patient characteristics

Characteristics	All patients (n=16)	C (n=9)	C+Len (n=7)	P
Age (years), median [range]	59 [39–68]	58 [39–64]	60 [41–68]	0.6330
Sex, n [%]				0.6330
Male	10 [63]	5 [56]	5 [71]	
Female	6 [37]	4 [44]	2 [29]	
Primary diagnosis, n [%]				0.4380
GCB	1 [6]	0	1 [14]	
Non-GCB	15 [94]	9 [100]	6 [86]	
Stage, n [%]				0.4380
I/II	1 [6]	0	1 [14]	
III/IV	15 [94]	9 [100]	6 [86]	
Status before leukapheresis, n [%]				1.0000
PR	7 [44]	4 [44]	3 [43]	
SD/PD	9 [56]	5 [56]	4 [57]	
IPI, n [%]				0.5500
0–2	3 [19]	1 [11]	2 [29]	
3–5	13 [81]	8 [89]	5 [71]	
Type, n [%]				
R/R	11 [69]	6 [67]	5 [71]	1.0000
Non-R/R	5 [31]	3 [33]	2 [29]	
CAR-T-cell origin, n [%]				1.0000
Autologous	15 [94]	8 [89]	7 [100]	
Donor	1 [6]	1 [11]	0	
CAR-T-cell targets, n [%]				0.0350*
CD19+CD20/CD22	10 [63]	8 [89]	2 [29]	
CD19	6 [37]	1 [11]	5 [71]	

C+Len, CAR-T therapy followed by lenalidomide; C, CAR-T; GCB, germinal center B cell; PR, partial response; SD, stable disease; PD, progressive disease; IPI, international prognostic index; R/R, refractory or relapsed; CAR-T, chimeric antigen receptor T cell.

To demonstrate the efficacy of lenalidomide maintenance after CAR-T therapy, patients were divided into a test group (n=7) and a control group (n=9). The test group received lenalidomide maintenance after C+Len, and the control group only opted for observation after CAR-T therapy. We compared the clinical features of these two cohorts, including age, sex, stage, international prognostic index (IPI), CAR-T cell targets, and other projects, and no significant differences were detected (P>0.05). The clinical characteristics of these subgroups are shown in *Table 1*.

Response

All 16 patients were included in the evaluation of efficacy. The total best overall response rate (ORR) was 81.3% (n=13); 6 patients achieved CR and seven patents achieved PR, respectively. Both cohorts achieved a relatively high ORR. Six of the seven patients (85.7%) achieved a major clinical response in the C+Len cohort, including three CRs and three PRs, compared with seven of nine (77.8%) achieving an objective response in the control group (P>0.05) (*Table 2*). With follow up to date, in the C+Len

Table 2 Clinical response and toxicities after trial therapy commenced in the two groups

Variables	CAR-T (n=9)	C+Len (n=7)	P
Clinical response			
CR	3	3	1.0000
PR	4	3	1.0000
NR	2	1	1.0000
ORR	7	6	1.0000
Toxicities, n (%)			
CRS			1.0000
1–2	3 (33.3)	2 (28.6)	
3–4	4 (44.4)	3 (42.9)	
Hematological toxicity			
Leukopenia (III/IV)	7 (77.8)	5 (71.4)	1.0000
Anemia (III/IV)	5 (55.6)	1 (14.3)	0.2870
Thrombocytopenia (III/IV)	4 (44.4)	1 (14.3)	0.5800
Organ toxicity			
Total bilirubin (III/IV)	1 (11.1)	0	1.0000
ALT (III/IV)	0	3 (42.9)	0.0630
Creatinine (III/IV)	2 (22.2)	0	0.2080

CR, complete remission; PR, partial remission; NR, non-remission; ORR, overall response rate; CRS, cytokine release syndrome; ALT, alanine-aminotransferase; C+Len, CAR-T therapy followed by lenalidomide; CAR-T, chimeric antigen receptor T cell.

cohort, one patient is in ongoing PR and two patients are in ongoing CR, while three patients are in ongoing CR in the control group. These unexpected results indicated that lenalidomide might not have a significant contribution in further improving the response rate of CAR-T-treated patients with R/R DLBCL.

In addition, the CAR-T copies of all living patients' peripheral blood were regularly measured during the follow-up period, and were observed to have remained at relatively high levels in both groups by qPCR (*Figure 1A*). One patient achieved CR1 after CAR-T treatment, but suffered a relapse shortly after receiving lenalidomide, and subsequently achieved CR2 accompanied by a significant increase in the CAR-T copies number (*Figure 1B*).

Survival

At a median follow-up of 9 months (range, 0.2–22.0 months), most members were alive in the test cohort: two patients were in ongoing PR, two patients experienced continuous CR, and one patient suffered lymphoma progression at 4 months. Meanwhile, five of nine patients (55.6%) died due to lymphoma relapse or progression in the control cohort (*Figure 2A*). Median OS was not reached in the C+Len cohort, while it was estimated at 3.5 months in the control group. The 1-year OS was improved from 33.3% to 100% in the test group (Gehan-Breslow-Wilcoxon test, P=0.0254) (*Figure 2B*). However, the 1-year progression free survival (PFS) in the test group was not significantly different from that of the control group (P=0.1208) (*Figure 2C*). None of patients received further treatment, but continued to be followed when the disease relapsed or progressed following commencement of the trial therapy.

In vitro test

We cultured anti-CD19 CAR-T cells in the presence or absence of 10 μmol/L lenalidomide (named the C+Len and C cohorts, respectively). Flow cytometer analysis showed that the growth of CAR-T cells in the presence of

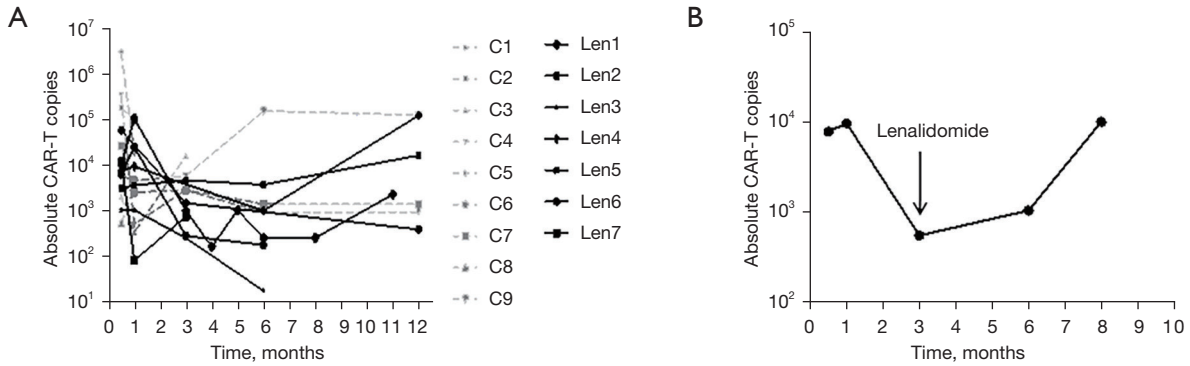

Figure 1 CAR-T copies in DLBCL patients after CAR-T therapy. (A) CAR-T copies detected by qPCR of alive DLBCL patients in both cohorts had no significant difference (*t*-test, P>0,05). (B) A patient's CAR-T level increased dramatically after lenalidomide exposure. CAR-T, chimeric antigen receptor T cell; DLBCL, diffuse large B-cell lymphoma; qPCR, quantitative real-time polymerase chain reaction.

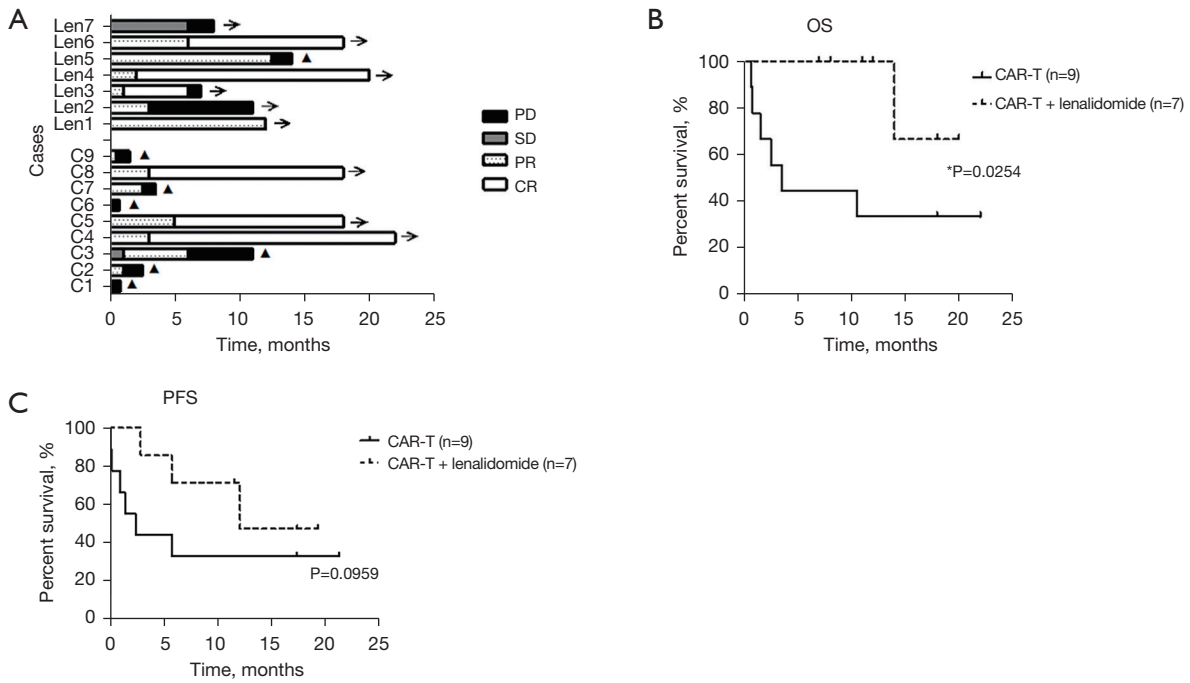

Figure 2 Clinical efficacy and survival in both groups of DLBCL patients ineligible for ASCT. (A) Duration of response and survival after CAR-T; (B) OS of the C+Len cohort was higher than that of the control group (Gehan-Breslow-Wilcoxon test, P=0.0254); (C) no statistically significant difference was achieved in PFS in both cohorts (Gehan-Breslow-Wilcoxon Test, P>0.05). ▲, death (time to death after CAR-T infusion); →, response ongoing. DLBCL, diffuse large B-cell lymphoma; ASCT, autologous stem cell transplantation; CAR-T, chimeric antigen receptor T cell; OS, overall survival; PFS, progression free survival; C+Len, CAR-T therapy followed by lenalidomide.

lenalidomide was faster than that in the C cohort (*Figure 3A*). Furthermore, we found that the ratio of $CD4^+$ and $CD8^+$ cells in C+Len cohort declined slower, indicating that the lenalidomide might delay the exhaustion of CAR-T cells (*Figure 3B*).

Safety

Among all patients, seven cases (43.8%) developed grade 3 CRS, but none died of severe CRS due to timely administration of symptomatic treatments consisting of antifebrile, high dose vasopressor and tocilizumab, as well as

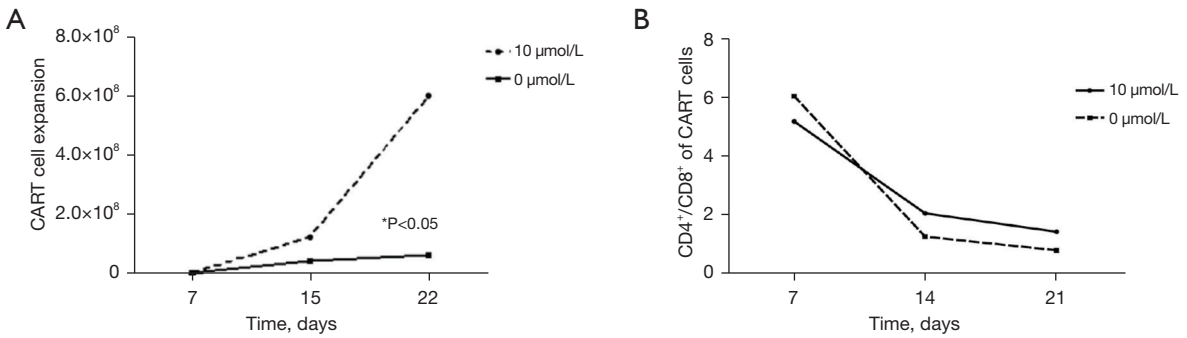

Figure 3 Lenalidomide delays the exhaustion of CAR-T. (A) Growth of the number of CAR-T cells at different time points. The CAR-T cells were labeled with the L-FITC protein (ACRO Biosystems, Beijing, China) and analyzed using an Attune NxT flow cytometer (Thermo Fisher). (B) The ratio of $CD4^+$ and $CD8^+$ T cells at different time points was analyzed using an Attune NxT flow cytometer (Thermo Fisher). CAR-T, chimeric antigen receptor T cell.

steroids. Due to the delayed administration of lenalidomide, the incidence and severity of CAR-T-related toxicity in both groups before lenalidomide administration were similar, including CRS grade, hematological toxicity and organ toxicity (P>0.5, *Table 2*), and the adverse reactions during CAR-T treatment were not aggravated. Moreover, the test group did not develop serious treatment-related toxicities after lenalidomide administration because of regular blood detection and timely corresponding treatment.

Discussion

To our knowledge, this is the first report about a significant benefit in OS with lenalidomide maintenance after CAR-T treatment in R/R DLBCL patients. We also observed an interesting phenomenon for the first time that lenalidomide reversed the downward trend of CART copies in a DLBCL patient.

With the widespread use of rituximab, the overall outcome of patients with DLBCL has been significantly improved (16). Meanwhile, the prognosis of approximately 30% of DLBCL patients remains poor due to a failure in durable remission or chemoresistance (4). Conventional standard treatments for R/R DLBCL include second-line intensive chemotherapy and autologous hematopoietic stem cell transplantation (ASCT) (4,17). However, only about 53% patients eventually proceed to ASCT (18), and the outcomes of those ineligible for ASCT are extremely poor. Van Den Neste *et al.* reported the real response rate of such group: the ORR of third-line chemotherapy was 39%, the median OS was 4.4 months, and the 1-year OS was only 23% (19). Notably, ABC-DLBCL had distinctly lower survival (20), as well as cases in early relapse and double-expressor which was defined as overexpression of MYC and BCL2 proteins (21). In the present study, the majority of patients were refractory lymphoma and non-GCB subtype, half of them had disease progression or were in SD status before CAR-T treatment, and even worse, two cases failed to achieve CR after ASCT and allogeneic hematopoietic stem cell transplantation, respectively. In other words, the general prognosis of our cases was expected to be extremely poor and required urgent improvement.

Immunotherapy approaches have played an important role in R/R DLBCL, such as anti-CD47 antibody, bispecific T-cell engager antibody, antibody drug conjugates, and so on, among which CAR-T have the best therapeutic effect (22). A large number of CAR-T-related clinical trials have confirmed the stunning efficacy of CAR-T treatment in R/R DLBCL patients (7). However, almost all CAR-T-related clinical trials had the same problem; that is, a proportion of patients treated with CAR-T will ultimately relapse or progress. To improve the treatment of R/R DLBCL, we should focus on preserving the efficacy of CAR-T therapy and prolonging the overall survival (OS) of patients.

It is well known that target antigen loss and exhaustion of CAR-T cells are the main reasons for disease progression or relapse in patients treated with CAR-T therapy (9). In our study, two patients underwent second biopsy pathology at the time of disease progression after CAR-T therapy, but none of them showed a loss of the target antigens including CD19/CD20/CD22. As a result, delaying the exhaustion of CAR-T cells and prolonging the survival of these patients became an urgent problem to be solved in our institution.

Lenalidomide, which acting mainly through direct anti-

tumor, immune and tumor microenvironment regulation, is widely used in patients with newly diagnosed or relapsed multiple myeloma and has achieved therapeutic efficacy (10,23,24). Previous studies have reported that lenalidomide enhances the function of CAR-T cells against multiple myelomas through co-stimulates T cells. Due to the pleiotropic effects of lenalidomide, researchers have tried to apply it to other diseases, including lymphoma. Lenalidomide was active in R/R NHL regardless of whether a single-agent or combination therapy was used (12,13). Thieblemont *et al.* found that lenalidomide maintenance after obtaining an ORR to Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (R-CHOP) could significantly prolong the PFS of elderly patients with DLBCL (25). Hernandez-Ilizaliturri *et al.* reported that patients with R/R non-GCB DLBCL were more sensitive to lenalidomide and achieved better results (26).

In our study, even though the majority of patients were the non-GCB subtype, relapsed in early time, or were in PD status before pretreatment, both groups receiving CAR-T therapy had achieved a relatively high ORR. The best ORR of the C+Len and control cohorts were 85.7% and 77.8%, respectively, and the best CR rate of the two group was 42.9% and 33.3%, respectively. This result was consistent with previous reports (27,28) Although there was no significant difference in the ORR and PFS between the two groups, our results still exhibited a trend of improvement in the C+Len cohort. To confirm the true influence of lenalidomide, further studies in more cases are needed, as the failure of ORR raising or PFS improvement cannot exclude the impact of sample size limitation.

Notably, OS benefit was observed in the C+Len cohort. A previous study using mice null for lenalidomide found that lenalidomide could provide OS benefits only in concurrent lenalidomide group (whose lenalidomide was given on the first day of CAR-T infusion), and the concurrent lenalidomide could enhance the secretion of various cytokines, thereby increasing the antitumor effect of CAR cells but meanwhile also augmenting the CRS (14). Unlike these results, we first took DLBCL patients as the study objects, and the administration of lenalidomide was started on the 15th day after CAR-T fusion in order to avoid aggravating CAR-T-related adverse effects. Our results showed that OS improvement existed in the delayed lenalidomide group, too (*Figure 2B*).

During the follow-up period, we detected the CAR-T copies in the peripheral blood of the patients. As all patients' CAR-T copies remained at relatively high levels when they were alive, there was no significant difference between the two groups. However, interestingly, we found that one patient's CAR-T level increased dramatically after lenalidomide exposure (*Figure 1B*). This phenomenon suggested that lenalidomide might significantly increase the copies of CAR-T cells.

To further understand the possible mechanisms of lenalidomide in anti-CD19 CAR-T therapy, an *in vitro* test was also conducted. A previous study reported that lenalidomide enhanced the function of anti-CS1 CAR-T cells against multiple myeloma (13). We cultured anti-CD19 CAR-T cells from healthy donors *in vitro* in the presence of lenalidomide (10 μmol/L). Our results showed that the immunomodulatory drug worked via a direct effect on CAR-T cells, the growth of CAR-T cells in the presence of lenalidomide was faster than that of C cohort, and the ratio of $CD4^+$ and $CD8^+$ cells in C+Len cohort declined slower. These results suggested that the lenalidomide might augment the number of CAR-T cells and delay their function exhaustion (*Figure 3*).

In conclusion, we found that lenalidomide maintenance after CAR-T therapy in DLBCL patients is a safe, feasible, and effective approach. We first confirmed that lenalidomide maintenance can improve the OS of patients with R/R DLBCL. The delayed exhaustion of CAR-T cells by lenalidomide maintenance may have contributed to this OS benefit. Further studies are needed to identify the mechanisms of OS benefit in patients administrated with lenalidomide maintenance after CAR-T therapy.

Acknowledgments

Funding: This work was supported by the National Natural Science Foundation of China (No. 81400112, No. 81400155, No. 81600114); the National Key R&D Program of China (No. 2016YFC0902800); the Natural Science Foundation of Jiangsu Province (No. BK20160342, No. BK20140374); Top-notch Young Health Talents, 5th Suzhou Health Professionals Program (No. GSWS2019035); and National Clinical Research Center for Blood Diseases Program (No. 2021ZKMC01, No. 2021ZKMB03).

Footnote

Reporting Checklist: The authors have completed the TREND reporting checklist. Available at https://atm.

amegroups.com/article/view/10.21037/atm-22-20/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-20/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-20/coif). LK and LY are from Shanghai Unicar-Therapy Bio-medicine Technology Co., Ltd. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the First Affiliated Hospital of Soochow University (FAHSU) (No. 2017053) and informed consent was taken from all the patients.

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(English Language Editor: C. Betlazar-Maseh)

Cite this article as: Ping N, Qu C, Li M, Kang L, Kong D, Chen X, Wu Q, Xia F, Yu L, Yao H, Yan L, Wu D, Jin Z. Overall survival benefits provided by lenalidomide maintenance after chimeric antigen receptor T cell therapy in patients with refractory/relapsed diffuse large B-cell lymphoma. Ann Transl Med 2022;10(6):298. doi: 10.21037/atm-22-20