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ORIGINAL RESEARCH ARTICLE

Predictors and Management of Relapse to Axicabtagene Ciloleucel in Patients with Aggressive B-cell Lymphoma☆

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Abstract

Objective/background: **Despite the success of chimeric antigen receptor (CAR) T-cell therapy in patients with aggressive non-Hodgkin lymphoma (aNHL), some patients still fail treatment, and their prognosis is dismal.**

Methods: **We performed a retrospective study of aNHL patients treated with axicabtagene ciloleucel (axi-cel) at two Mayo Clinic centers between 2018 and 2020. We evaluated predictive factors, toxicities, and responses to salvage regimens after CAR T-cell therapy.**

Results: **Thirty-four patients received axi-cel with a median length of hospitalization of 14 days. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome of any grade occurred in 91% and 41% of patients, respectively. Furthermore, 71% of patients responded to therapy, with 53% achieving a complete response (CR). The CRS grade and absolute lymphocyte count at leukapheresis (ALC_{Leuk}) correlated with CR and overall survival (OS), respectively. After a median follow-up of 6.8 months (interquartile range [IQR] 4.6−14.9), 15 patients (44%) showed progressive disease (PD). Most patients (60%) progressed during the first 3 months and had persistent CD19 tumor expression. Elevated C-reactive protein at baseline increased the risk of PD, whereas elevated ferritin increased PD and mortality risk. Twelve patients received salvage therapy, but only three responded. Median OS of relapsed/refractory patients to axi-cel was 3 months (IQR 1.3−5.1).**

Conclusion: **The grade of CRS and ALC_{Leuk} correlated with better outcomes to axi-cel therapy. In addition, elevated inflammatory markers at baseline were associated with PD and shorter survival. Relapses after treatment frequently occur within months after axi-cel infusion; they confer a poor prognosis and create an urgent need for novel and effective treatment options in this patient population.**

Keywords: Adoptive immunotherapy, Axicabtagene ciloleucel, Lymphoma non-Hodgkin, Neoplasm recurrence, Salvage therapy, Response predictor

1. Introduction

The treatment with chimeric antigen receptor (CAR) T-cells is considered a breakthrough in cancer therapy, particularly for patients with B-cell malignancies [1]. There are currently four anti-CD19 products commercially available (axicabtagene ciloleucel [axi-cel], tisagenlecleucel, brexucabtagene

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autoleucel, and most recently lisocabtagene maraleucel). Despite the success of CAR T-cell therapy, durable responses are not universal, and there are no effective salvage therapies for these patients [2–5].

Axi-cel is indicated for adult patients with relapsed/refractory (R/R) large B-cell lymphoma (diffuse large B-cell lymphoma [DLBCL] not otherwise specified, primary mediastinal B-cell lymphoma [PMBCL], high-grade B-cell lymphoma, and DLBCL transformed from follicular lymphoma [tFL]), after two or more lines of treatment [2,6].

ZUMA-1 is a Phase 2 study of axi-cel treatment in patients with refractory aggressive NHL (aNHL) that showed an overall (ORR) and complete (CR) response rates of 82% and 54%, respectively, and similar rates have been reported in the standard-of-care setting [7–9]. Median overall survival (OS) was not reached after a median follow-up of 15.4 months, leading to a 73% reduction in the risk of death in ZUMA-1 relative to the historical data from the SCHOLAR-1 study [7,10].

Despite the success of CAR T-cell therapies like axi-cel, the group of patients that progress after this treatment or those who have primary refractory disease represent a high-risk population with limited treatment options [7,8,11]. Still, some questions remain about patients who fail CAR T-cell therapy: their clinical characteristics and response to salvage therapies, general approach recommended to manage these patients, and predictors of response. Here, we analyze a cohort of patients treated with axi-cel, propose novel predictors of relapse to be validated, and describe outcomes of different current therapies after relapse to cellular therapy.

Methods

Study design and participants

We performed a retrospective study of adult patients with R/R aggressive B-cell NHL treated with standard-of-care axi-cel at two Mayo Clinic campuses (Arizona and Florida, USA). We included all patients infused between June 2018 and August 2020, if they had at least one response assessment or if they progressed/died during the first 30 days after infusion of CAR T-cells.

Patient selection, supportive care, toxicity assessment/management, and response assessment followed institutional practice guidelines. All patients received axi-cel infusion in an inpatient setting, and they remained hospitalized for at least 10 days of observation following CAR T-cell infusion. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were graded by modified Lee criteria, CARTOX-10, or the American Society for Transplantation and Cellular Therapy (ASTCT) grading system [12–14]. Response assessments were performed after axi-cel infusion on Days 30, 90, 180, and every 6 months after that or whenever there was a clinical suspicion of disease progression.

This study was approved by the Mayo Clinic Institutional Review Board and was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

Patient demographics, baseline characteristics, adverse events, and efficacy outcomes were summarized using frequencies and corresponding percentages. Continuous variables were summarized as median with interquartile range (IQR). Patient characteristics were compared using Mann–Whitney U test for continuous variables and Fisher's exact or chi-square test for categorical variables. A two-sided p value of less than 0.05 was considered significant.

Survival time was defined as the time from CAR T-cell therapy infusion until death of any cause. Progression-free survival (PFS) was defined as the time from CAR T-cell infusion until relapse, progression, or death for any reason. Receiver Operator Characteristic curve analysis and Youden's index were used to determine the optimal predictive cutoffs for absolute lymphocyte count at leukapheresis (ALC_{Leuk}), C-reactive protein (CRP), and ferritin levels in terms of disease progression and OS (See Supplementary Fig. 1). Time-to-event curves were established using the Kaplan-Meier method, and log-rank was used for group comparisons.

We evaluated several factors that could affect response to CAR T-cell therapy and OS by logistic regression analysis. A multivariable logistic regression model was constructed in which variables with $p < .05$ were included. Analyses were performed using GraphPad Prism (version 9.0.0; GraphPad Software Inc., San Diego, CA, USA).

Results

Patients

A total of 34 patients received axi-cel that was prescribed as standard-of-care treatment (Table 1). The median age at the time of infusion was 52 years

Table 1. Descriptive characteristics of the study groups.

Characteristic	CAR T response $n = 19$	CAR T failure $n = 15$	p
Age (yrs)	51 [39–62]	53 [45–62]	0.64
Male	11 (58)	10 (67)	0.73
ECOG status			0.86
0	5 (26)	5 (33)	
1	12 (63)	9 (60)	
2	2 (11)	1 (7)	
Lymphoma subtype			0.70
DLBCL	13 (68)	9 (60)	
tFL	5 (26)	4 (27)	
PMBCL	1 (5)	2 (13)	
Double hit[a]	3/16 (19)	1/14 (7)	0.60
Double expressor[b]	7/15 (47)	4/15 (27)	0.45
Stage of disease			0.52
I	0	1 (6)	
II	5 (26)	4 (27)	
III	3 (16)	4 (27)	
IV	11 (58)	6 (40)	
Number of lines before leukapheresis			0.58
2	8 (42)	8 (53)	
3	10 (53)	7 (47)	
5	1 (5)	0	
ALC_{Leuk} (10^9 cells/L)	0.68 [0.43–1.23]	0.74 [0.47–0.84]	0.75
CRP at Day 0 (mg/L)	11 [6–28]	19 [14–51]	0.05
Peak CRP within 14 days (mg/L)	**11 [6–28]**	**78 [34–156]**	**<0.001**
Ferritin at Day 0 (μg/L)	381 [287–1,129]	762 [409–2,153]	0.05
Peak ferritin within 14 days (μg/L)	1265 [531–1,489]	2321 [898–5,075]	0.11
Prior autologous transplant	6 (32)	3 (20)	0.70
Received bridging therapy	5 (26)	3 (20)	>0.99
CRS, all grades	19 (100)	12 (80)	0.08
CRS, grade ≥ 3	1 (5)	0	>0.99
ICANS, all grades	10 (53)	4 (27)	0.17
ICANS, grade ≥ 3	5 (26)	3 (20)	>0.99
Cerebral edema	1 (5)	1 (6)	>0.99
Received tocilizumab	11 (58)	5 (33)	0.19
Received steroids	6 (32)	3 (20)	0.70
Required ICU care	7 (37)	5 (33)	>0.99
Time from leukapheresis to infusion (days)	28 [26–29]	28 [26–32]	0.62
Time from last therapy to infusion (days)	86 [55–192]	114 [51–145]	0.58
FC conditioning regimen	19 (100)	15 (100)	0.57
Baseline CD19 tumor expression			
Positive	10/11 (91)	7/9 (78)	N/A
Negative	1/11 (9)	2/9 (22)	N/A
CD19 tumor expression after CAR T			
Positive	N/A	6/8 (75)	N/A
Negative	N/A	2/8 (25)	N/A

Note. Data are presented as median [interquartile range] or n (%). ALC_{Leuk} = absolute lymphocyte count at leukapheresis; CAR = chimeric antigen receptor; CRP = C-reactive protein; CRS = cytokine release syndrome; DLBCL = diffuse large B-cell lymphoma; ECOG = Eastern Cooperative Oncology Group; FC = Fludarabine Cyclophosphamide; ICANS = immune effector cell-associated neurotoxicity syndrome; ICU = intensive care unit; IHC = immunohistochemistry; PMBCL = primary mediastinal large B-cell lymphoma; tFL = transformed follicular lymphoma.

[a] Presence of MYC rearrangement and BCL2 or BCL6 rearrangement on fluorescence in situ hybridization.

[b] Immunohistochemical detection of MYC and BCL2 or BCL6 overexpression.

(IQR 41.5–62), 62% of patients were male, all patients had Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, and no significant comorbidities. There were seven (21%) patients with refractory disease and eight (23%) patients relapsed after CAR T-cell treatment. Furthermore, 65% of treated patients were diagnosed with DLBCL, and other diagnoses included tFL (26%) and PMBCL (9%). Most patients who responded to CAR T-cell therapy had double-hit lymphoma or double-expressor lymphoma (Table 1), but this difference did not reach statistical significance. Seventy

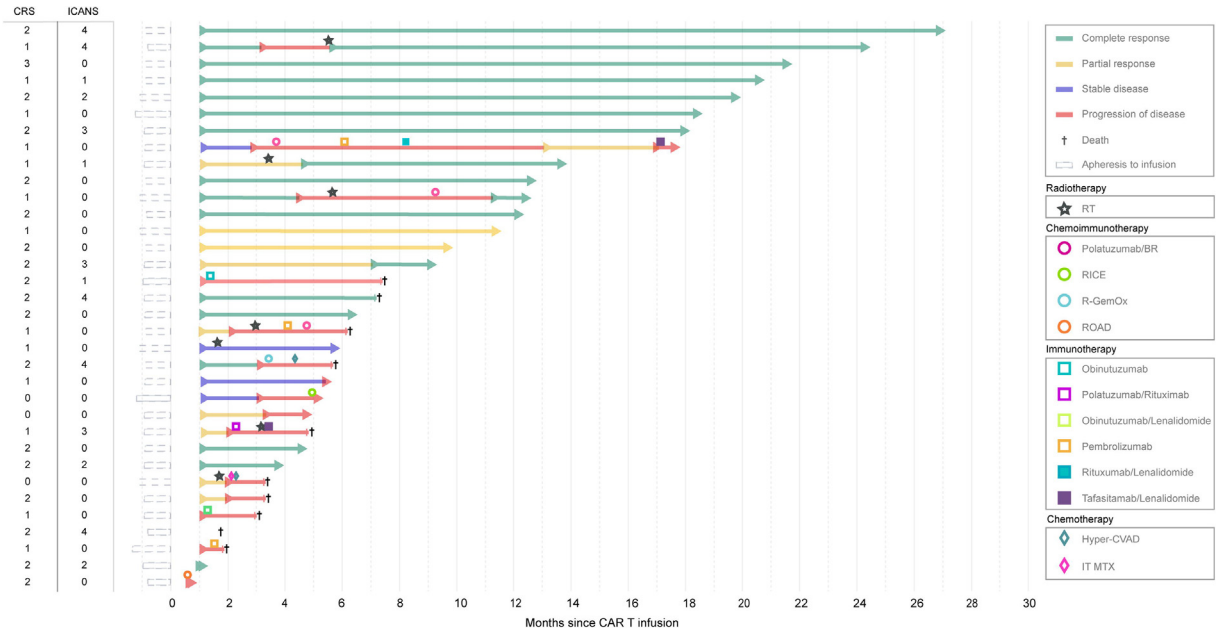

Fig. 1. A swimmer plot of patients with aggressive non-Hodgkin lymphoma who received axicabtagene ciloleucel. Median follow-up time was 6.8 months (IQR 4.6–14.9). Note. BR = Bendamustine Rituximab; CAR = Chimeric Antigen Receptor; CRS = Cytokine Release Syndrome; Hyper-CVAD = Cyclophosphamide, Vincristine sulfate, doxorubicin hydrochloride (Adriamycin), and Dexamethasone; ICANS = Immune Effector Cell-Associated Neurotoxicity Syndrome; IT MTX = Intrathecal Methotrexate; R-GemOx = Rituximab Gemcitabine and Oxaliplatin, ROAD = Rituximab, Oxaliplatin, cytosine Arabinoside, and Dexamethasone; RT = Radiotherapy.

percent of patients had stage III or IV, and half of the patients received at least three prior lines of therapy. The median time from the last treatment to axi-cel infusion was 3.2 months (IQR 1.8–5.6), and the time from leukapheresis to infusion was similar among groups (average of 28 days). All patients received cyclophosphamide and fludarabine as lymphodepleting conditioning regimen, and 23.5% received bridging therapy. The median ALC_{Leuk} was 0.71×10^9/L (IQR 0.45–1.00). All patients received a dose of 2×10^6 $CD3^+$ CAR^+ cells per kilogram, with a maximum total dose of 2×10^8 cells.

Safety

In total, 91% of patients developed CRS (3% grade $\geq$ 3). This adverse event and the use of tocilizumab were more frequent among the patients with response to axi-cel (100% vs. 80% and 53% vs. 33%, respectively). ICANS occurred in 41% of patients (24% grade $\geq$ 3). One patient developed grade 4 ICANS, received multiple doses of steroids and tocilizumab, was found to have a hypoplastic bone marrow biopsy with nearly absent hematopoiesis, and subsequently died of disseminated fungal and

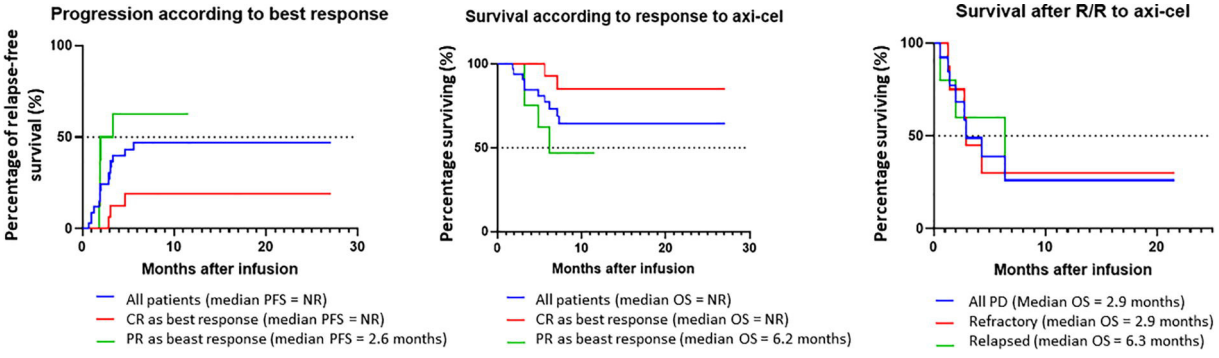

Fig. 2. Kaplan-Meier curves for survival and progression according to best response. Note. axi-cel = axicabtagene ciloleucel; CR = complete response; NR = not reached; OS = overall survival; PD = progressive disease; PFS = progression-free survival; PR = partial response; R/R = relapse/refractory.

mycobacterial infections. Thus, it was considered therapy-related death due to opportunistic infections in the setting of pancytopenia post-CAR T-cell therapy. The median time was 2 days (IQR 1–4) to any grade CRS event and 6 days (IQR 4–8) to any grade ICANS. The median duration was 4 days (IQR 1–7) for CRS symptoms and 9 days (IQR 7–12) for ICANS.

Treatment efficacy and CD19 expression

At a median follow-up of 6.8 months, the best ORR and CR to axi-cel were 71% and 53%, respectively (Fig. 1). Twelve patients have been followed for a minimum of 1 year, and 10 patients (29%) died before this benchmark. The median OS in the full cohort of patients was not reached, whereas in the R/R to axi-cel group, the median OS after progression was 3 months (IQR 1.3–5.1). Patients considered refractory to axi-cel had worse OS after progressive disease (PD) than relapsed patients (2.9 vs. 6.3 months), and patients who only achieved partial response had a median OS of 6.2 (IQR 4.4–9.9) months (Fig. 2). The median PFS in the full cohort was not reached, and the PFS rate at 6 months was 55% (95% confidence interval [CI] 34–68) (Fig. 2).

Fifteen (44%) patients treated with axi-cel had PD, 60% of whom progressed during the first 3 months after infusion. Among these patients, the median time to progression was 2 months (IQR 1.5–3) (Fig. 2).

All patients had baseline biopsies confirming aggressive B-cell NHL diagnosis, and CD19 status was assessed in 59% of the cases (Table 1). Eight of 15 patients with PD after axi-cel had CD19 expression reports available on their biopsies. Notably, six (75%) were still positive for CD19, and the two patients who were CD19 negative lacked expression of this receptor on baseline biopsies (Table 1). Therefore, there was no documented loss of CD19 expression in our cohort of patients with PD.

Therapies used after failure to CAR T-cell therapy

Twelve patients received additional salvage treatment. Six of them received one subsequent treatment line; three patients received two, and three patients received more than two lines of treatment. The median time to new treatment was 2.5 months (IQR 1.7–3.6). The most common salvage therapies were radiotherapy, polatuzumab plus bendamustine and rituximab (Pola-BR), and pembrolizumab (Table 2 and Fig. 1). None of the patients in this cohort received a second dose of CAR T-cell therapy or a hematopoietic stem cell transplant.

Table 2. Subsequent lines of therapy after Chimeric Antigen Receptor T-cell failure.

Treatment	*n*
Radiotherapy	7
Chemoimmunotherapy	
Polatuzumab plus bendamustine and rituximab	3
RICE	1
R-GemOx	1
ROAD	1
Immunotherapy	
Obinutuzumab	1
Polatuzumab plus rituximab	1
Obinutuzumab plus lenalidomide	1
Pembrolizumab	3
Rituximab plus lenalidomide	1
Tafasitamab plus lenalidomide	2
Chemotherapy	
Hyper-CVAD	2
Intrathecal methotrexate	1

Note: Hyper-CVAD = Cyclophosphamide, Vincristine sulfate, doxorubicin hydrochloride (Adriamycin), and Dexamethasone; R-GemOx = Rituximab Gemcitabine and Oxaliplatin; RICE = Rituximab, Ifosfamide, Carboplatin and Etoposide; ROAD = Rituximab, Oxaliplatin, cytosine Arabinoside, and Dexamethasone.

Only three (25%) patients who received salvage therapy had evidence of response (Table 2 and Fig. 1). One patient received radiotherapy and Pola-BR (ongoing CR at 1.2 months). Another received radiotherapy (ongoing CR at 19 months), and the third patient had three subsequent therapy lines, including Pola-BR pembrolizumab, and rituximab + lenalidomide, achieving a PR. Unfortunately, this last patient progressed 4 months later and is currently receiving tafasitamab plus lenalidomide.

Predictors of CAR T-cell therapy response

Our exploratory subgroup analyses showed that patients with higher CRS grade and higher number of tocilizumab doses were more likely to respond to CAR T-cell therapy in the unadjusted analysis. However, in the multivariable model, only the higher CRS grade (odds ratio [OR] 0.26, 95% confidence interval (CI) 0.05–0.94) remained statistically significant (Table 3).

We also explored factors affecting CR achievement and found that CRs were more likely in patients with lower pre-infusion levels of ferritin, more intense immune effector cell (IEC)-related toxicities, and higher number of tocilizumab doses. None of these factors showed a statistically significant association on multivariable analysis (Table 4).

Since we identified numerical differences in pro-inflammatory biomarkers between patients who

Table 3. Factors Affecting Any Response by Logistic Regression Analysis.

Parameters	OR	95% CI	*p*	OR	95% CI	*p*
	Univariate logistic regression			Multivariate logistic regression		
Age	0.98	0.98–1.04	0.52			
Sex (male vs. female)	1.45	0.36–6.24	0.60			
ECOG status	0.72	0.21–2.34	0.59			
Lymphoma subtype (DLBCL vs. PMBCL vs. tFL)	1.12	0.51–2.46	0.77			
Double hit[a] (yes vs. no)	0.33	0.015–3.0	0.37			
Double expressor[b] (yes vs. no)	0.42	0.08–1.86	0.25			
Stage of disease	0.69	0.32–1.44	0.33			
Number of lines before leukapheresis	0.57	0.16–1.69	0.34			
CRP at Day 0	**1.03**	**1.01–1.09**	**0.02**	1		
Peak CRP within 14 days	0.99	0.99–1.01	0.54			
Ferritin at Day 0	1.00	0.99–1.00	0.84			
Peak ferritin within 14 days	1.00	0.99–1.00	0.23			
Baseline ALC	0.49	0.09–1.76	0.29			
Peak ALC within 14 days	1.18	0.35–4.16	0.77			
Peak ALC within 31 days	1.57	0.62–4.56	0.35			
AUC ALC 15 days	1.11	0.90–1.42	0.34			
AUC ALC 31 days	1.06	0.96–1.20	0.24			
Prior autologous transplant (yes vs. no)	0.54	0.09–2.55	0.44			
Received bridging therapy (yes vs. no)	0.70	0.12–3.48	0.66			
CRS, any grade (yes vs. no)	—	—	—			
CRS, grade	**0.23**	**0.06–0.71**	**0.01**	**0.26**	**0.05–0.94**	**0.05**
ICANS, any grade (yes vs. no)	0.33	0.07–1.34	0.12			
ICANS, grade	0.77	0.46–1.22	0.27			
Cerebral edema (yes vs. no)	1.29	0.05–34.4	0.86			
Number of doses of tocilizumab received	**0.48**	**0.20–0.93**	**0.02**	0.67	0.26–1.38	0.32
Number of doses of steroids received	0.91	0.67–1.13	0.40			
Required ICU care (yes vs. no)	0.86	0.20–3.55	0.83			
Time from leukapheresis to infusion	1.06	0.88–1.30	0.50			
Time from last therapy to infusion	1.00	0.99–1.01	0.28			
Baseline CD19 tumor expression (yes vs. no)	0.35	0.01–4.36	0.41			

Note. ALC_{Leuk} = absolute lymphocyte count at leukapheresis; CI = confidence interval; CRP = C-reactive protein; CRS = cytokine release syndrome; DLBCL = diffuse large B-cell lymphoma; ECOG = Eastern Cooperative Oncology Group; ICANS = immune effector cell-associated neurotoxicity syndrome; ICU = intensive care unit; OR = odds ratio; PMBCL = primary mediastinal large B-cell lymphoma; tFL = transformed follicular lymphoma. Statistically significant OR formatted in bold.

[a] Presence of MYC rearrangement and BCL2 or BCL6 rearrangement on fluorescence in situ hybridization.

[b] Immunohistochemical detection of MYC and BCL2 or BCL6 overexpression.

responded to CAR T-cell therapy, and those with R/R to axi-cel disease, we explored the optimal cutoffs to predict disease progression and OS (Supplemental Fig. S1). Compared with patients with lower pro-inflammatory biomarkers, patients with a baseline CRP greater than 12 mg/L and ferritin higher than 392 μg/L had a hazard ratio to progression of 9.5 and 5.5, respectively (Fig. 3). Similarly, before infusion, patients with ferritin levels greater than 411 μg/L and peak levels during the first 14 days post-infusion greater than 1517 μg/L had shorter survival than patients with lower baseline or peak ferritin levels (Fig. 3).

Finally, after multivariate analyses, we found that patients with high ALC_{Leuk} have improved OS (OR 0.01, 95% CI 0.01–0.37) (see Table 5). Patients with an ALC_{Leuk} higher than 0.54×10^9/L showed longer survival (hazard ratio [HR] 0.11, 95% CI 0.03–0.44, $p = .001$) (Fig. 3 and Supplemental Fig. S1). We did not find correlation between ALC_{Leuk} and PFS or CR.

Discussion

In this retrospective cohort of patients treated under standard-of-care with axi-cel, we found similar ORR and CR rates to those who received axi-cel in the clinical trial setting [7,15]. Most patients with R/R disease after axi-cel showed PD early after axi-cel treatment (<3 months post-infusion); unfortunately, only 25% of subsequently treated patients responded to salvage treatment.

There are some differences in patients' characteristics from our cohort compared with the ZUMA-1 study. Overall, 21% of our patients would not have been eligible for the ZUMA-1 trial, mainly because of cytopenias. Patients here reported were younger (≥65 years: 6% vs. 24%), had an earlier stage of disease (stage III–IV: 71% vs. 85%), and had a higher prevalence of tFL (26% vs. 16%). However, we did not find statistically significant differences in ORR or CR rates between our study and ZUMA-1 [7,15].

Table 4. Factors Affecting Complete Response by Logistic Regression Analysis.

Parameters	OR	95% CI	*p*	OR	95% CI	*p*
	Univariate logistic regression			Multivariate logistic regression		
Age (yrs)	1.01	0.95–1.06	0.86			
Sex (male vs. female)	0.94	0.23–3.81	0.93			
ECOG status	0.43	0.11–1.42	0.17			
Lymphoma subtype (DLBCL vs. PMBCL vs. tFL)	1.15	0.53–2.56	0.73			
Double hit[a] (yes vs. no)	0.86	0.09–8.00	0.89			
Double expressor[b] (yes vs. no)	1.90	0.43–9.60	0.39			
Stage of disease	0.85	0.40–1.77	0.66			
Number of lines before leukapheresis	1.12	0.39–3.46	0.83			
CRP at Day 0	0.98	0.94–1.00	0.10			
Peak CRP within 14 days	1.01	1.00–1.02	0.09			
Ferritin at Day 0	**0.99**	**0.98–0.99**	**0.03**	1		
Peak ferritin within 14 days	1.00	0.99–1.00	0.11			
Baseline ALC	2.39	0.65–13.1	0.24			
Peak ALC within 14 days	2.32	0.66–13.9	0.21			
Peak ALC within 31 days	1.37	0.54–3.98	0.51			
AUC ALC 15 days	1.01	0.81–1.26	0.93			
AUC ALC 31 days	0.99	0.90–1.10	0.83			
Prior autologous transplant (yes vs. no)	2.17	0.46–12.1	0.33			
Received bridging therapy (yes vs. no)	0.86	0.17–4.35	0.85			
CRS, any grade (yes vs. no)	—	—	—			
CRS, grade	**5.26**	**1.62–22.6**	**0.01**	3.49	0.50–40.5	0.24
ICANS, any grade (yes vs. no)	**6.81**	**1.54–38.4**	**0.02**	0.71	0.01–77.2	0.88
ICANS, grade	**1.80**	**1.09–3.45**	**0.02**	4.91	0.78–127	0.17
Cerebral edema (yes vs. no)	—	—	—			
Number of doses of tocilizumab received	**4.81**	**1.78–21.5**	**<0.001**	18.3	2.10–425	0.10
Number of doses of steroids received	1.16	0.92–1.64	0.22			
Required ICU care (yes vs. no)	2.40	0.57–11.3	0.23			
Time from leukapheresis to infusion	0.90	0.73–1.09	0.28			
Time from last therapy to infusion	1.00	0.99–1.01	0.23			
Baseline CD19 tumor expression (yes vs. no)	—	—	—			

Note. ALC_{Leuk} = absolute lymphocyte count at leukapheresis; CI = confidence interval; CRP = C-reactive protein; CRS = cytokine release syndrome; DLBCL = diffuse large B-cell lymphoma; ECOG = Eastern Cooperative Oncology Group; ICANS = immune effector cell-associated neurotoxicity syndrome; ICU = intensive care unit; OR = odds ratio; PMBCL = primary mediastinal large B-cell lymphoma; tFL = transformed follicular lymphoma. Statistically significant OR formatted in bold.

[a] Presence of MYC rearrangement and BCL2 or BCL6 rearrangement on fluorescence in situ hybridization.

[b] Immunohistochemical detection of MYC and BCL2 or BCL6 overexpression.

In terms of safety, the most common adverse events were related to CRS and ICANS and occurred in 91% (3% grade $\geq$ 3) and 41% (24% grade $\geq$ 3) of patients, respectively. One patient died as a consequence of prolonged hospitalization with cytopenias and opportunistic fungal infection. Overall, our patients presented a lower frequency of severe IEC-associated toxicities than reports from other clinical trials and real-world experiences [7].

The prognosis of patients progressing after axi-cel treatment is poor. The median OS after progression was 2.9 months (95% CI 1.5 to not reached). Other authors have reported a median OS of 5.3 months for patients who progress after CAR T-cell therapy and a shorter OS of 3.75 months if PD occurs within 30 days [16]. At a median follow-up of 6.8 months, 44% of our patients progressed, most of them within the first 3 months after axi-cel infusion. Of the 15 patients who progressed after axi-cel, 12 received salvage treatment; only three had evidence of response. We did not observe a clear pattern of responses and correlation with specific therapies.

There are no established guidelines for the management of R/R aNHL patients after CAR T-cell therapy. The consensus is that the results with conventional treatments have been very disappointing, and this highlights the need for additional effective therapies [16,17]. A cohort with 100 patients treated after failure to axi-cel showed a longer PFS trend in patients treated with checkpoint inhibitor-based treatments than other therapies (lenalidomide-based regimens radiotherapy and chemotherapy) [16,17].

The median time to progression was of 2 months (IQR 1.5–3.1). Therefore, early assessment of these patients during the initial months after CAR T-cell infusion is critical to identify the patients who will show PD. However, early diagnosis without effective therapies may not impact the overall prognosis

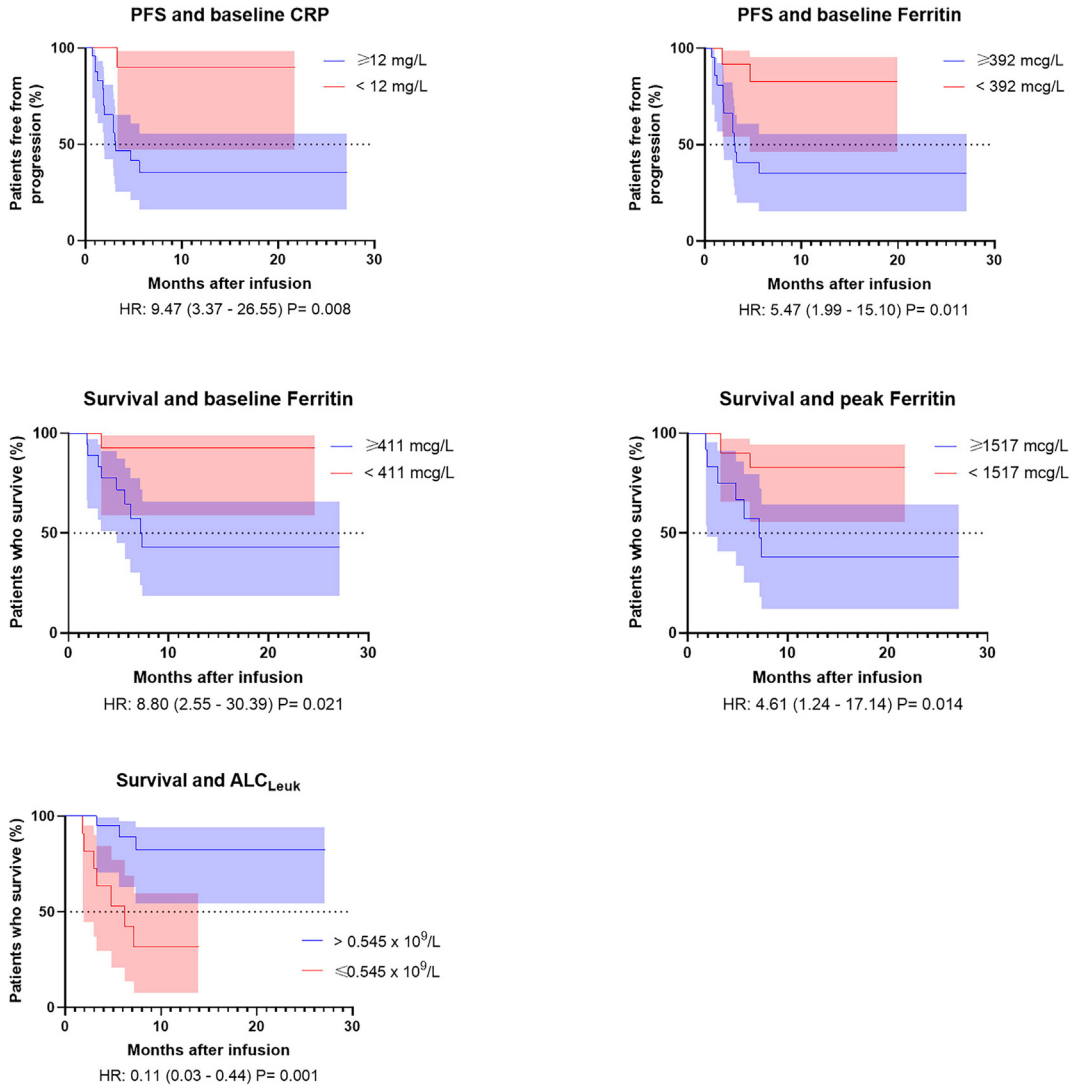

Fig. 3. Kaplan-Meier curves for survival and progression according to acute-phase reactant's thresholds and ALC_{Leuk}. Note. ALC_{Leuk} = absolute lymphocyte count at leukapheresis; CRP = C-reactive Protein; HR = hazard ratio; PFS = progression-free survival.

of R/R patients after CAR T-cell treatment. This is why effective treatment alternatives for R/R patients are urgently needed.

In our cohort, most patients in which CD19 expression data were available showed $CD19^{+}$ relapse, highlighting that antigen escape may not be the most relevant factor implicated in disease progression in these patients. Other factors such as T-cell expansion, proliferation, persistence, infiltration to the tumor microenvironment, tumor inhibitory mechanisms, and exhaustion could also be responsible for failure to therapy after CAR T-cell infusions [18]. Similarly, the two pivotal trials of the first anti-CD19 CAR T products, ZUMA1 and JULIET, showed a low incidence of CD19 negative relapse [7,19].

Our analyses show that higher ALC_{Leuk} is a strong predictor of survival after axi-cel treatment. Peak ALC after treatment has been reported as a surrogate marker of CAR T-cell expansion and correlates with response to therapy and IEC-related toxicities [8,20]. Also, others had shown a correlation between ALC at the time of treatment (ALC_{TT}) and OS, although not significant through a multivariate analysis [21]. To our knowledge, this is the first report of ALC_{Leuk} as a variable that directly correlates with survival, making it a very useful predictor of positive outcomes before initiating manufacturing processes. ALC_{TT} and ALC_{Leuk} are two different prognostic markers; they may complement each other. However, we think measuring the ALC earlier before interventions such as leukapheresis and lymphodepletion chemotherapy could represent a more reliable assessment of the patient's cellular immunity fitness [22]. Moreover, an

Table 5. Factors Affecting Overall Survival by Logistic Regression Analysis.

Parameters	OR	95% CI	*p*	OR	95% CI	*p*
	Univariate logistic regression			Multivariate logistic regression		
Age (yrs)	0.99	0.93–1.05	0.73			
Sex (male vs. female)	0.50	0.11–2.28	0.36			
ECOG status	2.46	0.67–11.6	0.18			
Lymphoma subtype (DLBCL vs. PMBCL vs. tFL)	0.64	0.22–1.54	0.34			
Double hit[a] (yes vs. no)	—	—	—			
Double expressor[b] (yes vs. no)	0.31	0.04–1.60	0.17			
Stage of disease	1.24	0.55–3.02	0.61			
Number of lines before leukapheresis	2.01	0.65–7.75	0.23			
CRP at Day 0	1.01	0.99–1.03	0.35			
Peak CRP within 14 days	0.99	0.98–1.00	0.25			
Ferritin at Day 0	1.01	1.01–1.02	0.03	1		
Peak ferritin within 14 days	1.00	1.00–1.01	0.01	1		
Baseline ALC	**0.03**	**0.01–0.40**	**<0.001**	**0.01**	**0.01–0.37**	**<0.001**
Peak ALC within 14 days	1.18	0.35–4.16	0.77			
Peak ALC within 31 days	1.57	0.62–4.56	0.35			
AUC ALC 15 days	0.92	0.67–1.16	0.49			
AUC ALC 31 days	0.88	0.74–1.01	0.10			
Prior autologous transplant (yes vs. no)	1.29	0.22–6.47	0.76			
Received bridging therapy (yes vs. no)	0.27	0.01–1.87	0.20			
CRS, any grade (yes vs. no)	0.82	0.07–18.9	0.88			
CRS, grade	0.81	0.28–2.40	0.70			
ICANS, any grade (yes vs. no)	1.67	0.37–7.65	0.50			
ICANS, grade	1.32	0.82–2.15	0.24			
Cerebral edema (yes vs. no)	2.56	0.09–69.4	0.53			
Number of doses of tocilizumab received	0.84	0.41–1.53	0.60			
Number of doses of steroids received	0.99	0.75–1.24	0.96			
Required ICU care (yes vs. no)	2.43	0.53–11.6	0.25			
Time from leukapheresis to infusion	1.04	0.85–1.27	0.68			
Time from last therapy to infusion	0.99	0.99–1.00	0.88			
Baseline CD19 tumor expression (yes vs. no)	1.09	0.08–26.4	0.95			

Note. ALC_{Leuk} = absolute lymphocyte count at leukapheresis; CI = confidence interval; CRP = C-reactive protein; CRS = cytokine release syndrome; DLBCL = diffuse large B-cell lymphoma; ECOG = Eastern Cooperative Oncology Group; ICANS = immune effector cell-associated neurotoxicity syndrome; ICU = intensive care unit; OR = odds ratio; PMBCL = primary mediastinal large B-cell lymphoma; tFL = transformed follicular lymphoma. Statistically significant OR formatted in bold.

[a] Presence of MYC rearrangement and BCL2 or BCL6 rearrangement on fluorescence in situ hybridization.

[b] Immunohistochemical detection of MYC and BCL2 or BCL6 overexpression.

earlier ALC evaluation using ALC_{Leuk} could help clinicians make important decisions about patient selection and distribute limited resources regarding costly treatments such as CAR T-cell therapy.

This finding suggests that ALC_{Leuk} is a robust and early prognostic marker that correlates independently with OS. Because time is of the essence in managing critically ill patients candidates for CAR T-cell therapy, we believe that an earlier and reliable prognostic maker such as ALC_{Leuk} represents a vital tool to guide clinicians' decisions efficiently and cost-effectively.

We found an association between the severity of IEC-related toxicities and the probability of achieving a CR in univariate analyses. Still, there was no statistical evidence of an independent association (Table 4). These trends may be subject to our sample size limitations, and more extensive studies will allow us to explore the significant associations of these variables with the clinical outcome after treatment with axi-cel (Table 3).

Our data suggest that the degree of immune system stimulation plays a role in achieving responses to CAR T-cell therapy and that the use of tocilizumab or steroids does not appear to interfere with CAR T-cell activity. Therefore, administering these medications to compromised patients with IEC-associated adverse events should be provided as needed and without hesitation.

We observed that patients with lower levels of pro-inflammatory biomarkers (baseline CRP or baseline ferritin) were less likely to progress after axi-cel therapy. We optimized the cutoffs for these variables and found that patients with low pre-infusion blood levels of CRP ($\leq$12 mg/L) and ferritin ($\leq$392 μg/L) had significantly better PFS. Moreover, patients that had low ferritin levels either at baseline (<411 μg/L) or a peak level during the 14 days post-

infusion (<1517 mcg/L) show a 4.6 times improvement in OS (Fig. 3 and Supplemental Fig. S1).

These findings are consistent with other reports [8,18,22]. The underlying mechanism(s) and precise molecular pathways associated with this process are unclear. However, a dysregulated pro-inflammatory state, reminiscent of myeloid cell hyperactivity (as seen in hemophagocytic lymphohistiocytosis syndrome) at baseline or after CAR T-cell infusion may induce immunosuppression that can directly impact the effector T-cell activation, expansion, and persistence, thus hampering the clinical benefit of CAR T-cell therapy [18]. Efforts to optimize allogeneic source of the product will bypass this downside of starting with autologous T-cells from diseased patients.

There is also evidence that cancer-associated inflammation, which is supported mainly by innate immune cells, contributes to tumor growth, therapy resistance, and the overall creation of a persistent immunosuppressive state [23]. Pre-existing high levels of cytokines that participate in inflammation and tissue regeneration can significantly shape the immune response against the tumor [23]. In the particular case of CAR T-cell therapy, it may lead to either overstimulation of the immune system with an increase in the frequency and severity of adverse events such as CRS and ICANS, and may also decrease the effectiveness of the infused cells due to inadequate expansion, persistence, tissue penetration, or deficient cytotoxic effect. Therefore, our data suggest that the patients' inflammatory state before CAR T-cell therapy should be a factor to be considered with particular attention. However, the most appropriate inflammatory parameters that should be evaluated to establish the patient prognosis remain to be defined.

Conclusions

Overall, combinatorial immunotherapy approaches that will include antibodies, cellular therapy, vaccinations, and the use of alternative IECs like macrophages and dendritic cells may be necessary to overcome the biological pathways associated with the immunosuppressive effects of the tumor microenvironment, and in general, the resistance to CAR T-cell therapy.

We found that the grade of CRS and that early assessment of the ALC at the time of leukapheresis, ALC_{Leuk}, correlated with better outcomes in patients treated with axi-cel. Particularly, we think that ALC_{Leuk} is a cost-effective and easy-to-obtain OS prognostic marker that provides a helpful assessment of the patient's immune status and can become a valuable tool for patient selection, management, and distribution of costly resources involved in CAR T-cell therapy.

More extensive clinical prospective studies evaluating ALC_{Leuk}, other prognostic makers, and the point-of-care technologies will be required to identify optimal tools that can help us select the patients most likely to benefit from CAR T-cell therapy and predict the response to salvage therapy depending on the specific mechanism(s) of relapse.

Novelty and impact

Predictors of positive outcomes after axi-cel include higher ALC at leukapheresis, lower CRP, lower ferritin, and a higher grade of CRS. Relapses after axi-cel often occur within months and confer a poor prognosis with limited response to salvage therapy.

Authors' contributions

JEC: study design, patient enrollment and treatment. PALD and JVFF: study design, data collection, and data analysis. ACR and MAKD: patient enrollment and treatment. EFM, MM, and ZAR: data collection. All authors participated in data interpretation, manuscript writing, and approval of the final submitted version.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JEC has received research funding from Fate Therapeutics, Kite Pharma, and Pharmacyclics. MAKD provides consultancy for Daiichi Sankyo and Pharmacyclics. All other authors declare no competing financial interests.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hemonc.2021.09.001.

References

[1] Moreno-Cortes E, Forero-Forero JV, Lengerke-Diaz PA, Castro JE. Chimeric antigen receptor T cell therapy in oncology – pipeline at a glance: analysis of the ClinicalTrials.gov database. Crit Rev Oncol Hematol 2021;159.

[2] Bouchkouj N, Kasamon YL, de Claro RA, George B, Lin X, Lee S, et al. FDA approval summary: axicabtagene ciloleucel

for relapsed or refractory large B-cell lymphoma. Clin Cancer Res 2019;25:1702–8.
[3] O'Leary MC, Lu X, Huang Y, Lin X, Mahmood I, Przepiorka D, et al. FDA approval summary: tisagenlecleucel for treatment of patients with relapsed or refractory B-cell Precursor acute lymphoblastic leukemia. Clin Cancer Res 2019;25:1142–6.
[4] Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. N Engl J Med 2020;382:1331–42. https://doi.org/10.1056/NEJMoa1914347.
[5] Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet 2020;396:839–52.
[6] Boyiadzis MM, Dhodapkar MV, Brentjens RJ, Kochenderfer JN, Neelapu SS, Maus MV, et al. Chimeric antigen receptor (CAR) T therapies for the treatment of hematologic malignancies: clinical perspective and significance. J ImmunoTher Cancer 2018;6:137.
[7] Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377:2531–44.
[8] Jacobson CA, Hunter BD, Redd R, Rodig SJ, Chen PH, Wright K, et al. Axicabtagene ciloleucel in the non-trial setting: outcomes and correlates of response, resistance, and toxicity. J Clin Oncol 2020;38:3095–106.
[9] Sermer D, Batlevi C, Palomba ML, Shah G, Lin RJ, Perales MA, et al. Outcomes in patients with DLBCL treated with commercial CAR T cells compared with alternate therapies. Blood Adv 2020;4:4669–78.
[10] Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood 2017;130:1800–8.
[11] Byrne M, Oluwole OO, Savani B, Majhail NS, Hill BT, Locke FL. Understanding and managing large B cell lymphoma relapses after chimeric antigen receptor T cell therapy. Biol Blood Marrow Transplant 2019;25:e344–51.
[12] Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy – assessment and management of toxicities. Nat Rev Clin Oncol 2018;15:47–62.
[13] Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant 2019;25:625–38.
[14] Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood 2014;124:188–95.
[15] Kochenderfer JN, Somerville RP, Lu T, Yang JC, Sherry RM, Feldman SA, et al. Long-duration complete remissions of diffuse large B cell lymphoma after anti-CD19 chimeric antigen receptor T cell therapy. Mol Ther 2017;25:2245–53.
[16] Chow VA, Gopal AK, Maloney DG, Turtle CJ, Smith SD, Ujjani CS, et al. Outcomes of patients with large B-cell lymphomas and progressive disease following CD19-specific CAR T-cell therapy. Am J Hematol 2019;94:E209–13.
[17] Spiegel JY, Dahiya S, Jain MD, Tamaresis JS, Nastoupil L, Jacobs MT, et al. Outcomes of patients with large B-cell lymphoma progressing after axicabtagene ciloleucel. Blood 2020.
[18] Locke FL, Rossi JM, Neelapu SS, Jacobson CA, Miklos DB, Ghobadi A, et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. Blood Adv 2020;4:4898–911.
[19] Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 2018;380:45–56.
[20] Novo M, Sidiqi MH, Paludo J, Gandhi S, Kubusek J, Truong T, et al. Peak lymphocyte count after CAR T Infusion is a clinically accessible test that correlates with clinical response in axicabtagene ciloleucel therapy for lymphoma. Blood 2019;134:4106.
[21] Korell F, Laier S, Sauer S, Veelken K, Hennemann H, Schubert ML, et al. Current Challenges in providing good leukapheresis products for manufacturing of CAR-T cells for patients with relapsed/refractory NHL or ALL. Cells 2020;9:1225.
[22] Sesques P, Ferrant E, Safar V, Wallet F, Tordo J, Dhomps A, et al. Commercial anti-CD19 CAR T cell therapy for patients with relapsed/refractory aggressive B cell lymphoma in a European center. Am J Hematol 2020;95:1324–33.
[23] Shalapour S, Karin M. Pas de Deux: Control of anti-tumor immunity by cancer-associated inflammation. Immunity 2019;51:15–26.