BRIEF REPORT

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Correlation of Peripheral Chimeric Antigen Receptor T-cell (CAR-T Cell) mRNA Expression Levels with Toxicities and Outcomes in Patients with Diffuse Large B-cell Lymphoma

Diffüz Büyük B-hücreli Lenfomalı Hastalarda Periferik Kimerik Antijen Reseptör T-hücresi (CAR-T Hücresi) mRNA Ekspresyon Düzeylerinin Toksisiteler ve Sonuçlarla Korelasyonu

Christian Messerli[1], Gertrud Wiedemann[2], Naomi Porret[2], Michael Nagler[3], Katja Seipel[4], Barbara Jeker[1], Urban Novak[1], Sacha Zeerleder[2], Ulrike Bacher[2]*, Thomas Pabst[1]*

[1]*University Hospital and University of Bern, Department of Medical Oncology, Bern, Switzerland*
[2]*University Hospital and University of Bern, Department of Hematology and Central Hematology Laboratory, Bern, Switzerland*
[3]*University Institute of Clinical Chemistry, University Hospital and University of Bern, Bern, Switzerland*
[4]*University of Bern, Department for Biomedical Research, Bern, Switzerland*
**These authors contributed equally to this work.*

Abstract

Cytokine-release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are significant complications in patients with relapsed/refractory diffuse large B-cell lymphoma undergoing chimeric antigen receptor T-cell (CAR-T cell) therapy. However, it remains unclear whether CAR-T cell expression itself is clinically relevant. We assessed CAR-T cell mRNA expression and DNA concentration by digital droplet PCR in peripheral blood from 14 sequential CAR-T cell recipients. Patients were grouped according to CAR-T cell peak expression. Patients with high CAR-T cell peak expression (8 patients; 57%) had higher rates of ICANS (p=0.0308) and intensive care unit admission (p=0.0404), longer durations of hospitalization (p=0.0077), and, although not statistically significant, a higher rate of CRS (p=0.0778). There was a correlation of CAR-T cell mRNA expression with DNA concentration, but CAR-T cell expression levels failed to correlate to response or survival. Our data suggest that higher CAR-T cell peak mRNA expression is associated with increased risk for ICANS and possibly CRS, requiring further investigation in larger studies.

Keywords: Non-Hodgkin lymphoma, Lymphomas, Neoplasia, Transplant-related toxicity, Stem cell transplantation, Molecular biology, CAR-T cell therapy, CAR-T cell expansion, Digital droplet PCR, Diffuse large B-cell lymphoma, Immune effector cell-associated neurotoxicity syndrome

Öz

Sitokin salımı sendromu (CRS) ve immün efektör hücre ile ilişkili nörotoksisite sendromu (ICANS), kimerik antijen reseptörü T-hücresi (CAR-T hücresi) tedavisi gören nüksetmiş/refrakter diffüz büyük B-hücreli lenfoma hastalarında önemli komplikasyonlardır. Bununla birlikte, CAR-T hücre ifadesinin klinikle ilişkili olup olmadığı belirsizliğini korumaktadır. Ondört CAR-T hücre alıcısından periferik kanda dijital damlacık PCR'ı ile CAR-T hücresi mRNA ekspresyonunu ve DNA konsantrasyonunu değerlendirdik. Hastalar, CAR-T hücre pik ifadesine göre gruplandırıldı. Yüksek CAR-T hücre pik ekspresyonu olan hastalar (8 hasta; %57) daha yüksek ICANS (p=0,0308) ve yoğun bakıma yatış (p=0,0404), daha uzun hastanede yatış süreleri (p=0,0077) ve istatistiksel olarak anlamlı olmasa da, daha yüksek CRS oranına sahipti (p=0,0778). CAR-T hücresi mRNA ekspresyonu ile DNA konsantrasyonu arasında bir korelasyon vardı, ancak CAR-T hücresi ekspresyon seviyeleri, yanıt veya hayatta kalma ile korelasyon göstermedi. Verilerimiz, daha yüksek CAR-T hücre zirvesi mRNA ekspresyonunun, daha büyük çalışmalarda daha fazla araştırmayı gerektiren, artan ICANS ve muhtemelen CRS riski ile ilişkili olduğunu göstermektedir.

Anahtar Sözcükler: Non-Hodgkin lenfoma, Lenfomalar, Neoplazi, Nakil ilişkili toksisite, Kök hücre nakli, Moleküler biyoloji, CAR-T hücre tedavisi, CAR-T hücre genişlemesi, Dijital damlacık PCR'ı, Diffüz büyük B-hücreli lenfoma, İmmün efektör hücre ilişkili nörotoksisite sendromu

Address for Correspondence/Yazışma Adresi: Thomas Pabst, M.D., University Hospital and University of Bern, Department of Medical Oncology, Bern, Switzerland
E-mail : thomas.pabst@insel.ch ORCID: orcid.org/0000-0002-6055-5257

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is curable with standard intensive immunochemotherapy in roughly two-thirds of all cases, but a significant proportion of cases are refractory or relapse after first- and second-line therapies [1,2,3]. Accumulating data suggest that chimeric antigen receptor T-cell (CAR-T cell) therapy targeting CD19 can cure a significant proportion of relapsing DLBCL patients [4,5,6,7,8,9,10]. Tisagenlecleucel (Kymriah®) and axicabtagene ciloleucel (Yescarta®) are approved for adults with relapsed or refractory DLBCL [11]. The general view is that in vivo expansion of CAR-T cells within the patient is crucial for CAR-T efficacy, and CAR-T cells can be detected in the peripheral blood and other organs [5,8,11,12,13]. However, the importance of CAR-T cell expansion for tumor regression and outcome remains controversial [5,6,7,13]. Specific complications after CAR-T cell infusion are cytokine-release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) [4,14,15,16]. There is still an unmet clinical need for laboratory-based markers to predict such toxicities and outcomes in a timely manner. Among these potential markers, the characterization of CAR-T cell expansion may contribute to improved monitoring of patients following CAR-T cell infusion [13].

Materials and Methods

We analyzed 14 sequential patients with relapsed or refractory DLBCL who underwent CAR-T cell therapy at Bern University Hospital. All patients provided written informed consent. The inclusion criteria were the availability of CAR-T cell assessment results during follow-up for CAR-T cell mRNA expression (CAR-T/*ABL1*) and for CAR-T cell DNA concentration (copies/µg DNA). Seven patients received tisagenlecleucel and seven patients received axicabtagene ciloleucel, while assessments were conducted regardless of the CAR-T cell product.

Quantification of transgene copy numbers was performed via droplet digital polymerase chain reaction (ddPCR) assay to quantify sequences of the intracellular domain of the CAR construct as described previously [11]. Results were given as CAR-T cell copy number per micrograms of DNA ("CAR-T cell concentration") in the peripheral blood. For the analysis of CAR-T cell mRNA expression in the peripheral blood, mRNA was extracted using the QIAamp RNA Blood Mini Kit (QIAGEN, Hombrechtikon, Switzerland) followed by reverse transcription using the Superscript System (Invitrogen, Carlsbad, CA, USA). Samples were processed as previously reported [11] and a CAR-T/*ABL1* expression ratio ("CAR-T cell expression") was calculated. Patients were categorized according to peak CAR-T cell expression during follow-up. Patients with CAR-T/*ABL1* ratios equal to or greater than 1.0 were considered as the high CAR-T cell peak expression group whereas patients with CAR-T/*ABL1* ratios below 1.0 constituted the low CAR-T cell peak expression group.

In addition, patients were grouped according to their CAR-T/*ABL1* expression ratios at three months following CAR-T cell infusion. Patients with CAR-T/*ABL1* ratios equal to or greater than 0.01 formed the high CAR-T cell persistence group and patients with CAR-T/*ABL1* ratios of less than 0.01 formed the low/no CAR-T cell persistence group.

The time points and frequencies of the measurements of CAR-T cells, C-reactive protein (CRP), and interleukin-6 (IL-6) were determined by the attending doctor of each individual patient according to clinical need, but as a general rule these measurements should be performed at least twice weekly. The median time to first CAR-T cell mRNA expression measurement after infusion was 3 days (mean: 4.9 days). Once CAR-T cell peak expression was reached, further measurements followed at 1, 3, and 6 months after infusion. CRS and ICANS were graded according to American Society for Transplantation and Cellular Therapy consensus scores [11,17,18].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.1.0 (GraphPad Software, San Diego, CA, USA), utilizing the Mann-Whitney U test for quantitative variables and the chi-square test for qualitative variables. Overall survival (OS) and progression-free survival (PFS) were analyzed using the Kaplan-Meier estimator and log-rank (Mantel-Cox) test.

Results

No significant differences were identified in patient characteristics regarding the groups with high versus low CAR-T cell peak expression (Supplementary Table S1). The best response after CAR-T cell therapy was complete remission (CR) in five patients (36%) (Supplementary Table S2). Five patients (36%) died during the observation period, all due to disease progression. Median OS and PFS were not yet reached after a median follow-up period of 26 weeks (Supplementary Figure S1).

We observed that the peak CAR-T cell mRNA expression of 1.012 CAR-T/*ABL1* (range: 0.0089-21.5411) was reached after a median of 9 days (range: 5-32) following CAR-T cell infusion (Supplementary Table S2). Subsequently, the median CAR-T cell expression decreased from a median of 0.1360 CAR-T/*ABL1* (range: 0.0009-1.4374) after 1 month to 0.0157 CAR-T/*ABL1* (range: 0-0.3176) after 3 months and 0.0088 CAR-T/*ABL1* (range: 0-0.0168) after 6 months.

The CAR-T cell DNA concentration, assessed in parallel, also peaked after a median of 9 days (range: 5-21) at a median level of 13,788 copies/µg DNA (range: 54-118,382). To compare these

two different measures of CAR-T cell mRNA expression and CAR-T cell DNA concentration, a linear regression analysis of corresponding peak values (log 10) was performed, resulting in a significant correlation (Figure 1A).

In the group with high CAR-T cell peak expression, the peak CAR-T/*ABL1* expression ratio showed a median of 1.749 (range: 1.0263-21.5411) as compared to 0.1638 CAR-T/*ABL1* (range: 0.0089-0.5403) in the low CAR-T cell peak expression group (p=0.0007; Table 1). Upon comparing the groups, there was no difference in the interval to peak expression (p≥0.9999). After 1 month, the high CAR-T cell peak expression group continued to have a significantly higher median CAR-T cell expression level (p=0.0293). The median CAR-T cell expression did not differ between the groups after 3 months (p=0.3268). The CAR-T/*ABL1* expression course for each individual following CAR-T cell therapy is depicted in Figure 1B.

No difference was observed between the groups regarding best response (p=0.3688; Table 1). This was also observed for the responses after 3 months (p=0.8721) and 6 months (p=0.9433).

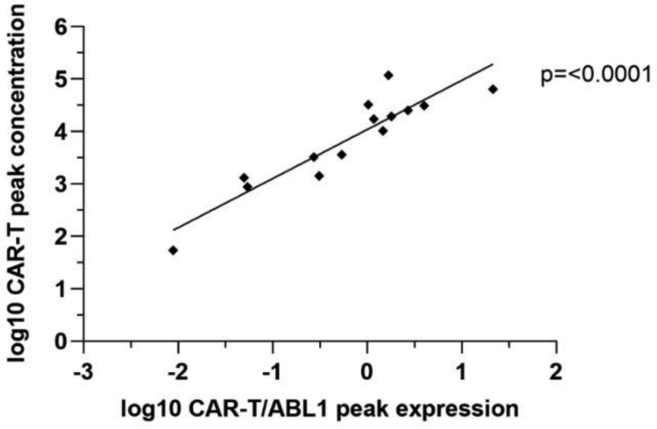

Figure 1A. Correlation of CAR-T/*ABL1* peak expression (based on mRNA measurements) and CAR-T cell peak concentration (based on DNA measurements), p≤0.0001.

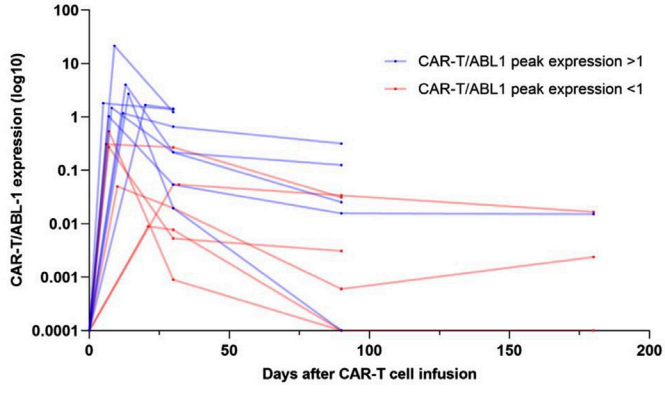

Figure 1B. CAR-T cell expression (based on mRNA measurements) following CAR-T cell infusion for each individual of the high (blue) and low (red) CAR-T cell expression (based on mRNA measurements) groups.

Similarly, OS and PFS did not differ between the groups (Supplementary Figure S2).

Expression levels 3 months after CAR-T cell infusion were available for 11 patients (two patients had died before this assessment and for one patient data were not available for technical reasons). There was no difference between the CAR-T cell persistence groups described above in terms of the achievement of best response or responses after 3 or 6 months (Supplementary Table S3). Similarly, OS and PFS did not differ between the groups (Supplementary Figure S2). The group with high CAR-T cell persistence tended to have more patients treated with tisagenlecleucel than axicabtagene ciloleucel (p=0.1030; Supplementary Table S3).

During hospitalization, several adverse events were observed (Supplementary Table S2). CRS occurred in 12/14 cases (86%). In two cases (14%), symptoms highly compatible with CRS occurred within 24 hours and were classified as such by the attending physician with the overall conditions of the patients being taken into consideration. ICANS was seen in 7/14 cases (50%). ICANS tended to occur later than CRS (p=0.0692; Supplementary Figure S3). Eleven patients (79%) were treated with tocilizumab during hospitalization and four patients (29%) needed admission to the ICU. The high CAR-T cell peak expression group tended to have more CRS (any grade, p=0.0778; Table 1). This group also had a higher rate of ICANS (p=0.0308), a higher median peak CRP level during hospitalization (63 mg/L versus 25 mg/L, p=0.0115), a higher rate of patients admitted to the ICU (four patients (50%) versus no patients (0%), p=0.0404; Table 1), and a longer median duration of hospitalization (34 days versus 19 days, p=0.0077). Furthermore, there were no significant differences in the occurrence of CRS (any grade, p=0.3180) or ICANS (any grade, p=0.1907) when the data were stratified for the two different CAR-T cell products.

Discussion

At present, no parameter has been identified for the reliable prediction of patients preferentially responding to CAR-T cell therapy or developing specific side effects [4,10,17,18,19,20,21,22,23]. It has been hypothesized that CAR-T cell expansion in the peripheral blood of CAR-T cell recipients may be indicative of the response to CAR-T cell therapy and may also correlate with the immunological side effects of this therapeutic approach [4,5,6,7,8,9,11,13,15,24,25].

Previous studies determined the presence of CAR-T cells in blood samples by qPCR based on DNA or by flow cytometry [5,8,13]. In the present study, we focused on CAR-T cell expression as measured by ddPCR based on mRNA assessments. We observed that CAR-T cell peak expression (based on mRNA measurements) and CAR-T cell peak concentration (based on DNA assessments)

Table 1. Descriptions of CAR-T cell expressions (based on mRNA measurements) and concentrations (based on DNA measurements), adverse events, laboratory parameters, response rates, and clinical courses following CAR-T cell infusion for subgroups stratified by peak CAR-T cell expression.

	Peak expression ratio of CAR-T/*ABL1* ≥1 (n=8)	Peak expression ratio of CAR-T/*ABL1* <1 (n=6)	p
Median CAR-T cell peak expression, CAR-T/*ABL1* (range)	1.749 (1.0263-21.5411)	0.1638 (0.0089-0.5403)	0.0007
Median duration to CAR-T cell peak expression, days (range)	10 (5-20)	8 (6-32)	>0.9999
Median CAR-T cell expression following CAR-T cell infusion, CAR-T/*ABL1* (range)			
1 month (n=14)	0.4383 (0.0196-1.4374)	0.0137 (0.0009-0.2714)	0.0293
3 months (n=11)	0.0254 (0-0.3176)	0.0031 (0-0.0339)	0.3268
Median CAR-T cell peak concentration, copies/µg DNA (range)	28,213 (10,327-118,382)	1,370 (54-3,602)	0.0007
Median duration to CAR-T cell peak concentration, days (range)	10 (5-20)	7 (6-21)	0.4682
Median CAR-T cell concentration following CAR-T cell infusion, copies/µg DNA (range)			
1 month (n=14)	3,766 (230-93,100)	82 (0-467)	0.0047
3 months (n=11)	164 (0-1,512)	38 (0-231)	0.3268
Occurrence of CRS, n (%)			0.0778
CRS (any grade)	8 (100)	4 (67)	
Grade 1	5 (63)	0 (0)	
Grade 2	1 (12)	4 (67)	
Grade 3	2 (25)	0 (0)	
Grade 4	0 (0)	0 (0)	
No CRS	0 (0)	2 (33)	
Occurrence of ICANS, n (%)			0.0308
ICANS (any grade)	6 (75)	1 (17)	
Grade 1	2 (25)	1 (17)	
Grade 2	1 (12)	0 (0)	
Grade 3	3 (38)	0 (0)	
Grade 4	0 (0)	0 (0)	
No ICANS	2 (25)	5 (83)	
Median duration of hospitalization, days (range)	34 (21-51)	19 (18-29)	0.0077
Median peak CRP level during hospitalization, mg/L (range)	63 (23-202)	25 (3-98)	0.0115
Median peak IL-6 level during hospitalization, pg/mL (range)	3,557 (36-42,209)	152 (12-5,060)	0.0813
Patients treated with tocilizumab, n (%)	7 (88)	4 (67)	0.3472
Patients treated in intensive care, n (%)	4 (50)	0 (0)	0.0404
Best response after CAR-T cell therapy, n (%)			0.3688
CR or PR	7 (88)	6 (100)	
RD	1 (12)	0 (0)	
Response 3 months after CAR-T cell therapy, n (%)			0.8721
CR or PR	5 (63)	4 (67)	
PD or DE	3 (37)	2 (33)	
Response 6 months after CAR-T cell therapy, n (%)			0.9433
CR or PR	3 (38)	2 (33)	
PD or DE	3 (38)	2 (33)	
NE	2 (24)	2 (33)	
Relapse (RD or PD) after CAR-T cell therapy, n (%)	3 (39)	2 (33)	0.8721
Mortality after CAR-T cell therapy, n (%)	3 (39)	2 (33)	0.8721

ICANS: Immune effector cell-associated neurotoxicity syndrome; CRS: cytokine-release syndrome: CRP: C-reactive protein; IL-6: interleukin-6; CR: complete remission; PR: partial remission; SD: stable disease; RD: refractory disease; PD: progressive disease; DE: death due to disease progression; NE: not evaluated (6 months of follow-up not yet reached).

correlated significantly. Given that the CAR-T cell peak expression groups (high versus low) did not differ in terms of CAR-T cell persistence at 3 months, one may suggest that a high CAR-T cell peak alone does not guarantee higher persistence.

Although our cohort is limited in size and follow-up duration, the findings seem to be comparable to those obtained for larger series of CAR-T cell recipients [4,5,6,7,8,14,15,24,25,26]. However, it should be noted that we were not able to investigate CD19 expressions in the lymphoma tissues at the time of relapse. Response and survival rates did not differ between the high and low CAR-T cell peak expression groups as defined above. Awasthi et al. [13] investigated the cellular kinetics of tisagenlecleucel following CAR-T cell therapy by quantitative PCR (qPCR) and flow cytometry in the peripheral blood and bone marrow samples of DLBCL patients and reported similar findings. In some previous studies, neither maximum expansion nor the exposure as area under the curve within the first 28 days (AUC0-28) after CAR-T cell infusion correlated with response at 3 months after CAR-T infusion [6,13]. In contrast, Neelapu et al. [5] and Locke et al. [7] found that higher axicabtagene ciloleucel CAR-T cell levels (peak concentration of CAR-T cells in the peripheral blood or AUC0-28, based on qPCR measurements) were associated with stronger efficacy and better objective response [5,6,7,8,13].

It should be emphasized that ICANS of any grade was more common in the group with high CAR-T cell peak expression compared to the low CAR-T cell peak expression group. This most likely explains the longer duration of hospitalization and higher rate of ICU admission in the former group. Furthermore, the group with high CAR-T cell peak expression had a higher median peak CRP level and a higher median peak IL-6 level, although the latter finding did not reach statistical significance. This may suggest a contribution of CAR-T cell peak expansion to inflammation and endothelial activation [16]. Comparable to our results, Neelapu et al. [5] reported axicabtagene ciloleucel CAR-T cell levels to be associated with neurological events but not with CRS. In contrast, Schuster et al. [6] and Awasthi et al. [13] found tisagenlecleucel CAR-T cell expansion to be associated with CRS and tocilizumab use but not with neurological events. A correlation of CRS with enhanced in vivo cell expansion and higher tumor burden was suggested by a number of previous reports [4,5,13]. The lack of data on tumor burden is a limitation of the present study [4,5,13]. However, lactate dehydrogenase levels at the time of CAR-T cell therapy did not differ between the groups as separated by peak CAR-T expression. It remains to be clarified to what extent the pathophysiologies of CRS and neurological events are intertwined, but a complex pathophysiology leading to adverse events can be assumed [5,16,20].

Conclusion

Our data suggest that CAR-T cell expression based on mRNA and CAR-T cell concentration based on DNA are strongly correlated. CAR-T cell mRNA expression may be used as a surrogate measurement method with possibly better feasibility in routine laboratory practice and better comparability to other parameters as mRNA measurements become a more widespread method in lymphoma research. Difficulties in correlating CAR-T cell expansion in the peripheral blood with the response to CAR-T cell therapy and survival seem not to be due to differences between CAR-T cell mRNA expression and DNA concentration in the peripheral blood. Rather, these difficulties may be due to the fact that the lymph nodes (or other organs) may be more relevant compartments for the response to CAR-T cell therapy. Interestingly, a high level of CAR-T cell peak expression in the peripheral blood is associated with more adverse events, specifically a higher risk for neurotoxicity, and longer hospitalization. Finally, prospective studies will be needed to clarify whether the measurement of peak CAR-T cell mRNA expression or DNA concentration in the peripheral blood would be helpful in identifying candidates at increased risk of ICANS or higher grades of CRS who could eventually benefit from preemptive immunosuppressive therapy or more intensive monitoring in the days and weeks following CAR-T cell infusion.

Ethics

Ethics Committee Approval: This study was approved (#BE-2020-00777) by the local ethics committee.

Informed Consent: All patients gave written consent.

Authorship Contributions

Surgical and Medical Practices- B.J., U.N., S.Z., T.P.; Concept- U.B., T.P.; Design- T.P.; Data Collection or Processing- C.M.; Analysis or Interpretation- C.M., G.W., N.P., M.N., K.S.; Literature Search- C.M., U.B., T.P.; Writing- C.M., U.B., T.P.

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Supplementary Table S1. Characteristics of previous therapies, disease, and CAR-T cell treatment of all 14 patients undergoing CAR-T cell therapy and of the subgroups stratified by their peak CAR-T cell expression (based on mRNA measurements).

	All 14 patients	Peak expression CAR-T/*ABL1* ≥1 (n=8)	Peak expression CAR-T/*ABL1* <1 (n=6)	p
Median age at diagnosis, years (range)	61 (38-73)	61 (38-73)	62 (45-70)	0.9754
Gender, female/male (ratio)	7/7 (1)	5/3 (1.7)	2/5 (0.5)	0.2801
Initial diagnosis, n (%)				0.3523
DLBCL	7 (50)	5 (62)	2 (33)	
Secondary DLBCL, transformed from				
FL	5 (36)	2 (25)	3 (50)	
CLL	1 (7)	1 (12)	0 (0)	
MZL	1 (7)	0 (0)	1 (17)	
Initial lymphoma stage, n (%)				0.3340
I-II	5 (36)	2 (25)	3 (50)	
II-IV	9 (64)	6 (75)	3 (50)	
CNS manifestation of the lymphoma, n (%)	1 (7)	1 (12)	0 (0)	0.3688
Number of treatment lines before CAR-T cell therapy, n (%)				0.7327
1-2	10 (71)	6 (75)	4 (67)	
≥3	4 (29)	2 (25)	2 (33)	
Previous ASCT, n (%)	8 (57)	5 (62)	3 (50)	0.6400
Previous radiotherapy, n (%)	4 (29)	1 (12)	3 (50)	0.1243
Bridging chemotherapy between lymphapheresis and CAR-T cell infusion, n (%)	6 (43)	3 (38)	3 (50)	0.6400
Bridging radiotherapy between lymphapheresis and CAR-T cell infusion, n (%)	1 (7)	1 (12)	0 (0)	0.3688
CAR-T cell product, n (%)				0.6456
Tisagenlecleucel	7 (50)	4 (50)	3 (50)	
Axicabtagene ciloleucel	7 (50)	4 (50)	3 (50)	
Median age at CAR-T cell therapy, years (range)	67 (39-77)	65 (39-76)	68 (52-77)	0.6620
Median LDH level before lymphodepleting chemotherapy, U/L (range)	528 (248-2407)	641 (248-2407)	425 (330-728)	0.7546
Remission status at the time of CAR-T cell therapy, n (%)				0.1190
PR or SD	6 (43)	2 (25)	4 (67)	
PD	8 (57)	6 (75)	2 (33)	
Median follow-up, weeks (range)	26 (4-45)	26 (4-45)	25 (20-45)	0.4136

DLCBL: Diffuse large B-cell lymphoma; FL: follicular lymphoma; CLL: chronic lymphocytic leukemia; MZL: marginal zone lymphoma; ASCT: autologous stem cell transplantation; CNS: central nervous system; LDH: lactate dehydrogenase; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease.

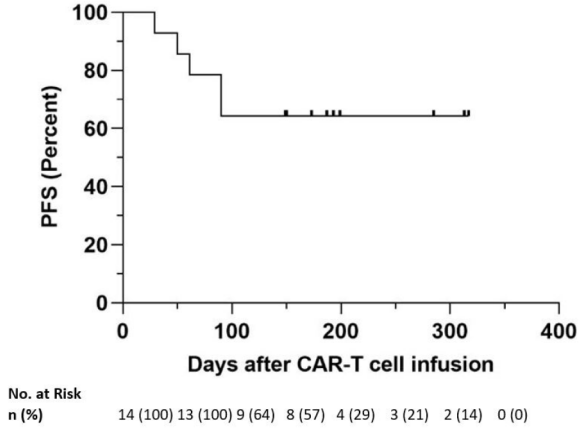
Supplementary Table S2. Description of the CAR-T cell expressions (based on mRNA measurements) and concentrations (based on DNA measurements), adverse events, laboratory parameters, response rates, and clinical courses following CAR-T cell infusion of all 14 patients.	
Median duration of hospitalization, days (range)	24 (18-51)
Median CAR-T cell peak expression, CAR-T/*ABL1* (range)	1.012 (0.0089-21.5411)
Median duration to CAR-T cell peak expression, days (range)	9 (5-32)
Median CAR-T cell expression following CAR-T cell infusion, CAR-T/*ABL1* (range)	
1 month (n=14)	0.1360 (0.0009-1.4374)
3 months (n=11)	0.0157 (0-0.3176)
6 months (n=4)	0.0088 (0-0.0168)
Median CAR-T cell peak concentration, copies/µg DNA (range)	13,788 (54-118,382)
Median duration to CAR-T cell peak concentration, days (range)	9 (5-21)
Median CAR-T cell concentration following CAR-T cell infusion, copies/µg DNA (range)	
1 month (n=14)	466 (0-93,100)
3 months (n=11)	70 (0-1,519)
6 months (n=6)	89.5 (0-585)
Occurrence of cytokine-release syndrome, n (%)	
Grade 1	5 (36)
Grade 2	5 (36)
Grade 3	2 (14)
Grade 4	0 (0)
Median duration to cytokine-release syndrome, days (range)	3 (0-12)
Occurrence of ICANS, n (%)	
Grade 1	3 (21)
Grade 2	1 (7)
Grade 3	3 (21)
Grade 4	0 (0)
Median duration to ICANS, days (range)	7 (3-15)
Median peak CRP level during hospitalization, mg/L (range)	42 (3-202)
Median duration to peak CRP, days (range)	4 (1-13)
Median peak IL-6 level during hospitalization, pg/mL (range)	2,880 (7-157,117)
Median duration to peak IL-6, days (range)	5 (1-13)
Patients treated with tocilizumab, n (%)	11 (79)
Patients treated in the intensive care unit, n (%)	4 (29)
Best response after CAR-T cell therapy, n (%)	
CR	5 (36)
PR	8 (57)
RD	1 (7)
Response 3 months after CAR-T cell therapy, n (%)	
CR or PR	9 (64)
PD or DE	5 (36)
Response 6 months after CAR-T cell therapy, n (%)	
CR or PR	5 (36)
PD or DE	5 (36)
NE	4 (28)
Relapse (RD or PD) after CAR-T cell therapy, n (%)	5 (36)
Median duration to relapse, days (range)	61 (29-90)
Mortality (all due to disease progression) after CAR-T cell therapy, n (%)	5 (36)
Median duration to death, days (range)	127 (29-201)

ICANS: Immune effector cell-associated neurotoxicity syndrome; CRP: C-reactive protein; IL-6: interleukin-6; CR: complete remission; PR: partial remission; SD: stable disease; RD: refractory disease; PD: progressive disease; DE: death due to disease progression; NE: not evaluated (6 months of follow-up not yet reached).

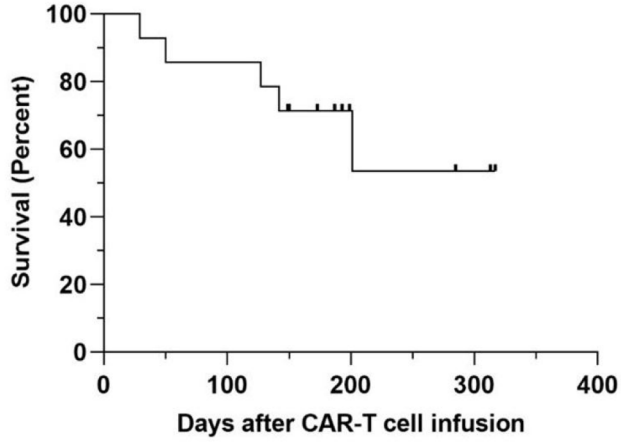
Supplementary Table S3. Description of response rates, follow-up, and CAR-T cell products of 11 patients stratified by CAR-T cell expression (based on mRNA measurements) 3 months after CAR-T cell infusion.

	CAR-T/*ABL1* persistence after 3 months ≥0.01 (n=6)	CAR-T/*ABL1* persistence after 3 months <0.01 (n=5)	p
Best response after CAR-T cell therapy, n (%)			>0.9999
CR or PR	6 (100)	5 (100)	
RD	0 (0)	0 (0)	
Response 3 months after CAR-T cell therapy, n (%)			0.3869
CR or PR	5 (83)	3 (60)	
PD or DE	1 (17)	2 (40)	
Response 6 months after CAR-T cell therapy, n (%)			0.3019
CR or PR	4 (66)	1 (20)	
PD or DE	1 (17)	2 (40)	
NE	1 (17)	2 (40)	
Median follow-up, weeks (range)	28 (18-45)	27 (20-41)	0.7922
CAR-T cell product, n (%)			0.1030
Tisagenlecleucel	4 (67)	1 (20)	
Axicabtagene ciloleucel	2 (25)	4 (80)	

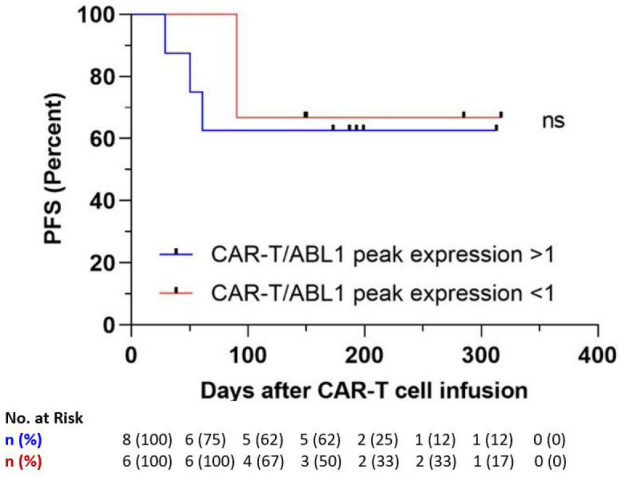
CR: Complete remission; PR: partial remission; SD: stable disease; RD: refractory disease; PD: progressive disease; DE: death due to disease progression; NE: not evaluated (6 months of follow-up not yet reached).



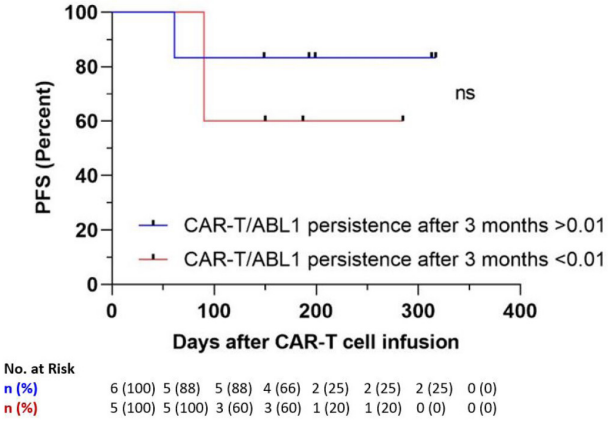
Supplementary Figure S1A. Progression-free survival (PFS) of all patients undergoing CAR-T cell therapy (median PFS not yet reached).



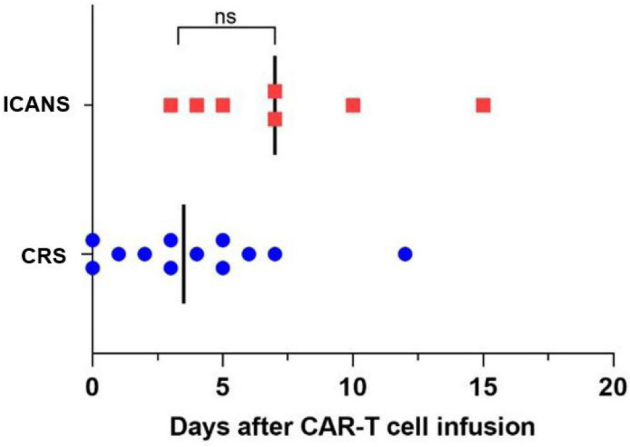
Supplementary Figure S1B. Overall survival (OS) of all 14 patients undergoing CAR-T cell therapy (median OS not yet reached).



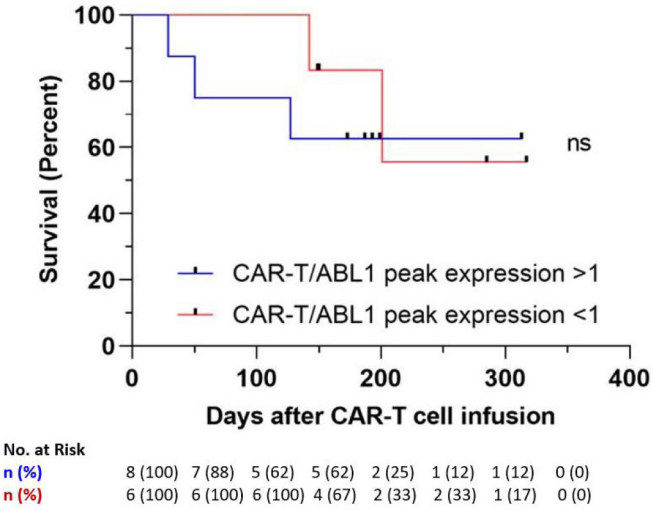
Supplementary Figure S2A. Progression-free survival (PFS) stratified for the high (blue) and low (red) CAR-T cell peak expression groups (based on mRNA measurements), p=0.6592.



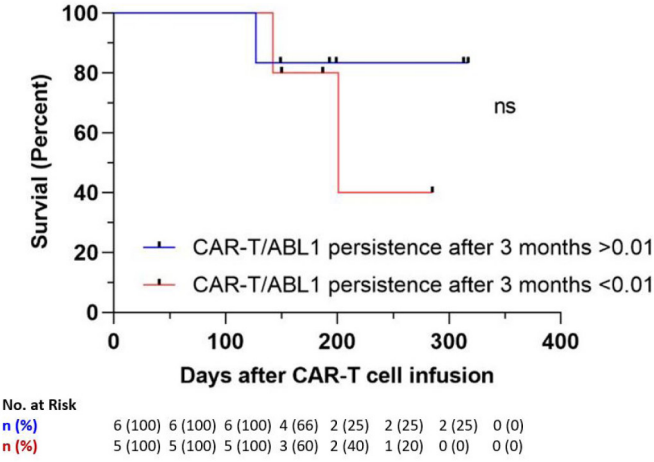
Supplementary Figure S2C. Progression-free survival (PFS) stratified for the high (blue) and low/no (red) CAR-T cell persistence groups (based on mRNA measurements), p=0.512.



Supplementary Figure S3. Comparison of the interval to cytokine-release syndrome (CRS) and the interval to immune effector cell-associated neurotoxicity syndrome (ICANS) after CAR-T cell infusion. ICANS (red squares, n=7) tended to occur later than CRS (blue circles, n=12), p=0.0692.



Supplementary Figure S2B. Overall survival (OS) stratified for the high (blue) and low (red) CAR-T cell peak expression groups (based on mRNA measurements), p=0.5278.



Supplementary Figure S2D. Overall survival (OS) stratified for the high (blue) and low/no (red) CAR-T cell persistence groups (based on mRNA measurements), p=0.5282.