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Eleven cases of laryngeal edema after tisagenlecleucel infusion: a 3-year single center retrospective study of CD19-directed chimeric antigen receptor T-cell therapy for relapsed and refractory B-cell lymphomas

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Authorship contributions

E.H. analyzed data and wrote the manuscript. J.A. planned the study, supplied clinical data, and provided scientific discussions. S.K. performed flow cytometric analysis and

analyzed data. Y.F. helped with writing the manuscript. Y.T. performed qPCR. Y.A. helped with writing the manuscript. T.M., E.S., H.T., Y.T., N.W., T.T., H.Y., Y.H., and M.S. provided clinical information and scientific discussions. S.N. helped with statistical analysis. M.I. performed qPCR and analyzed data. M.A. planned and directed the study, analyzed data, and wrote the manuscript.

Declaration of interests

J.A. received a research grant and honoraria from AbbVie. T.T. received research funding from Bristol Myers Squibb and Sysmex. M.A. received research funding from Century Therapeutics, received research grants from Sumitomo Pharma, Chugai Pharmaceutical, Kyowa Kirin, and received honoraria from Novartis and AbbVie. All the other authors have no conflicts of interest to disclose.

Data-sharing statement

Clinical data are available upon request from the corresponding author.

Letter to the editor

CD19-directed chimeric antigen receptor T (CAR-T) therapy showed promising results in refractory and relapsed (R/R) B-cell lymphomas, leading to re-working of the therapeutic landscape[1-5]. We conducted a single-center retrospective analysis of 59 patients with R/R B-cell lymphoma enrolled for CAR-T therapy with tisagenlecleucel (tisa-cel) between September 2020 and September 2023. The study was approved by the Ethics Committee of Juntendo University School of Medicine (E21-0057) in accordance with the Declaration of Helsinki. Figure 1A is a schema of our study design. Supplementary Table 1A shows the patient characteristics of 53 diffuse large B-cell lymphoma (DLBCL) and 6 follicular lymphoma (FL) patients enrolled for tisa-cel therapy. Forty-one study patients (38 DLBCL, 3 FL) received an infusion of tisa-cel. Supplementary Table 1B shows patient status before infusion. Response assessments were completed in 37 DLBCL and 1 FL (Figure 1B). The overall response rate (ORR) in 37 DLBCL was 65% (Figure 1C). The first patient who was infused with tisa-cel at our institution has sustained remission for over 36.6 months. Survival estimates for DLBCL patients are shown in Figure 1D. Overall survival (OS) and progression-free survival (PFS) rates at 12 months were 73.8% and 49.6%, respectively. Median OS was not reached and median PFS was 10.6 months (Figure 1D). We also conducted subgroup

analyses. Patients with bulky disease showed significantly poorer ORR than those without bulky disease (p=0.0007) (Supplementary Figure 1A). Increased LDH level at lymphodepletion and presence of bulky disease presaged significantly shorter OS and PFS (Supplementary Figure 1B).

Safety outcomes were assessed in all 41 infused patients (Supplementary Table 1C). Cytokine release syndrome (CRS) was observed in 30 patients (73%), with grade ≥3 in 14 (34%). No treatment-related mortality occurred. Of the 30 patients with CRS, patients with grade ≥3 CRS had onset of fever significantly earlier than patients with grade 1-2 CRS (median 31.5 hours *vs* 53.5 hours, p=0.0182) (Figure 1E). The patients with grade ≥3 CRS were infused with significantly higher numbers of CAR-positive cells (p=0.0034) (Figure 1F).

Of importance is that in this study we encountered distinct adverse events not commonly reported: 11 patients (27%) developed laryngeal edema (Figure 2A). Supplementary Table 1D shows the details that characterized patients with laryngeal edema and our management routes. Edema appeared regardless of the presence of neck tumour (5 patients with *vs*. 6 patients without neck tumour). The mean duration from infusion to onset of laryngeal edema was 3.4 days. All 11 patients were admitted to our intensive care unit; 9 (82%) required emergency airway management due to the

risk of airway obstruction. Interestingly, 1 patient with laryngeal edema did not present with any systemic symptoms (grade 0 CRS). Laryngeal edema improved within 14 days after tisa-cel infusion in all cases.

Although a few case reports of laryngeal edema with tisa-cel (but not with axi-cel and liso-cel) for B-cell lymphoma have appeared[6], its mechanism is still unknown and no established management guidelines exist in spite of the major risk of life-threatening airway obstruction[6, 7]. Therefore, we assessed risk factors for the occurrence of laryngeal edema and the influence of steroid treatment on both CAR-T cell expansion and clinical outcome. Furthermore, to the best of our knowledge, there has been no report of laryngeal edema with tisa-cel outside Japan[6, 8]. The reason for this is unclear, but it may be attributed to ethnic differences in T-cell biology. One such example is T-cell chronic active Epstein-Barr virus disease (CAEBV), which is more common in Asia than in the United States[9]. This regional predisposition to CAEBV suggested that genetic polymorphisms concerning the EBV immune response and impaired EBV-specific cytotoxic T-cell activity are responsible for development of CAEBV[9, 10].

Patients with laryngeal edema were infused with significantly higher numbers of CAR-positive cells than were those without edema (median 4.0×10^8 vs 3.3×10^8 cells, p=0.0411) (Figure 2B). A CAR-positive cell number of 3.4×10^8 was identified as the

cut-off point for prediction of laryngeal edema. The frequency of laryngeal edema was significantly higher in patients infused with ≥3.4×10^8 CAR-positive cells than in patients infused with <3.4×10^8 CAR-positive cells (p=0.0385) (Figure 2C).

We also analyzed the memory T-cell phenotype of available infused products (n=20). CAR-T cells of patients with laryngeal edema (n=5) showed a significantly higher proportion of effector memory T-cells than did those of patients without edema (n=15) (p=0.0107) (Figure 2D). OS in DLBCL patients with laryngeal edema was 100% at a maximum period of 12 months, while OS without laryngeal edema was 67.1% (p=0.1415) (Figure 2E). Other factors such as infusion reaction, CRS grade, and the use of steroids were not significantly correlated with ORR (Supplementary Figure 1C).

Our impression clinically was that the frequency of laryngeal edema after tisa-cel infusion rose in the summer of 2022. We thus attempted to coordinate date of tisa-cel manufacture with occurrence of laryngeal edema. Patients infused with tisa-cel manufactured after June 2022 had a high frequency of grade ≥3 CRS occurrence, whereas no event of grade ≥3 CRS was seen in patients infused with products manufactured before May 2022 (Figure 2F). We then compared product characteristics before and after June 2022. Interestingly, CAR-positive cell numbers after June 2022 were significantly higher than in products manufactured before June 2022 (p<0.0001)

(Figure 2G). ORR also improved from 50% (before June 2022) to 76% (after June 2022) (Supplementary Figure 1D). Several possible reasons for this change have been considered. We previously reported that $CD4^+$T-cell numbers were decreased in patients treated with bendamustine-including regimens [11]. Therefore, we decided to avoid using bendamustine before apheresis, a decision perhaps confirmed as correct by our high manufacturing success rate of 100% since June 2022 compared to 78% before June 2022. Another possible factor may be changes in manufacturing company protocols, although we have no access to such information.

To investigate *in vivo* persistence of CAR-T cells after treatment, CAR copy number was monitored long-term by qPCR to detect peripheral blood CAR-T cells[12-14] (Figure 3A). For a maximum period of 36.6 months of follow-up, the CAR transgene continued detectable in patients with complete response (CR) or partial response (PR). The mean peripheral blood CAR copy number at 14 days, 1 month, and 3 months after tisa-cel infusion tended to decrease further in patients with stable disease (SD) or progressive disease (PD) than in patients with CR or PR, although the difference did not reach statistical significance (Figure 3B and Supplementary Figure 2A). C_{max} and $AUC_{0\text{-}28d}$ of CAR copy number in the peripheral blood also did not significantly differ between CR/PR and SD/PD patients, nor in patients with or without durable remission

(Supplementary Figure 2B and 2C). We also investigated the relationships between C_{max} and $AUC_{0\text{-}28d}$ of CAR copy number and CRS grades. As expected, higher C_{max} and $AUC_{0\text{-}28d}$ of CAR copy number significantly correlated with higher frequency of grade ≥3 CRS (Supplementary Figure 2D).

Steroid treatment was used predominantly in patients with laryngeal edema. To investigate the impact of steroid treatment on CAR copy number in peripheral blood, we compared peripheral blood CAR copy numbers immediately before and one day after steroid treatment in patients with available samples (n=9). The ratio of CAR copy number ranged from 0.57 to 4.00 (Figure 3C). No clear decrease after administration of dexamethasone was observed (0.96 - 4.00). However, methylprednisolone administration significantly decreased the ratio of CAR copy number (0.57 - 1.41) compared to that of dexamethasone administration (p=0.0417) (Figure 3D).

Peripheral blood CAR-T cells were analyzed by flow cytometry for a period of over 12 months in our patients (Figure 3E). Expression of exhaustion markers (LAG3, PD-1, TIGIT, TIM-3)[15] on CAR-T cells clearly differed between patients with CR and PD. High expression of exhaustion markers was observed at 1 month after tisa-cel infusion and continued for over 12 months in a patient with PD. In contrast, these markers showed relatively low expression in a patient with CR (Figure 3F). In PD patients, although

opportunity for long-term monitoring is rare (they need to start salvage therapy as soon as possible), we additionally analyzed the expression of exhaustion markers on CAR-T cells (2 CR patients and 2 PD patients) at 1 month and 3 months after tisa-cel infusion. Similarly, the expression of PD-1 and TIGIT was higher in PD than in CR patients (Supplementary Figure 2E). From these results, despite the limited size of our PD patient sample, we conclude that expression of exhaustion markers (PD-1 and TIGIT) on CAR-T cells in peripheral blood of PD patients tended to be higher than on those of CR patients.

In conclusion, our data on tisa-cel treatment for R/R B-cell lymphoma show high efficacy with tolerable toxicity and provide valuable insights into risk factors and management drawn from multiple patients with laryngeal edema. More importantly, we have identified that high CAR-positive cell number ($\geq 3.4\times10^8$ CAR-positive cells) and high proportion of effector memory T-cells within infused tisa-cel products were risk factors for laryngeal edema. Therefore, utmost caution is required when these risk factors are present in CAR-T products. As laryngeal edema occurred regardless of the presence of neck tumours and systemic CRS, close monitoring during treatment is required even if neck lymphoma lesions are lacking. Since steroid administration did not impair ORR without effect on CAR copy number, treatment with dexamethasone

should immediately be initiated when signs of edema are found on laryngoscopic examinations. A significant increase in CAR-positive cell numbers was observed after June 2022 in tisa-cel products, associated with higher frequency of grade ≥3 CRS alongside improved ORR. We believe that our analyses encompass both efficacy and safety with tisa-cel therapy in real-world settings.

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Figure Legends

Figure 1. Patient characteristics, overall response rates, overall survival, and safety outcome

(A) Flow chart and schematic illustration of patients enlisted for chimeric antigen receptor T (CAR-T) therapy. 53 with diffuse large B-cell lymphoma (DLBCL) and 6 with follicular lymphoma (FL) enrolled in tisa-cel therapy at Juntendo University Hospital between September 2020 and September 2023 were recruited. Data from medical

records were reviewed and collected up to December 31, 2023. Information on numbers of CAR-positive T-cells was collected from final manufacturing product reports. The empty bag after infusion was washed with phosphate-buffered saline to obtain residual cells. Peripheral blood samples from patients were also collected at approximate points after infusion (days 7 and 14, 1 month, and every 3 to 6 months. (B) Swimmer plot with day 0 representing the day of tisa-cel infusion. Response assessments were completed in 37 DLBCL patients and 1 FL patient (1 DLBCL patient and 2 FL patients were treated before assessment) (C) Overall response rates (ORR) of patients infused with tisa-cel. Patients with response are in pink (complete response, CR; partial response, PR), and patients with no response are in blue (stable disease, SD; progressive disease, PD). (D) Kaplan-Meier outcome estimates of overall survival (OS) (top) and progression-free survival (PFS) (bottom). Median follow-up from infusion to data cut-off was 8.4 months (2.8 - 36.6 months) for infused patients. NR, not reached. (E) Relationship between cytokine release syndrome (CRS) and time to onset of fever. *, $p<0.05$ by unpaired *t*-test. (F) Infused CAR-positive cell number, patients with grade 0-2 CRS (black) and grade ≥3 CRS (white). **, $p<0.01$ by unpaired *t*-test.

Figure 2. Analysis of patients with laryngeal edema

(A) Laryngoscopic findings on day 3 (left) and day 4 (right). (B) Relationship between chimeric antigen receptor (CAR) positive cell number in patients with and without laryngeal edema. *, $p<0.05$ by unpaired *t*-test. (C) Laryngeal edema occurrence between patients infused with ≥ 3.4×10^8 and < 3.4×10^8 CAR-positive cells. *, $p<0.05$ by Fisher's exact test. (D) Representative flow cytometrograms of memory-phenotype cells. Mean percentages of effector memory (EM) phenotype in patients with or without laryngeal edema are shown. *, $p<0.05$ by unpaired *t*-test. SCM, stem cell memory; CM, central memory. (E) Kaplan-Meier outcome estimates of overall survival (OS) in patients with or without edema. (F) Relationship between manufacturing date and cytokine release syndrome (CRS) grade. (G) Relationship between manufacturing date and CAR-positive cell number. ****, $p<0.0001$ by unpaired *t*-test.

Figure 3. Long-term monitoring of chimeric antigen receptor (CAR) transgene and impact of steroid treatment on CAR transgene

(A) Long-term monitoring of CAR copies / μg genomic DNA (Log 10) after infusion by qPCR. Genomic DNA was isolated from peripheral blood and CD19-CAR vector copy number was quantified using real-time PCR. Individual PCR reactions were normalized against GAPDH levels. Copies of transgene/microgram of DNA were calculated

according to the formula: copies calculated from CD19 standard curve/input DNA (ng) × correction factor (ng detected/ng input) × 1,000 ng. Patients with complete response (CR) and partial response (PR) in pink, patients with stable disease (SD) and progressive disease (PD) in blue, before assessment in grey.

(B) Mean CAR copy number of CR/PR patients in pink and of SD/PD patients in blue. (Products, p=0.3751; 3 months, p=0.0615) ns, not significant by unpaired t-test. (C) Change ratio of CAR copy number before and after steroid administration. Dexamethasone (DEX) in red, methylprednisolone (mPSL) in blue, and dexamethasone + methylprednisolone in green. Numbers represent the potency of glucocorticoids activity; hydrocortisone 1 mg is defined as 1. (D) Change ratio of CAR copy number with dexamethasone (DEX) and with methylprednisolone (mPSL). *, p<0.05 by Mann-Whitney U test. (E) Flow cytometric analysis of $F(ab')_2$-streptavidin expression on tisa-cel at 1 month, 3 months, 6 months, and 12 months after infusion. Complete response (CR) patients in red (top), progressive disease (PD) patients in blue (bottom), negative control in grey. (F) Flow cytometric analysis of LAG3, PD-1, TIGIT, and TIM-3 expression on tisa-cel at 1 month, 3 months, 6 months, and 12 months after infusion. All analyses show CR patients in red (top), PD patients in blue (bottom), negative control in grey.

Figure 1

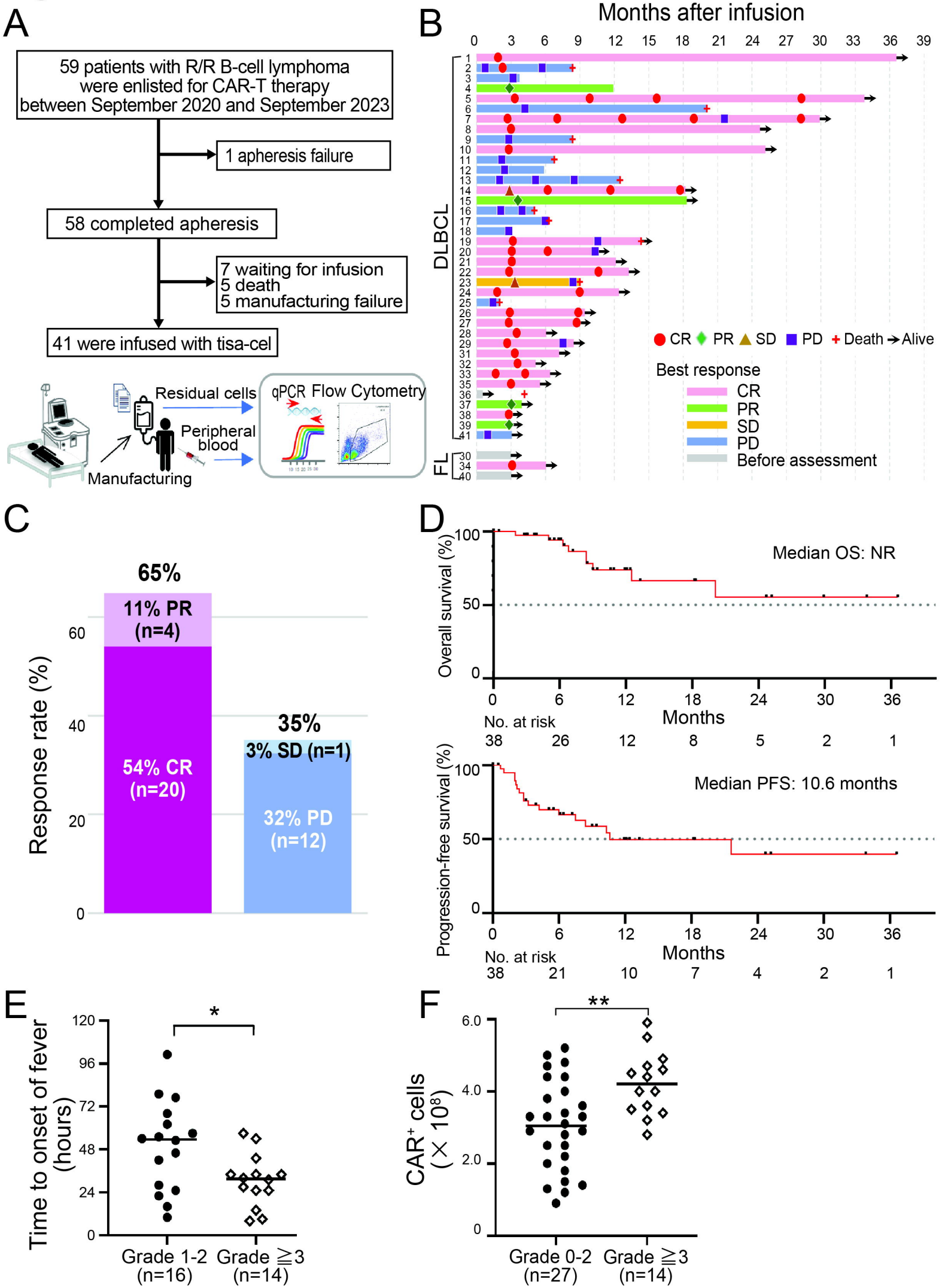

A
59 patients with R/R B-cell lymphoma were enlisted for CAR-T therapy between September 2020 and September 2023
1 apheresis failure
58 completed apheresis
7 waiting for infusion
5 death
5 manufacturing failure
41 were infused with tisa-cel
Residual cells
Peripheral blood
Manufacturing
qPCR Flow Cytometry
B
Months after infusion
DLBCL
FL
CR PR SD PD Death Alive
Best response
CR
PR
SD
PD
Before assessment
C
65%
11% PR (n=4)
54% CR (n=20)
35%
3% SD (n=1)
32% PD (n=12)
Response rate (%)
D
Overall survival (%)
Median OS: NR
No. at risk
38 26 12 8 5 2 1
Progression-free survival (%)
Median PFS: 10.6 months
No. at risk
38 21 10 7 4 2 1
Months
E
Time to onset of fever (hours)
Grade 1-2 (n=16)
Grade ≧3 (n=14)
F
CAR+ cells (× 10^8)
Grade 0-2 (n=27)
Grade ≧3 (n=14)

Figure 2

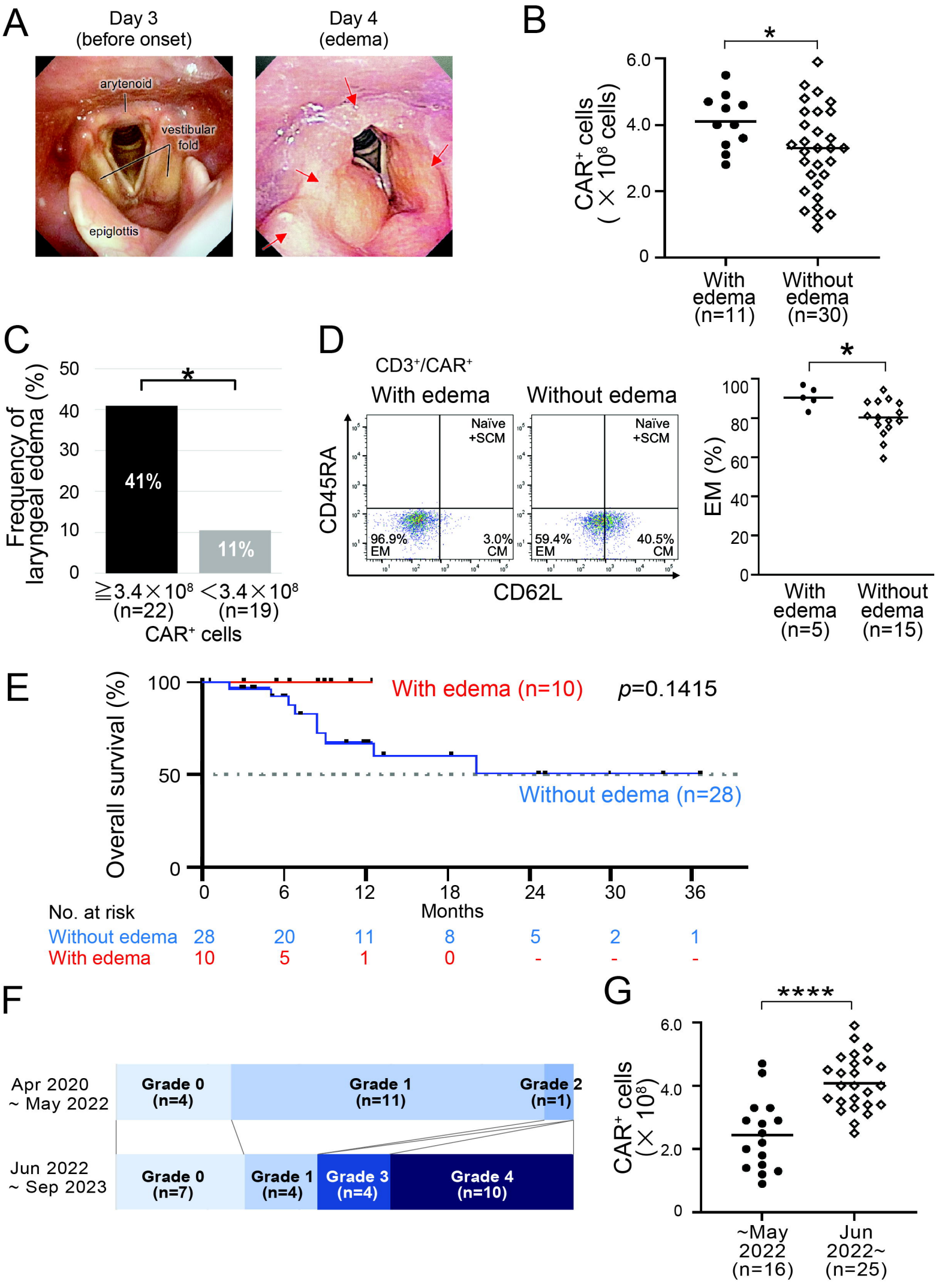
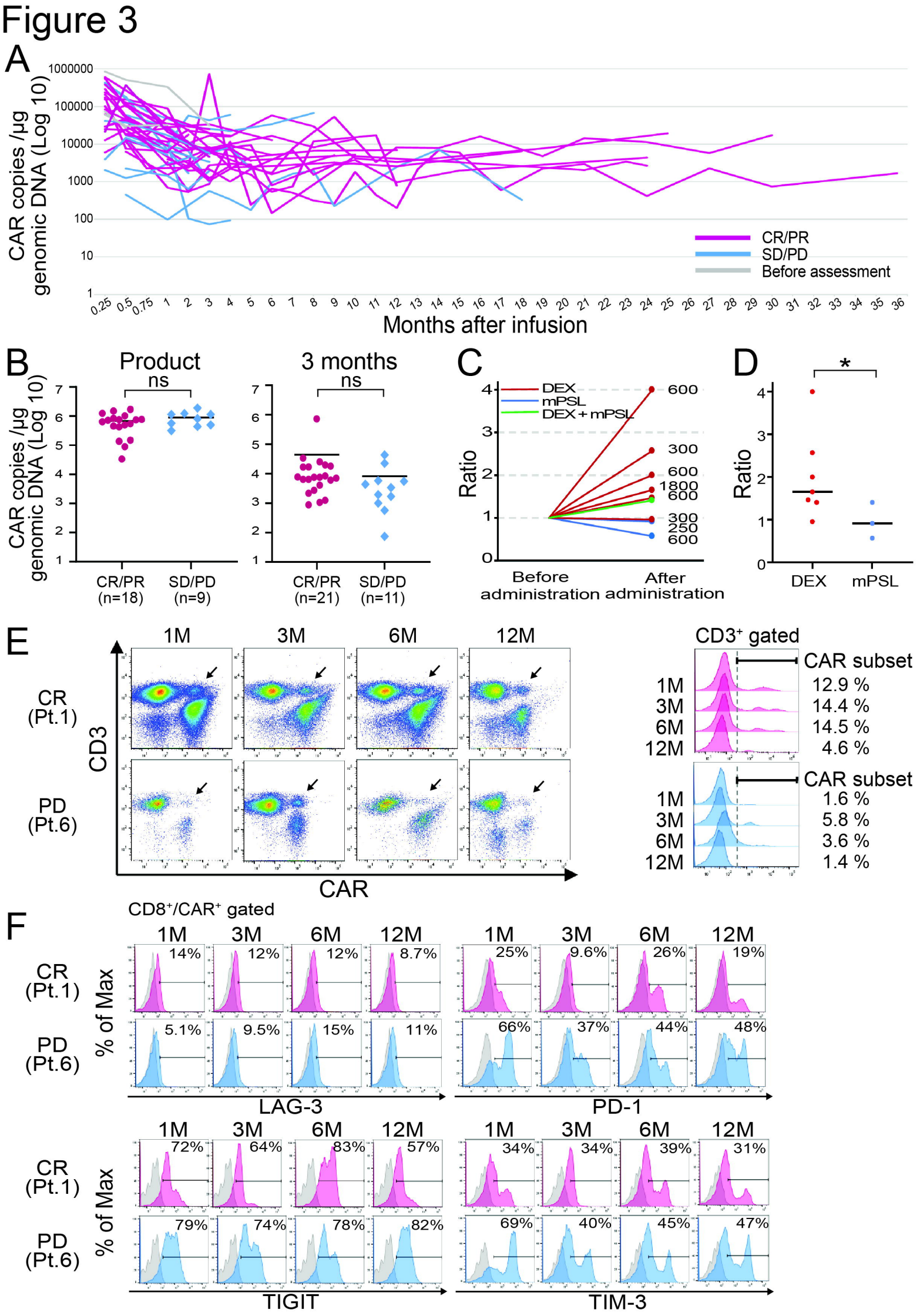

A
Day 3
(before onset)
Day 4
(edema)
arytenoid
vestibular fold
epiglottis
B
CAR+ cells (× 10^8 cells)
6.0
4.0
2.0
0
*
With edema (n=11)
Without edema (n=30)
C
Frequency of laryngeal edema (%)
50
40
30
20
10
0
*
41%
11%
≧3.4×10^8 (n=22)
<3.4×10^8 (n=19)
CAR+ cells
D
CD3+/CAR+
With edema
Without edema
CD45RA
CD62L
Naïve +SCM
96.9% EM
3.0% CM
59.4% EM
40.5% CM
EM (%)
100
80
60
40
20
0
*
With edema (n=5)
Without edema (n=15)
E
Overall survival (%)
100
50
0
With edema (n=10)
p=0.1415
Without edema (n=28)
0
6
12
18
24
30
36
Months
No. at risk
Without edema 28 20 11 8 5 2 1
With edema 10 5 1 0 - - -
F
Apr 2020 ~ May 2022
Grade 0 (n=4)
Grade 1 (n=11)
Grade 2 (n=1)
Jun 2022 ~ Sep 2023
Grade 0 (n=7)
Grade 1 (n=4)
Grade 3 (n=4)
Grade 4 (n=10)
G
CAR+ cells (× 10^8)
6.0
4.0
2.0
0
****
~May 2022 (n=16)
Jun 2022~ (n=25)

Figure 3


A
CAR copies /μg genomic DNA (Log 10)
1000000
100000
10000
1000
100
10
1
CR/PR
SD/PD
Before assessment
0.25 0.5 0.75 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Months after infusion
B
Product
ns
3 months
ns
CAR copies /μg genomic DNA (Log 10)
CR/PR (n=18)
SD/PD (n=9)
CR/PR (n=21)
SD/PD (n=11)
C
DEX
mPSL
DEX + mPSL
Ratio
600
300
600
1800
600
300
250
600
Before administration
After administration
D
*
Ratio
DEX
mPSL
E
1M
3M
6M
12M
CR (Pt.1)
PD (Pt.6)
CD3
CAR
CD3+ gated
CAR subset
1M 12.9 %
3M 14.4 %
6M 14.5 %
12M 4.6 %
CAR subset
1M 1.6 %
3M 5.8 %
6M 3.6 %
12M 1.4 %
F
CD8+/CAR+ gated
1M 3M 6M 12M
CR (Pt.1)
PD (Pt.6)
% of Max
14% 12% 12% 8.7%
5.1% 9.5% 15% 11%
LAG-3
25% 9.6% 26% 19%
66% 37% 44% 48%
PD-1
72% 64% 83% 57%
79% 74% 78% 82%
TIGIT
34% 34% 39% 31%
69% 40% 45% 47%
TIM-3

Supplementary Table 1

A. Patient characteristics

	DLBCL		FL	
	n=53		n=6	
Age				
median, years (range)	66	(35-79)	69	(60-74)
≥65, no. (%)	31	(58%)	5	(83%)
Sex				
Male	33	(62%)	3	(50%)
Female	20	(38%)	3	(50%)
ECOG performance status				
0	50	(94%)	5	(83%)
1	3	(6%)	1	(17%)
Disease stage at initial diagnosis				
I or II	8	(15%)	2	(33%)
III or IV	45	(85%)	4	(67%)
Histological subtypes				
de novo DLBCL	37	(70%)	-	-
transformed DLBCL	16	(30%)	-	-
Number of previous lines of therapy before apheresis				
2 lines	22	(42%)	2	(33%)
≥3 lines	31	(58%)	4	(67%)
median (range)	3	(2-10)	4	(2-7)
Previous ASCT	16	(30%)	0	(0%)
Primary refractory	20	(38%)	3	(50%)

DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; ECOG: Eastern Cooperative Oncology Group; ASCT: autologous stem cell transplantation

B. Patient status before infusion

	DLBCL		FL	
	n=38		n=3	
Bridging chemotherapy	33	(87%)	3	(100%)
Radiation therapy after apheresis	9	(24%)	1	(33%)
LDH at lymphodepletion >ULN	18	(47%)	1	(33%)
Bulky disease at infusion (>5 cm)	6	(16%)	1	(33%)
Status before infusion				
CR	7	(18%)	0	(0%)
PR	11	(29%)	1	(33%)
SD	16	(42%)	0	(0%)
PD	4	(11%)	2	(67%)

DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; LDH: lactate dehydrogenase; ULN: upper limit of normal; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease

C. Adverse events

	n=41	
Infusion reaction	7	(17%)
Cytopenia over 3 months, grade ≥3	8	(20%)
Hypogammaglobulinemia	5	(12%)
CRS		
Any grade	30	(73%)
Grade ≥3	14	(34%)
Median time to onset, day (range)	2	(0-5)
Details of manifestations		
Fever	30	(73%)
Hypotension	10	(24%)
Hypoxia	4	(10%)
Acute kidney injury	1	(2%)
Laryngeal edema	11	(27%)
ICANS		
Any grade	3	(7%)
Grade ≥3	2	(5%)
Median time to onset, day (range)	4	(4-7)
Details of manifestations		
Confusional state	2	(5%)
Seizure	1	(2%)
Aphasia	1	(2%)
Peripheral neuropathy	1	(2%)
Tocilizumab use	21	(51%)
Corticosteroid use	10	(24%)
Admission to intensive care unit	15	(37%)
Treatment-related mortality	0	(0%)

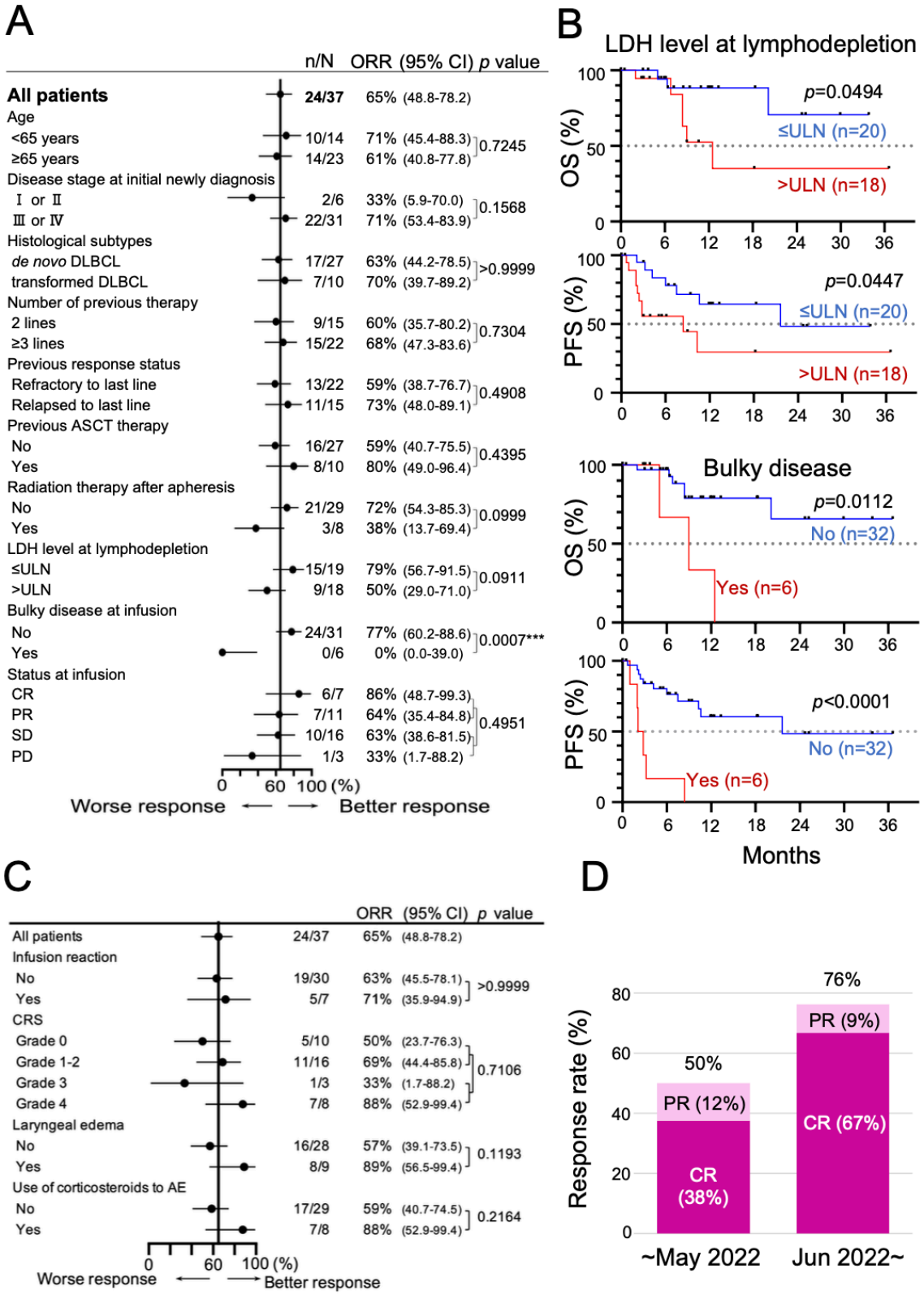
CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome

D. Characteristics of patients with laryngeal edema

		n=11	
CRS grade			
0		1	(9%)
1~2		0	(0%)
3~4		10	(91%)
Mean duration to onset, day (range)		3.4	(2~5)
Presence of cervical tumor		5	(45%)
Tocilizumab		11	(100%)
Corticosteroids		7	(64%)
DEX	10 mg/body×1 dose	2	(18%)
	10 mg/body×2 doses	2	(18%)
	20 mg/body×3 doses	1	(9%)
mPSL	2 mg/kg×1 dose	1	(9%)
Combination of DEX and mPSL		1	(9%)
Emergency airway management		9	(82%)
Admission to intensive care unit		11	(100%)

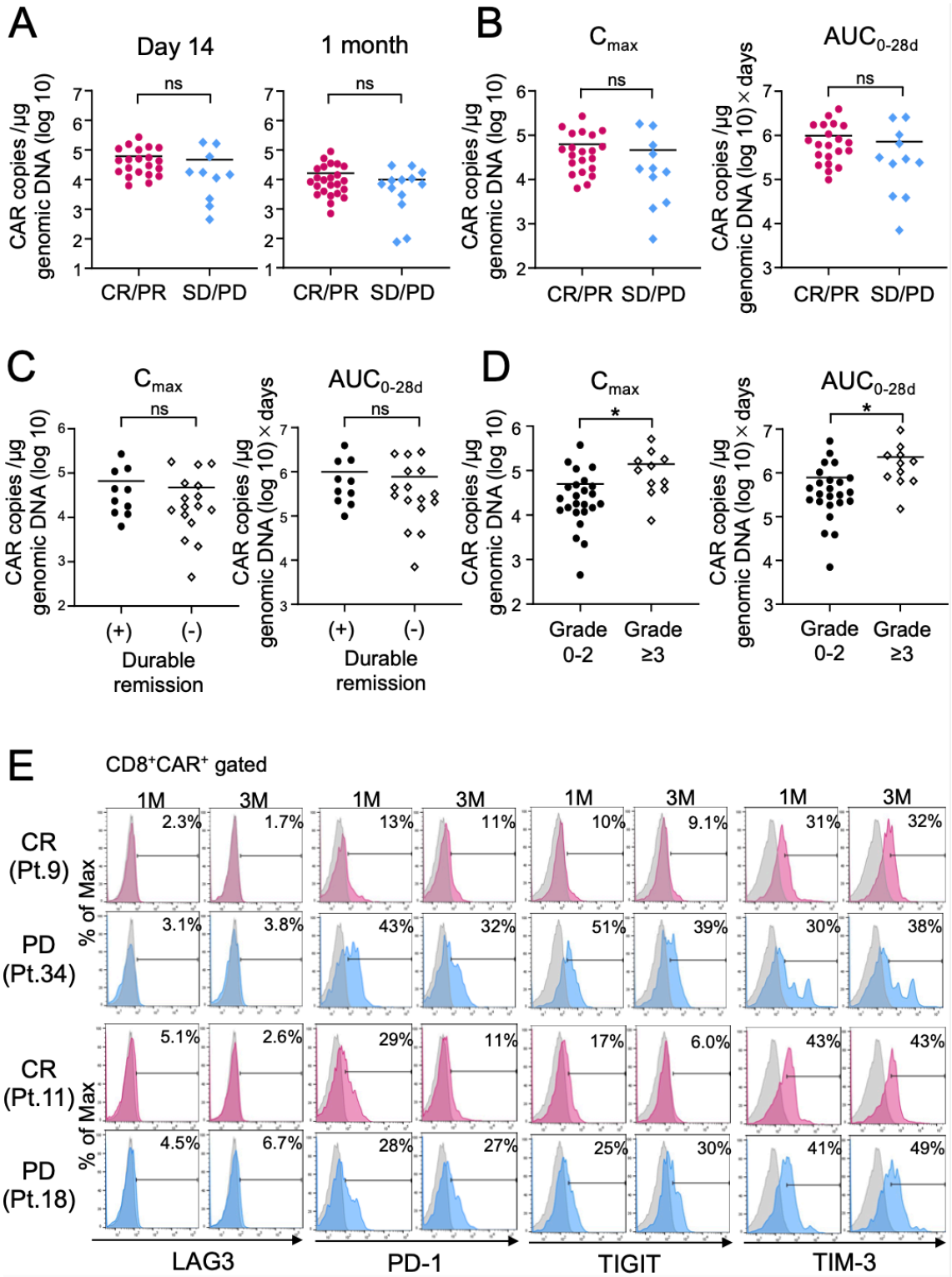
CRS: cytokine release syndrome; DEX: dexamethasone; mPSL: methylprednisolone

Supplementary Figure 1



(A) Univariate analyses affecting overall response rate (ORR). The vertical line represents the ORR for all patients. ***, $p<0.001$ by χ^2 / Fisher's exact test. (B) Subgroup analyses of overall survival (OS) and progression-free survival (PFS) classed by LDH level at onset of lymphodepletion and by bulky disease. (C) Univariate analyses affecting ORR. The vertical line represents the ORR for all patients. comparison by χ^2 / Fisher's exact test. (D) ORR of patients infused with tisa-cel before May 2022 and after Jun 2022.

Supplementary Figure 2



(A) Mean chimeric antigen receptor (CAR) copy number of complete response (CR)/ partial response (PR) patients in pink and of stable disease (SD)/ progressive disease (PD) patients in blue. (Day 14, p=0.1864; 1 month, p=0.4793) ns, not significant by unpaired t-test. (B) C_{max} (left) and $AUC_{0\text{-}28d}$ (right) of CAR copy number according to patient disease status. Patients with CR and PR in pink and patients with SD and PD in blue. Ns, not significant by unpaired t-test. (C) C_{max} (left) and $AUC_{0\text{-}28d}$ (right) of CAR copy number according to patient disease status (with durable remission or without durable remission). (D) C_{max} (left) and $AUC_{0\text{-}28d}$ (right) of patients with grade 0-2 cytokine release syndrome (CRS) (black) and grade ≥3 CRS (white). Maximum concentration of CAR copy number after infusion; C_{max}, Area under curve of CAR copy number from day 0 to day 28; $AUC_{0\text{-}28d}$ (C_{max}; p=0.0215, $AUC_{0\text{-}28d}$; p=0.0217) *, p<0.05 by unpaired t-test. (E) Flow cytometric analysis of LAG3, PD-1, TIGIT, and TIM-3 expression on tisa-cel at 1 month, 3 months after infusion. All analysis are CR patients in red, PD patients in blue, negative control in grey.