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

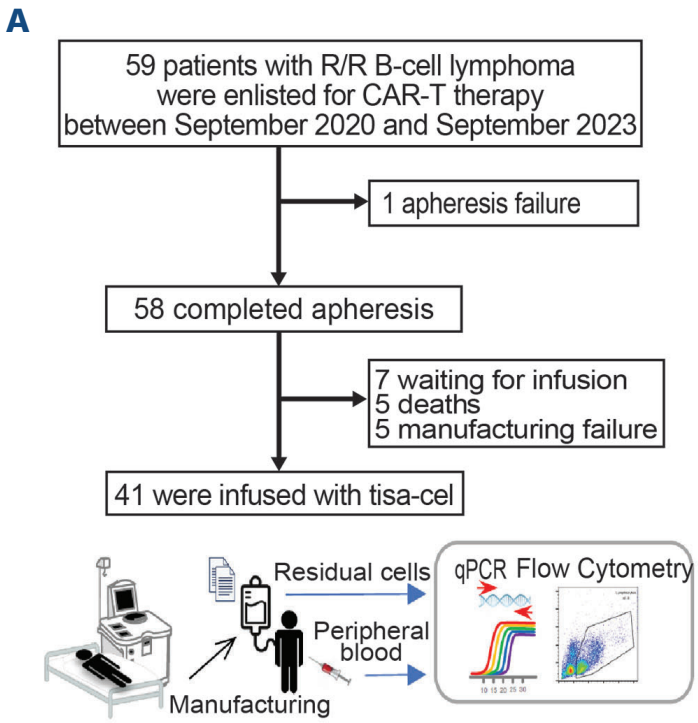
CD19-directed chimeric antigen receptor (CAR) T-cell therapy has shown promising results in refractory and relapsed B-cell lymphomas, leading to re-working of the therapeutic landscape.[1-5] We conducted a single-center, retrospective analysis of 59 patients with relapsed/refractory B-cell lymphoma enrolled for CAR T-cell 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. *Online Supplementary Table S1A* shows the characteristics of the 53 patients with diffuse large B-cell lymphoma (DLBCL) and six with follicular lymphoma (FL) enrolled for tisa-cel therapy. Forty-one study patients (38 DLBCL, 3 FL) received an infusion of tisa-cel. *Online Supplementary Table S1B* shows the patients' status before the infusion. Response assessments were completed in 37 patients with DLBCL and one with FL (Figure 1B). The overall response rate in the 37 DLBCL patients was 65% (Figure 1C). The first patient who was infused with tisa-cel at our institution has a sustained remission for over 36.6 months. Survival estimates for DLBCL patients are shown in Figure 1D. Overall survival and progression-free survival rates at 12 months were 73.8% and 49.6%, respectively. The median overall survival was not reached and the median progression-free survival was 10.6 months (Figure 1D). We also conducted subgroup analyses. Patients with bulky disease had a significantly poorer overall response rate than those without bulky disease (P=0.0007) (*Online Supplementary Figure S1A*). Increased lactate dehydrogenase level at lymphodepletion and presence of bulky disease presaged significantly shorter overall and progression-free survival (*Online Supplementary Figure S1B*).

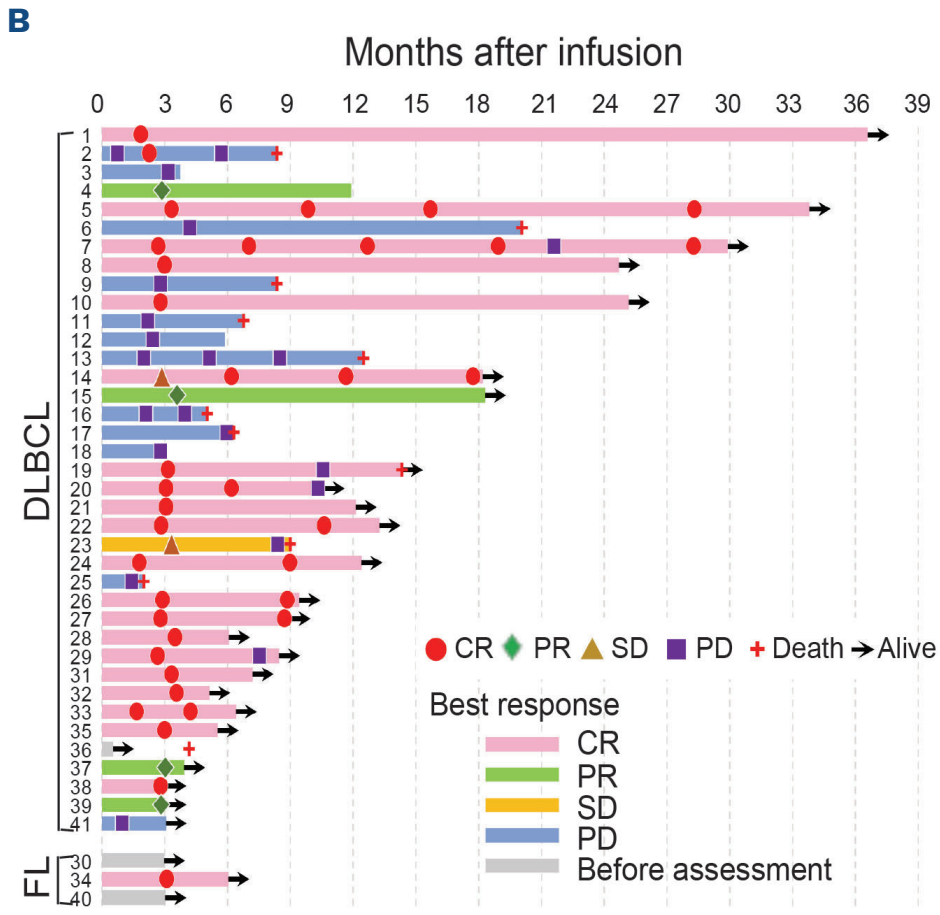
Safety outcomes were assessed in all 41 infused patients (*Online Supplementary Table S1C*). Cytokine release syndrome (CRS) was observed in 30 patients (73%), and was grade ≥3 in 14 (34%). No treatment-related deaths occurred. Of the 30 patients with CRS, patients with grade ≥3 CRS had onset of fever significantly earlier than patients with grade 1 or 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 T cells (P=0.0034) (Figure 1F).

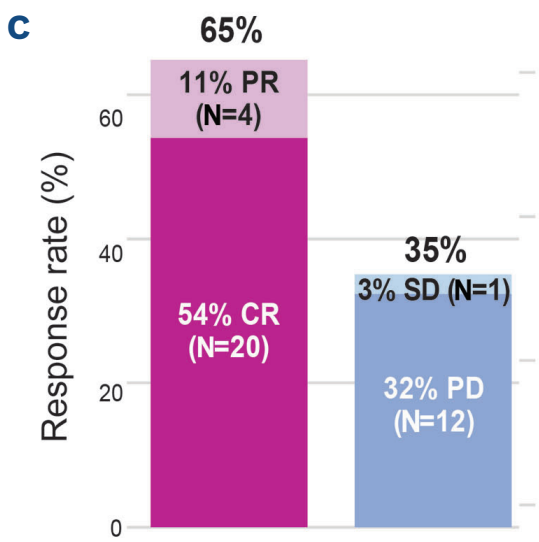
Of importance is that in this study we encountered distinct adverse events not commonly reported: 11 patients (27%) developed laryngeal edema (Figure 2A). *Online Supplementary Table S1D* shows the details that characterized patients with laryngeal edema and our management routes. Edema appeared regardless of the presence of neck tumor (5 patients with *vs.* 6 patients without neck tumor). The mean duration from infusion to onset of laryngeal edema was 3.4 days. All 11 patients were admitted to our intensive care unit; nine (82%) required emergency airway management due to the risk of airway obstruction. Interestingly, one 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 or liso-cel) treatment for B-cell lymphoma have appeared,[6] the mechanism of this phenomenon is still unknown and no established management guidelines exist in spite of the major risk of life-threatening airway obstruction.[6,7] We, therefore, 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. An example of this is T-cell chronic active Epstein-Barr virus (EBV) disease, which is more common in Asia than in the United States.[9] This regional predisposition to chronic active EBV disease suggests that genetic polymorphisms concerning the EBV immune response and impaired EBV-specific cytotoxic T-cell activity are responsible for development of the chronic active disease.[9,10]

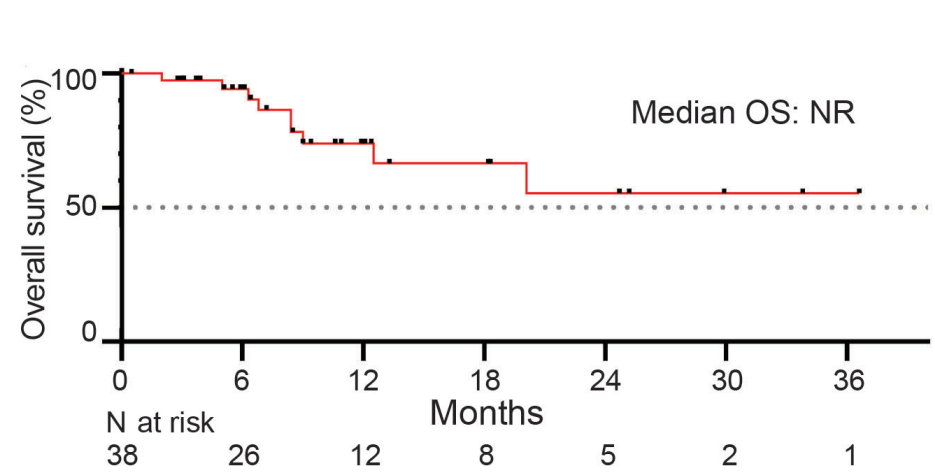
Patients with laryngeal edema were infused with significantly higher numbers of CAR-positive cells than were those without edema (median $4.0\text{x}10^8$ *vs*. $3.3\ \text{x}10^8$ cells, respectively; P=0.0411) (Figure 2B). A CAR-positive cell number of 3.4×10^8 was identified as the cut-off point for prediction of laryngeal edema. The frequency of laryngeal edema was significantly higher in patients infused with $\geq3.4\times10^8$ CAR-positive cells than in patients infused with $<3.4\times10^8$ CAR-positive cells (P=0.0385) (Figure 2C).

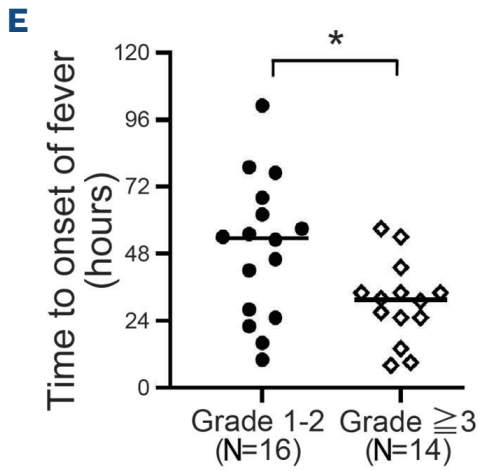
We also analyzed the memory T-cell phenotype of available infused products (N=20). CAR T cells of patients with laryngeal edema (N=5) showed a significantly higher proportion of effector memory T cells than did those of patients without edema (N=15) (P=0.0107) (Figure 2D). The overall

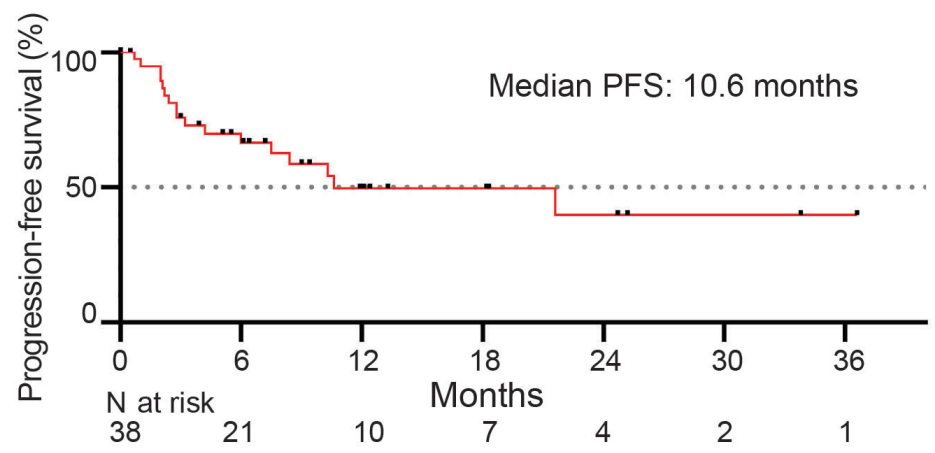












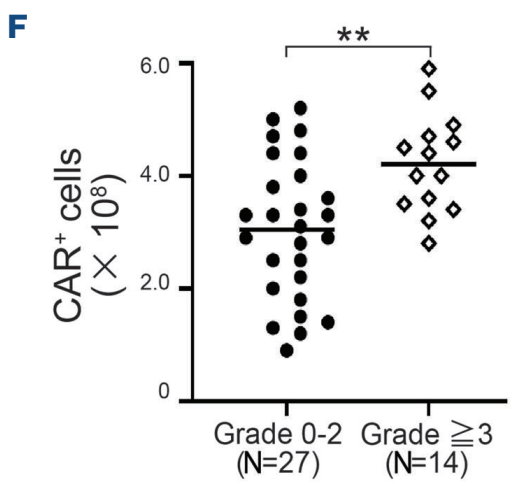


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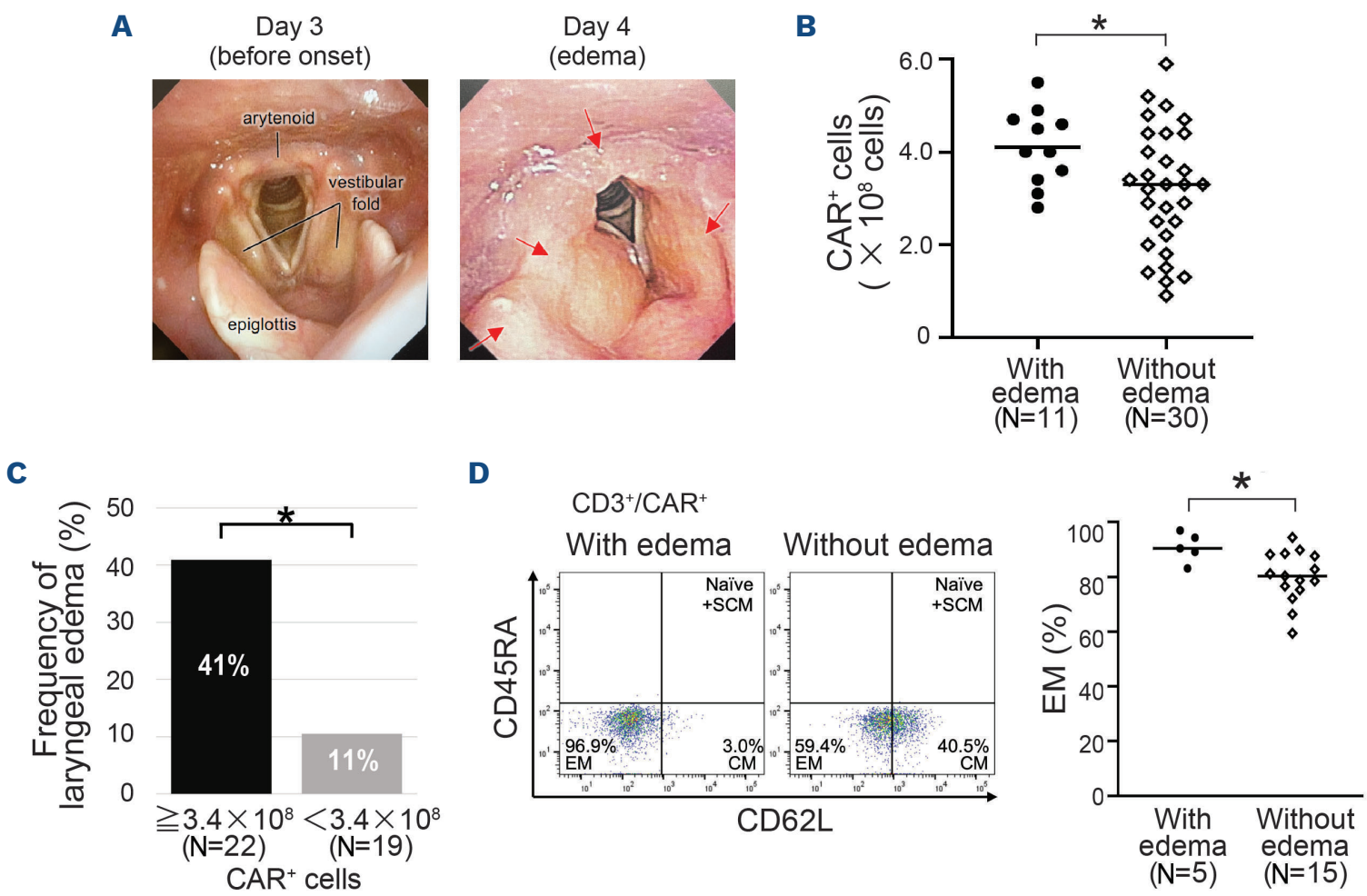
Figure 1. Patients' characteristics, overall response rates, overall survival, and safety outcome. (A) Flow chart and schematic illustration of patients enlisted for chimeric antigen receptor T-cell (CAR-T) therapy. Between September 2020 and September 2023, 53 patients with diffuse large B-cell lymphoma (DLBCL) and six with follicular lymphoma (FL) were recruited to receive tisagenlecleucel (tisa-cel) therapy at Juntendo University Hospital. 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 one FL patient (1 DLBCL patient and 2 FL patients were treated before assessment). (C) Overall response rates of patients infused with tisa-cel. Patients who responded are represented in pink (complete response, partial response), and patients with no response are represented in blue (stable disease, progressive disease). (D) Kaplan-Meier outcome estimates of overall survival (top) and progression-free survival (bottom). The median follow-up from infusion to data cut-off was 8.4 months (2.8-36.6 months) for infused patients. (E) Relationship between cytokine release syndrome (CRS) and time to onset of fever. *P<0.05 by an 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 an unpaired t test. R/R: relapsed/refractory; qPCR: quantitative polymerase chain reaction; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; OS: overall survival; NR: not reached; PFS: progression-free survival.

survival of DLBCL patients with laryngeal edema was 100% at a maximum period of 12 months, while that of patients 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 overall response rate (*Online Supplementary Figure S1C*). 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 grade ≥3 CRS events were seen in patients infused with products manufactured before May 2022 (Figure 2F). We then compared product characteristics before and after June 2022. Interestingly, the numbers of CAR-positive cells were significantly higher in products manufactured after June 2022 than in those manufactured before June 2022 (P<0.0001) (Figure 2G). The overall response rate improved from 50% (before June 2022) to 76% (after June 2022) (*Online Supplementary Figure S1D*). Several possible reasons for this change have been considered. We previously reported that the numbers of CD4-positive T cells were decreased in patients treated with regimens including bendamustine.[11] We, therefore, 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.



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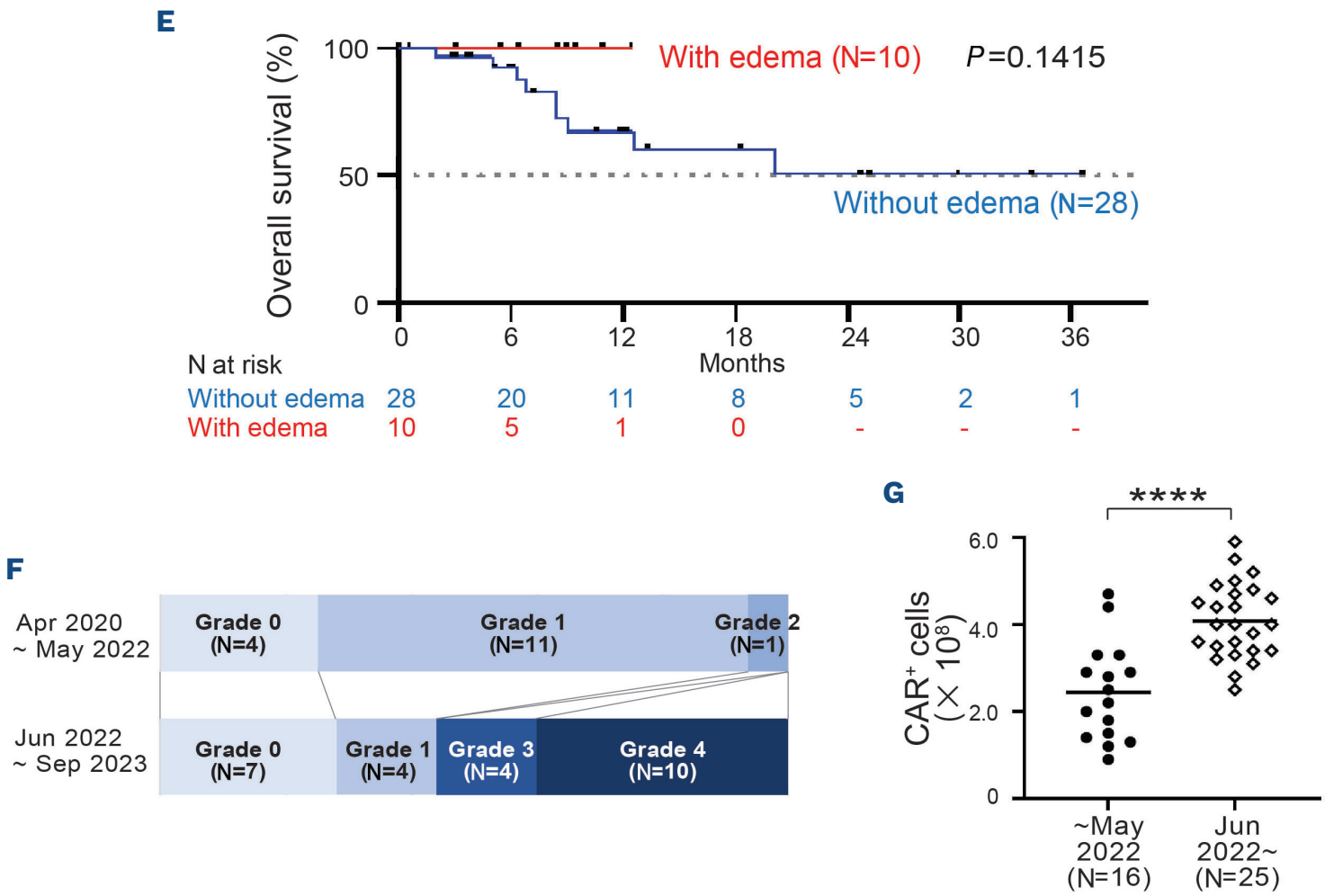

Figure 2. Analysis of patients with laryngeal edema. (A) Laryngoscopic findings on day 3 (left) and day 4 (right). (B) Relationship between chimeric antigen receptor (CAR)-positive T-cell number in patients with and without laryngeal edema. *P<0.05 by an unpaired t test. (C) Occurrence of laryngeal edema between patients infused with ≥3.4×10^8 and <3.4×10^8 CAR-positive cells. *P<0.05 by a Fisher exact test. (D) Representative flow cytometrograms of memory-phenotype cells. Mean percentages of effector memory phenotype in patients with or without laryngeal edema are shown. *P<0.05 by an unpaired t test. (E) Kaplan-Meier outcome estimates of overall survival 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 an unpaired t test. EM: effector memory T cells; SCM: stem cell memory T cells; CM: central memory T cells.

To investigate *in vivo* persistence of CAR T cells after treatment, CAR copy number was monitored long-term by quantitative polymerase chain reaction 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 to be detectable in patients with a complete or partial response. The mean peripheral blood CAR copy number at 14 days, 1 month, and 3 months after tisa-cel infusion tended to decrease more in patients with stable disease or progressive disease than in patients with a complete or partial response, although the difference did not reach statistical significance (Figure 3B, *Online Supplementary Figure S2A*). The time to reach maximum concentration (C_{max}) and the area under the curve ($AUC_{0\text{-}28d}$) of CAR copy number in the peripheral blood also did not differ significantly between patients with a complete or partial response and those with stable or progressive disease, nor in patients with or without durable remission (*Online Supplementary Figure S2B, C*). 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 correlated significantly with higher frequency of grade ≥3 CRS (*Online Supplementary Figure S2D*).

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 1 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 a complete response or progressive disease. High expression of exhaustion markers was observed at 1 month after tisa-cel infusion and continued for over 12 months in a patient with progressive disease. In contrast, the expression of these markers was relatively low in a patient who had a complete response (Figure 3F). Although opportunity for long-term monitoring is rare in patients with progressive disease (they need to start salvage therapy as

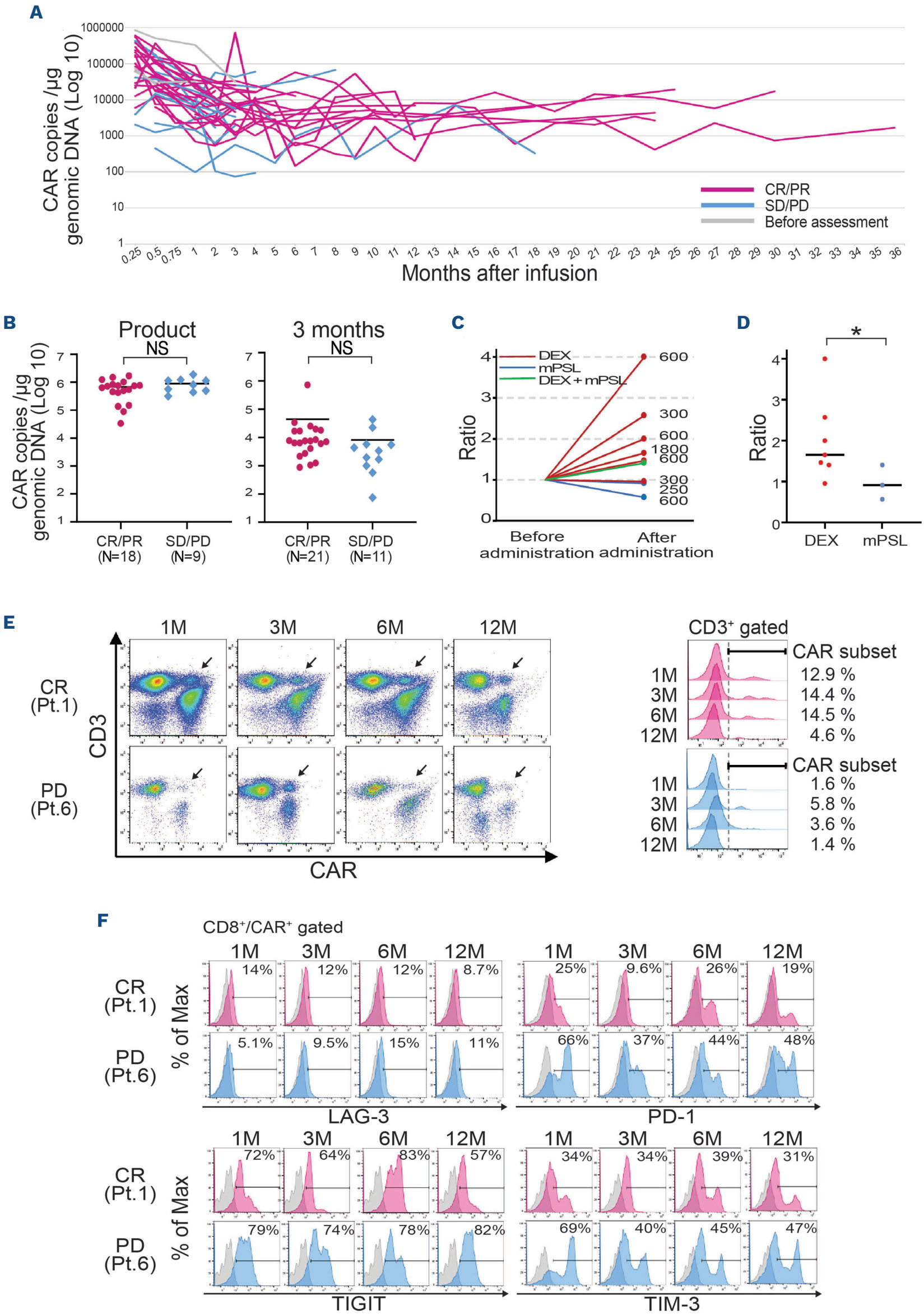

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Figure 3. Long-term monitoring of the chimeric antigen receptor (CAR) transgene and impact of steroid treatment on the transgene. (A) Long-term monitoring of CAR copies/μg genomic DNA (log 10) after infusion by real-time polymerase chain reaction (qPCR). 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/μg 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) are represented in pink, patients with stable disease (SD) and progressive disease (PD) in blue, those before assessment in gray. (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 significantly different by an unpaired t test. (C) Change in ratio of CAR copy number before and after steroid administration. Dexamethasone (DEX) in red, methylprednisolone (mPSL) in blue, and DEX + mPSL in green. Numbers represent the potency of glucocorticoid activity; hydrocortisone 1 mg is defined as 1. (D) Change in ratio of CAR copy number with DEX and with mPSL. *P<0.05 by a Mann-Whitney U test. (E) Flow cytometric analysis of F(ab')$_2$-streptavidin expression on tisagenlecleucel (tisa-cel) at 1 month, 3 months, 6 months, and 12 months after infusion. Patients in CR are represented in red (top), patients with PD in blue (bottom), and negative controls in gray. (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 controls in gray.

soon as possible), we additionally analyzed the expression of exhaustion markers on CAR T cells in such patients (2 with progressive disease and 2 with complete response) at 1 month and 3 months after tisa-cel infusion. Similarly, the expression of PD-1 and TIGIT was higher in patients with progressive disease than in those with a complete response (*Online Supplementary Figure S2E*). Despite the limited size of our sample of patients with progressive disease, from these results, we conclude that expression of exhaustion markers (PD-1 and TIGIT) on CAR T cells in peripheral blood of patients with progressive disease tended to be higher than the expression on those of patients who had a complete response.

In conclusion, our data on tisa-cel treatment for relapsed or refractory B-cell lymphomas 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 identified that a high number of CAR-positive cells (≥3.4×10^8 CAR-positive cells) and a high proportion of effector memory T cells within infused tisa-cel products were risk factors for laryngeal edema. Therefore, the utmost caution is required when these risk factors are present in CAR T-cell products. As laryngeal edema occurred regardless of the presence of neck tumors and systemic CRS, close monitoring during treatment is required even if neck lymphoma lesions are absent. Since steroid administration did not impair the overall response rate without effect on CAR copy number, treatment with dexamethasone should be initiated immediately 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 and this was associated with a higher frequency of grade ≥3 CRS alongside improved overall response rate. We believe that our analyses encompass both efficacy and safety with tisa-cel therapy in real-world settings.

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Contributions

EH analyzed data and wrote the manuscript. JA planned the study, supplied clinical data, and provided scientific discussions. SK performed flow cytometric analysis and analyzed data. YF helped with writing the manuscript. YT performed the quantitative

polymerase chain reaction analysis. YA helped with writing the manuscript. TM, ES, HT, YT, NW, TT, HY, YH, and MS provided clinical information and scientific discussions. SN helped with the statistical analysis. MI performed quantitative polymerase chain reaction and analyzed data. MA planned and directed the study, analyzed data, and wrote the manuscript.

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Data-sharing statement

Clinical data are available upon request from the corresponding author.

References

1. Schuster SJ, Tam CS, Borchmann P, et al. Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study. Lancet Oncol. 2021;22(10):1403-1415.
2. Fowler NH, Dickinson M, Dreyling M, et al. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. Nat Med. 2022;28(2):325-332.
3. Neelapu SS, Jacobson CA, Ghobadi A, et al. Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. Blood. 2023;141(19):2307-2315.
4. Jacobson CA, Chavez JC, Sehgal AR, et al. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. Lancet Oncol. 2022;23(1):91-103.
5. Abramson JS, Palomba ML, Gordon LI, 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(10254):839-852.
6. Inoue Y, Fujino T, Chinen S, et al. Cervical local cytokine release syndrome following chimeric antigen receptor T-cell therapy in patients with relapsed or refractory diffuse large B-cell lymphoma. Cureus. 2023;15(5):e38905.
7. Jin A, Feng J, Wang Z, et al. Severe dyspnea caused by rapid enlargement of cervical lymph node in a relapsed/refractory B-cell lymphoma patient following chimeric antigen receptor T-cell therapy. Bone Marrow Transplant. 2019;54(7):969-972.
8. Shima H, Kurosawa T, Oikawa H, et al. Cervical edema extending to the larynx as local cytokine release syndrome following chimeric antigen receptor T-cell therapy in a boy with refractory acute lymphoblastic leukemia. Case Rep Oncol. 2022;15(1):257-262.
9. Bollard CM, Cohen JI. How I treat T-cell chronic active Epstein-Barr virus disease. Blood. 2018;131(26):2899-2905.
10. Kimura H, Fujiwara S. Overview of EBV-associated T/NK-cell lymphoproliferative diseases. Front Pediatr. 2018;6:417.
11. Furuta Y. Lymphocyte analysis of apheresis products involved in CAR-T cell manufacturing failure. Jpn J Transfus Cell Ther. 2024;70(3):440-446.
12. Kalos M, Levine BL, Porter DL, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med. 2011;3(95):95ra73.
13. Harada S, Ando M, Ando J, et al. Dual-antigen targeted iPSC-derived chimeric antigen receptor-T cell therapy for refractory lymphoma. Mol Ther. 2022;30(2):534-549.
14. Milone MC, Fish JD, Carpenito C, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther. 2009;17(8):1453-1464.
15. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12(6):492-499.