

CAR T expansion and systemic inflammation: diverging impacts on large B-cell lymphoma therapy in the multicenter CART SIE study

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Abstract

Chimeric antigen receptor (CAR) T expansion has been linked to anti-tumor response in relapsed/refractory large B-cell lymphoma both in clinical trials and smaller real-world studies. Here, we present the largest multicenter real-world analysis to date, evaluating 262 patients treated with tisagenlecleucel or axicabtagene ciloleucel in second or subsequent relapse. Our findings underscore the complementary roles of multiparameter flow cytometry and droplet digital polymerase chain reaction in monitoring CAR T cells. While droplet digital polymerase chain reaction accurately quantifies transgene copies, multiparameter flow cytometry provides critical phenotypic details, revealing CAR T-cell subpopulations that are associated with its efficacy. Consistent with prior studies, we confirm the association of CAR T expansion with response rates, progression-free survival, and toxicities. However, we reveal that expansion alone does not ensure efficacy. Elevated markers of systemic inflammation, such as ferritin and C-reactive protein, are linked to poorer outcomes despite robust expansion. These markers correlate with reduced cytotoxic CD8⁺ T cells with central memory features among *in vivo* expanded CAR T-cell populations, with similar associations observed in manufactured and leukapheresis products. Importantly, patients with high baseline inflammation who achieved significant expansion demonstrated progression-free survival outcomes comparable to those with limited expansion, highlighting the negative impact of inflammation on CAR T-cell

efficacy. Interestingly, ferritin and C-reactive protein levels were similar among responding patients, regardless of differences in CAR T expansion. Collectively, our findings indicate that systemic inflammation is associated with the phenotypic quality of T and CAR T cells. While functional validation is warranted, these results underscore the need to address inflammatory pathways to improve treatment outcomes.

Introduction

Clinical response to CD19-directed T-cell therapy in relapsed/refractory (R/R) large B-cell lymphoma (LBCL) is strongly linked to its *in vivo* expansion and persistence as demonstrated in clinical trials.¹⁻⁴ This underscores the importance of monitoring circulating chimeric antigen receptor (CAR) T cells in peripheral blood (PB) and tumor tissues during clinical follow-up.

Despite the established significance of CAR T expansion, its clinical relevance in real-world settings has been explored primarily in monocentric studies with small patient cohorts.⁵⁻⁷ Multicenter studies assessing the efficacy of CD19-directed CAR T-cell therapy have been conducted worldwide,⁸⁻¹⁶ but systematic longitudinal data on *in vivo* CAR T expansion remain scarce. This gap is partly attributable to the absence of standardized methods for CAR T-cell monitoring,¹⁷ which hampers efforts to correlate CAR T-cell kinetics with clinical outcomes and toxicity profiles. Additionally, although expansion kinetics have been shown to correlate with CAR T-product characteristics, pre-treatment tumor burden and systemic inflammation,^{5,18} the precise interplay of these factors on circulating CAR T expansion remains unclear.¹⁹

In our study, we discuss how the current monitoring methodologies, namely multiparameter flow cytometry (MFC) and droplet digital polymerase chain reaction (ddPCR), provide complementary yet distinct information useful for optimizing CAR T-cell therapy. Furthermore, we perform real-world correlative studies on CAR T expansion kinetics in a multicenter cohort of 262 patients, and investigate the relevance of CAR T subpopulations in mediating its therapeutic efficacy. Importantly, we also examine the role of inflammatory markers on CAR T expansion to understand its possible impact on CAR T activity.

Methods

CART SIE is an ongoing multicenter prospective observational study coordinated by the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, conducted across 21 Italian hematology centers (*clinicaltrials.gov. Identifier: NCT06339255*).

For this analysis, 262 LBCL patients were selected from the CART SIE cohort based on the availability of biological samples and paired clinical data as shown (*Online Supplementary Figure S1*). Patients received either tisa-genlecleucel

(tisa-cel) or axicabtagene ciloleucel (axi-cel) as standard of care across 12 participating Italian centers, between December 2019 and May 2024. All patients received fludarabine/cyclophosphamide lymphodepletion, according to clinical practice.

Response to treatment was assessed at day 90 post-infusion using the Lugano 2014 classification.²⁰ Patients achieving complete response (CR) or partial response (PR) were classified as responders (RE). Patients with stable disease (SD), progressive disease (PD), or those who died due to progression were considered non-responders (NR). Biological samples were centralized at the Hematology Division of the Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy), where the study was conducted. This study was approved by the local ethics committee (INT 180/19) and by the institutional review boards at each site (*clinicaltrials.gov. Identifier: NCT06339255*), and adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants.

CAR T enumeration was performed by MFC and an in house ddPCR and real time quantitative PCR (RT-qPCR) method (see the *Online Supplementary Appendix* for details; *Online Supplementary Figures S2 and S5-8; Online Supplementary Table S3*).

Additional methods including immunophenotyping of T cells and CAR T cells, genomic DNA and cell-free DNA extraction, enzyme-linked immunosorbent assays and statistical analyses, are reported in the *Online Supplementary Appendix*.

Results

CAR T expansion dynamics

The pharmacokinetic profiles of CAR T cells were studied in 262 R/R LBCL patients, enrolled in the CART SIE study. Detailed patient characteristics and clinical outcomes are summarized in *Online Supplementary Table S1*. Patients received either tisa-cel or axi-cel as standard of care in second or subsequent relapse. Patient characteristics were representative of the broader LBCL cohort of the CAR T SIE study (*Online Supplementary Table S2*).

Circulating CAR T cells were monitored using MFC until CAR T were no longer detectable. The peak of CAR T expansion occurred within the first month after infusion and median time to maximal expansion (T_{max}) was day 10. Median CAR T count at day 10 post-infusion (C_{10}) was 21.03 c/μL (range, 0-932.19), at peak expansion (C_{max}) was 35.55 c/μL (range, 0-1,626.8), and the median cumulative CAR T level within

the first month (AUC_{0-30}) was 79.49 (range, 0-1,964) (*Online Supplementary Figure S3*). No CAR T cells were detected in one patient at any time point post infusion. Given the strong correlations among kinetic parameters ($P<0.0001$; *Online Supplementary Figure S4*), cumulative CAR T levels within the first month (AUC_{0-30}) were prioritized for subsequent analyses. This approach provided a comprehensive assessment of CAR T dynamics, accommodating patients with fewer available time points for monitoring.

CAR T dynamics were also monitored using a molecular ddPCR method capable of detecting the CAR transgene in both tisa-cel and axi-cel (*Online Supplementary Appendix; Online Supplementary Table S3; Online Supplementary Figures S5-8*). We found strong correlations between pharmacokinetic parameters ($P<0.0001$; *Online Supplementary Figure S9*) and between MFC and ddPCR ($P<0.0001$; $N=114$; *Figure 1; Online Supplementary Figure S10*). This validated the utility of both techniques for monitoring CAR T expansion. Nonetheless, ddPCR was particularly useful for assessing CAR T presence in low-cellularity samples where MFC was impractical or unsuitable. These included bone marrow samples, tumor biopsies, ascites and plasmatic cell-free DNA. CAR copy levels were detected in all specimen types (*Online Supplementary Table S4*) and were highly variable across samples (range, 0-2.79E+04 copies/ μ g DNA), demonstrating significant CAR T trafficking to various local sites. We then analyzed the CAR T content of infusion product leftovers ($N=30$) using both MFC and ddPCR. Consistent with our previous findings,⁵ flow cytometry revealed that axi-cel products contained a higher percentage of CAR⁺ cells among CD3⁺ and among CD45⁺ populations (*Figure 2A, B*). Similarly, ddPCR showed that axi-cel infusion products were over ten-times enriched also in CAR copies/ μ g DNA compared to tisa-cel (5.2E+05 vs. 0.66E+05 copies/ μ g DNA respectively; $P<0.01$) (*Figure 2C*). Of note, when we calculated the transduction efficiency of T lymphocytes by integrating MFC and ddPCR data, to estimate the CAR vector copy number (VCN) per CAR T cell, we discovered that VCN was significantly higher in axi-cel (5.7 copies per CAR T cell; range, 3.2-9.8) compared to tisa-cel (2.8 copies per CAR T-cell; range, 1.8-4.9; $P<0.0001$; *Figure 2D*). These findings suggest that while molecular assays are relevant to assess CAR T bio-distribution, they may overestimate CAR T counts relative to flow cytometry due to differences in VCN.

Correlation of CAR T expansion with clinical parameters and toxicities

We assessed the relationship between CAR T expansion kinetics using MFC data, and a range of baseline clinical parameters, including age, sex, histology, stage, Eastern Cooperative Oncology Group (ECOG) performance status, International Prognostic Index (IPI), bulky disease, extranodal involvement, prior lines of therapy and history of autologous stem cell transplantation (ASCT). Among these, a significant

association was observed only with prior ASCT. Patients with a history of ASCT exhibited higher cumulative CAR T levels within the first month (AUC_{0-30}) compared to those without ASCT (median AUC_{0-30} : 136.8 vs. 72.85; $P<0.05$; *Online Supplementary Figure S11A-J*). Importantly, no significant

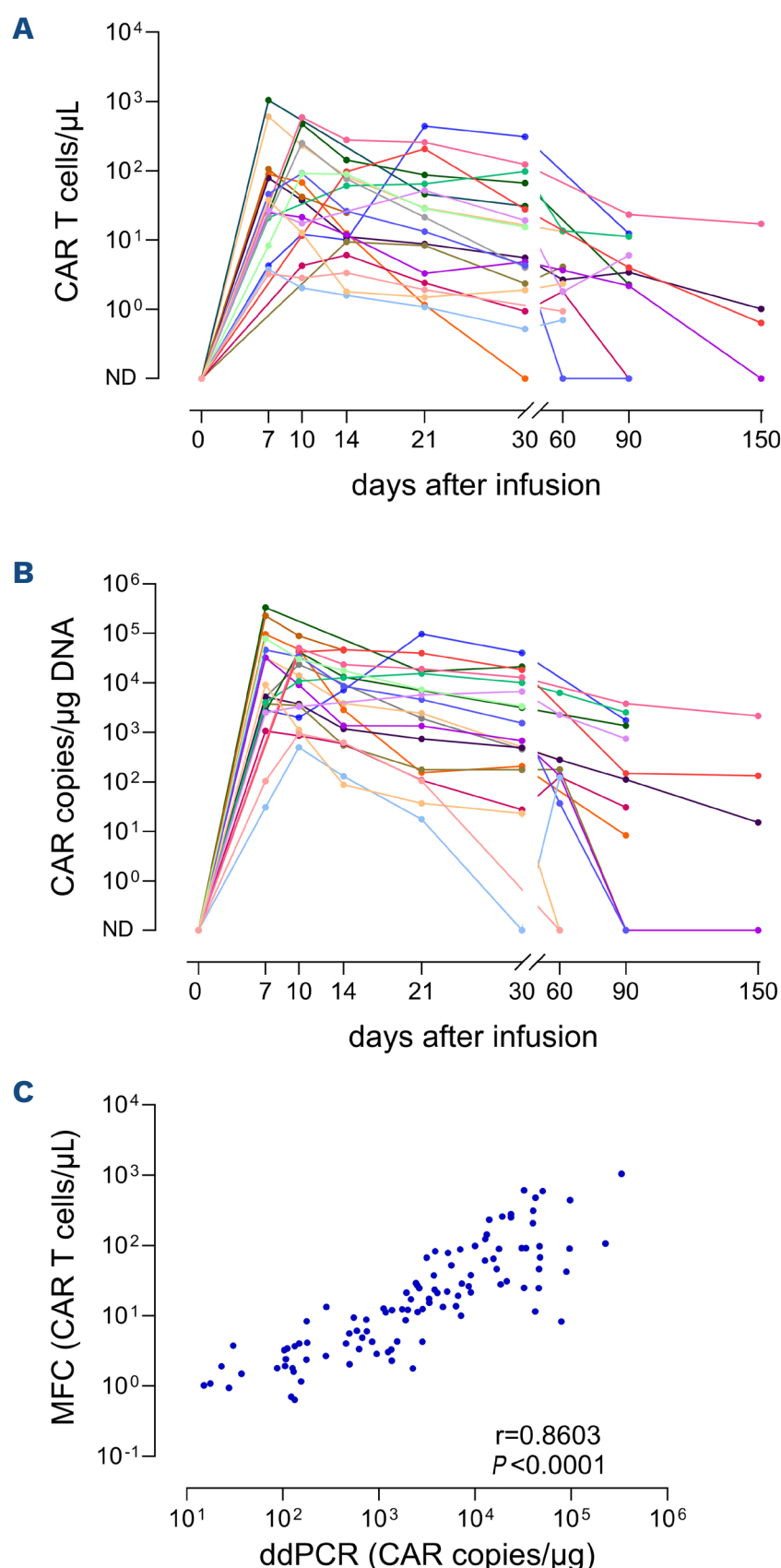


Figure 1. CAR T expansion monitoring by multiparameter flow cytometry and droplet digital polymerase chain reaction. Longitudinal chimeric antigen receptor (CAR) T expansion kinetics assessed by both (A) multiparameter flow cytometry (MFC) and (B) droplet digital polymerase chain reaction (ddPCR) at the indicated time points, on peripheral blood (PB) samples from 20 lymphoma patients. (C) Scatter dot plots showing the Spearman correlation between CAR T enumerations performed by MFC and ddPCR ($N=114$ paired samples).

differences in CAR T expansion were found between axi-cel and tisa-cel products (*Online Supplementary Figure S11K*). Furthermore, expansion did not correlate with response to bridging therapy, disease status (relapsed vs. refractory) or the LBCL subtype (*Online Supplementary Figure S11L-O*). Next, we explored the relationship between CAR T expansion and infusion-related toxicities. Higher CAR T expansion rates were associated with cytokine release syndrome (CRS) occurrence across all grades (*Online Supplementary Figure S12A, B*), and univariable logistic models confirmed this (*Online Supplementary Table S5*). On the other hand, no significant differences in CAR T expansion were found in patients experiencing immune effector cell-associated neurotoxicity syndrome (ICANS) (*Online Supplementary Figure S12C, D*), but univariable models suggested that all kinetic parameters were predictive of ICANS (*Online Supplementary Table S6*).

To evaluate the potential impact of CAR T expansion on hematological toxicities, we analyzed cytopenias at various time points. Early assessment at day 30 post-infusion revealed that thrombocytopenia, especially severe thrombocytopenia (grade ≥ 3), but not anemia, leukopenia, or neutropenia, was significantly associated with higher CAR T expansion ($P < 0.05$; *Online Supplementary Figure S13A-E*). By day 90, however, CAR T expansion no longer showed a significant impact on cytopenias (*Online Supplementary Figure S13F-I*).

Together, these findings confirm that immune cell activation driven by CAR T expansion plays a key role in the development of infusion-related toxicities. However, the impact of CAR T expansion on hematotoxicities seems to be limited to early thrombocytopenia, with no detectable long-term effects.

CAR T expansion and its association with day 90 response and progression-free survival

We identified a significant association of CAR T expansion with treatment response at day 90. Responders (RE) demonstrated higher CAR T-cell levels compared to non-responders (NR) across kinetic parameters (median C_{10} : 26.68 CAR T/ μ L vs. 14.16 CAR T/ μ L for RE and NR [$P < 0.05$]; median C_{max} : 43 CAR T/ μ L vs. 23.94 CAR T/ μ L for RE and NR [$P < 0.01$]; median AUC_{0-30} : 105.2 vs. 55.76 for RE and NR [$P < 0.01$]); (*Online Supplementary Figures 3A and S14A-F*), confirmed by univariable logistic models (*Online Supplementary Table S7*). To investigate the prognostic impact of CAR T expansion, patients were classified into “expanders” and “poor expanders” based on median values of C_{10} , C_{max} , and AUC_{0-30} . Expanders exhibited significantly longer progression-free survival (PFS) compared to poor expanders (C_{10} : median PFS not reached vs. 135 days; $P < 0.05$; C_{max} : median PFS not reached vs. 135 days; $P < 0.01$; AUC_{0-30} : median PFS 561 vs. 168 days; $P < 0.05$) (Figure 3B; *Online Supplementary Figure S14G-H*). Accordingly, univariable Cox models showed a strong association of C_{max} and AUC_{0-30} with PFS whereas

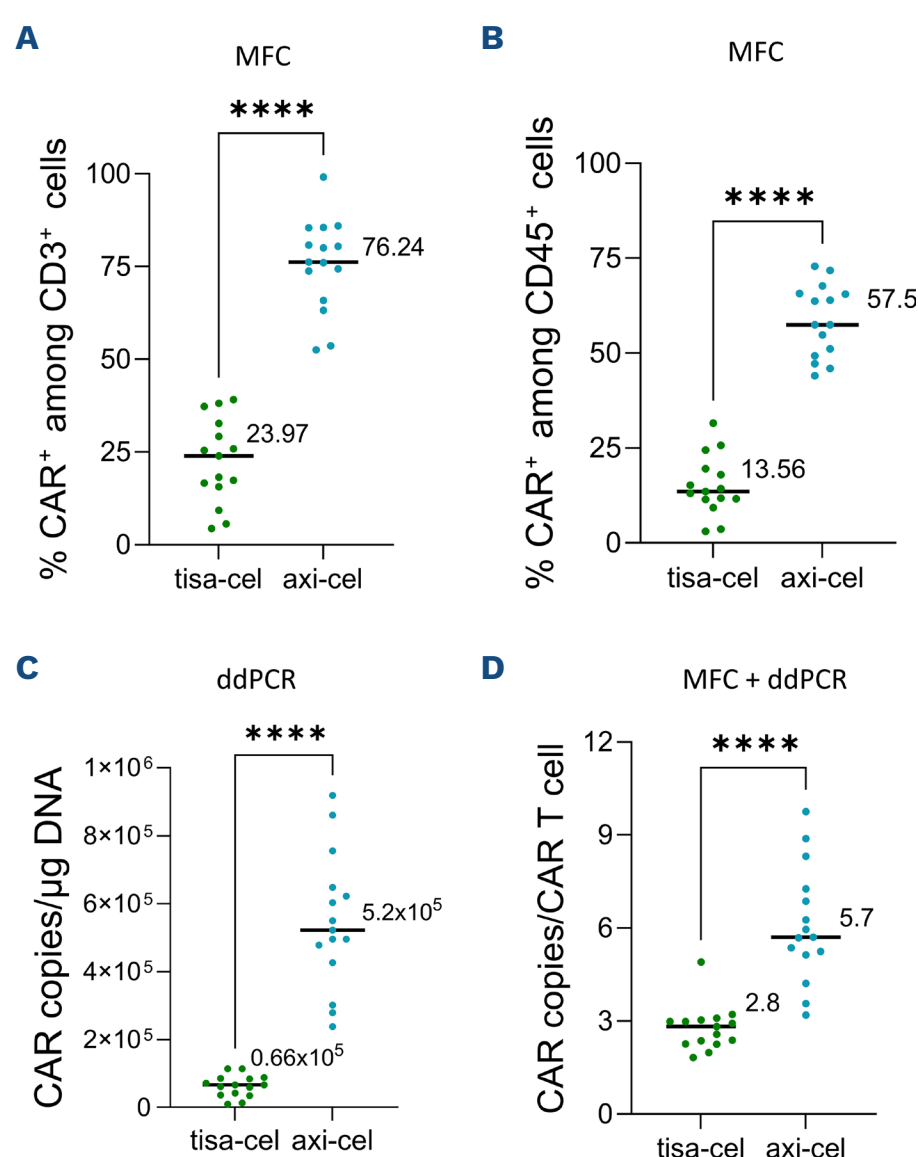


Figure 2. Study of tisa-cel and axi-cel infusion products using multiparameter flow cytometry and droplet digital polymerase chain reaction. Dot plots showing the differences in the percentage of chimeric antigen receptor-positive (CAR⁺) cells among (A) CD3⁺ and (B) CD45⁺ cells between tisagenlecleucel (tisa-cel) or axicabtagene ciloleucel (axi-cel) infusion products (N=30). (C) Dot plot showing the difference in CAR copy number/ μ g DNA between tisa-cel or axi-cel infusion products (N=30). (D) Dot plot showing the difference in CAR copy number/CAR T cell between tisa-cel and axi-cel infusion products (N=30). The calculation was performed as described in the *Online Supplementary Methods*. P values were calculated applying the Mann-Whitney test; **** $P < 0.0001$. MFC: multiparameter flow cytometry; ddPCR: droplet digital polymerase chain reaction.

C_{10} was not predictive (*Online Supplementary Table S8*). Of note, none of these kinetic parameters were able to predict overall survival (*Online Supplementary Table S9*).

To determine the independent contribution of CAR T expansion to clinical outcomes, we performed multivariable analyses using clinical parameters identified from univariable models (*Online Supplementary Tables S10 and S11*). These analyses confirmed that CAR T expansion significantly influenced the day 90 response ($P = 0.0017$), along with pre-infusion ferritin levels ($P = 0.0149$), CAR T product type ($P = 0.0431$), and age ($P = 0.0217$) (Figure 3C). Similarly, CAR T expansion was a predictor of PFS ($P = 0.002$), together with pre-infusion lactate dehydrogenase (LDH) levels ($P = 0.0002$) (Figure 3D).

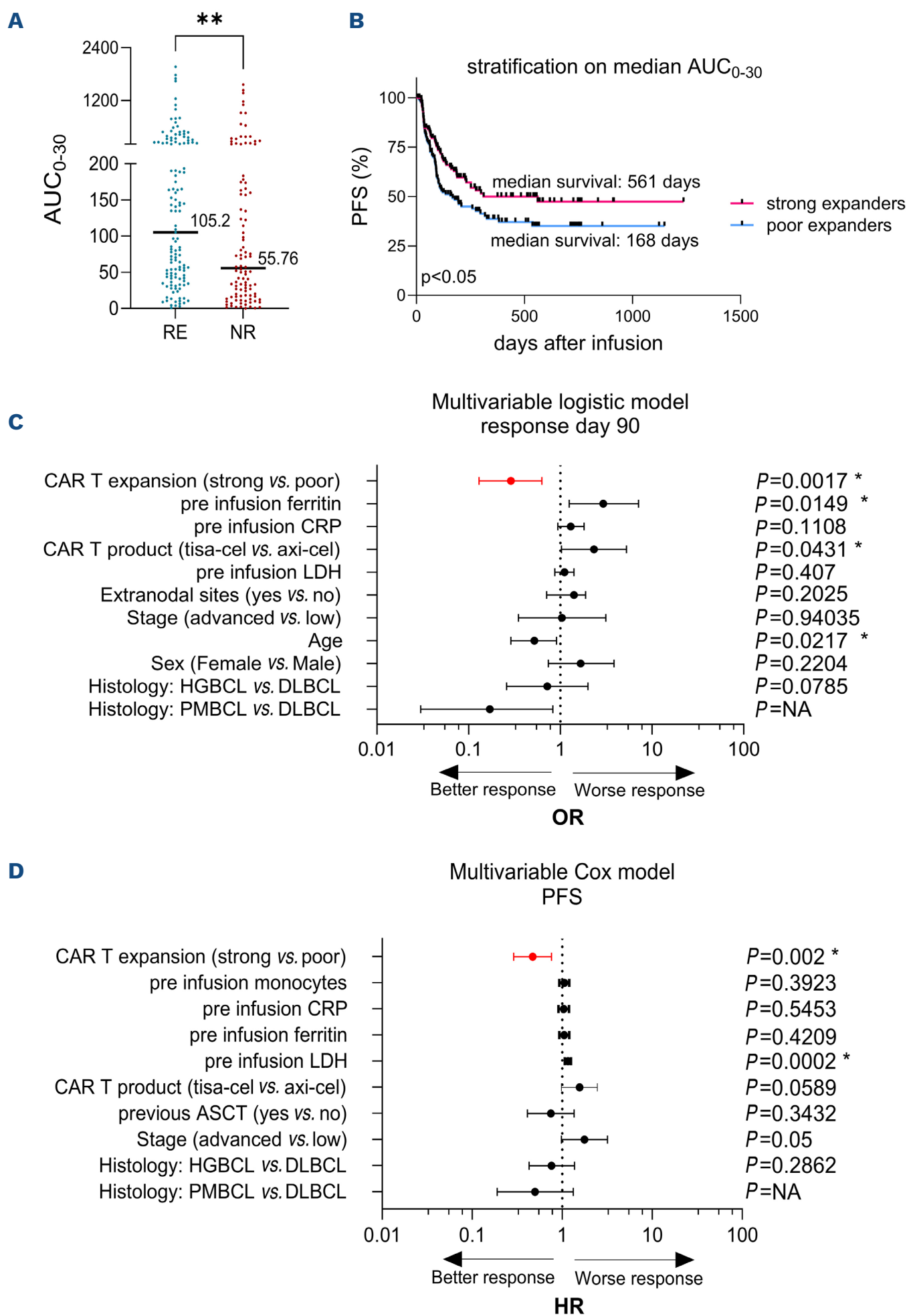


Figure 3. CAR T expansion significantly impacts day 90 response and progression-free survival. (A) Magnitude of chimeric antigen receptor (CAR) T expansion within the first month after infusion (AUC_{0-30}) in responders (RE) and non-responders (NR) by day 90 (N=235). Exact median values are reported. P value was calculated applying the Mann-Whitney test; $**P<0.01$. (B) Kaplan-Meier curve showing progression-free survival (PFS) according to CAR T expansion (N=262). Patients were dichotomized into strong and poor expanders based on the AUC_{0-30} . Comparisons were made applying the log-rank test. (C) Forest plot reporting the results of a multivariable logistic model assessing potential clinical and biological risk factors for response at day 90 (N=160). (D) Forest plot showing results of multivariate Cox regression model assessing potential clinical and biological risk factors for progression-free survival (PFS) (N=147). CRP: C reactive protein; LDH: lactate dehydrogenase; ASCT: autologous stem cell transplantation; HGBCL: high grade B-cell lymphoma; DLBCL: diffuse large B-cell lymphoma; PMBCL: primary mediastinal B-cell lymphoma; OR: odds ratio; HR: hazard ratio; NA: not applicable.

Moreover, to understand the association between expansion and outcomes among CAR T products, we estimated logistic and Cox regression models with an interaction term between CAR T product and expansion. For overall response rate (ORR), the odds ratio (OR) for the effect of expansion was comparable between axi-cel (OR=1.67) and tisa-cel (OR=2.35), and the interaction term was not statistically significant. A similar pattern was observed for PFS (hazard ratio [HR] in axi-cel =0.68; HR in tisa-cel =0.63), again with a non-significant interaction term, thus suggesting that the expansion effect were similar across the two products (*Online Supplementary Tables S12 and S13*).

Taken together, these data confirm the clinical relevance of CAR T expansion as a critical determinant of treatment response and PFS in a large multicenter, real-world setting, irrespective of the product.

Differences in T-cell composition as predictors of CAR T-cell *in vivo* expansion and efficacy

To analyze the effect of T-cell phenotypes on CAR T expansion, we examined circulating CAR-positive (CAR⁺) and CAR-negative (CAR⁻) CD4⁺ and CD8⁺ T cells during the expansion period. Expanders showed higher levels of CAR⁺CD8⁺ cells at days 7, 10, 14, and peak expansion, with an increase in CAR⁺CD8⁺ central memory T cells (T_{CM}) and a decrease in exhausted CAR⁺CD8⁺ T cells (PD1⁺LAG3⁺TIM3⁺) at peak expansion (*Online Supplementary Figure S15*). The CAR⁺CD4/CD8 ratio was lower in expanders at all time points but significantly differed only at day 10 and peak expansion (*Online Supplementary Figure S16*). In contrast, no major differences were observed in the CAR⁻ population except for higher CAR⁻CD8⁺ levels in expanders at day 7 (*Online Supplementary Figure S17*). These results suggest that CD8⁺ cytotoxic T cells are key to robust CAR T expansion.

To account for product-specific differences among the composition of expanding CAR T cells, we also compared the frequencies of CAR⁺ subpopulations among axi-cel and tisa-cel products on the peak day of expansion. Importantly, we observed higher levels of CAR⁺CD4⁺ T cells ($P=0.0007$) and lower CAR⁺CD4/CD8 ratio ($P=0.02$) in axi-cel patients, as well as higher frequencies of CAR⁺CD8⁺ T_{CM} ($P=0.0113$) in tisa-cel patients (*Online Supplementary Figure S18A-C*). However, none of these compositional differences correlated with response or survival (*data not shown*). Moreover, since expansion was not affected by the CAR T product, all consequent analyses assessing the effects of CAR T phenotypes on expansion and outcomes were performed on both products combined.

Next, we evaluated whether T-cell phenotypes in leftover cells from infusion bags influenced expansion. While CAR⁺CD4⁺ and CAR⁺CD8⁺ levels did not differ significantly between strong and poor expanders (*data not shown*), CD8⁺ CAR T-cell levels and CAR⁺CD4/CD8 ratios in infusion products correlated with their levels at peak expansion

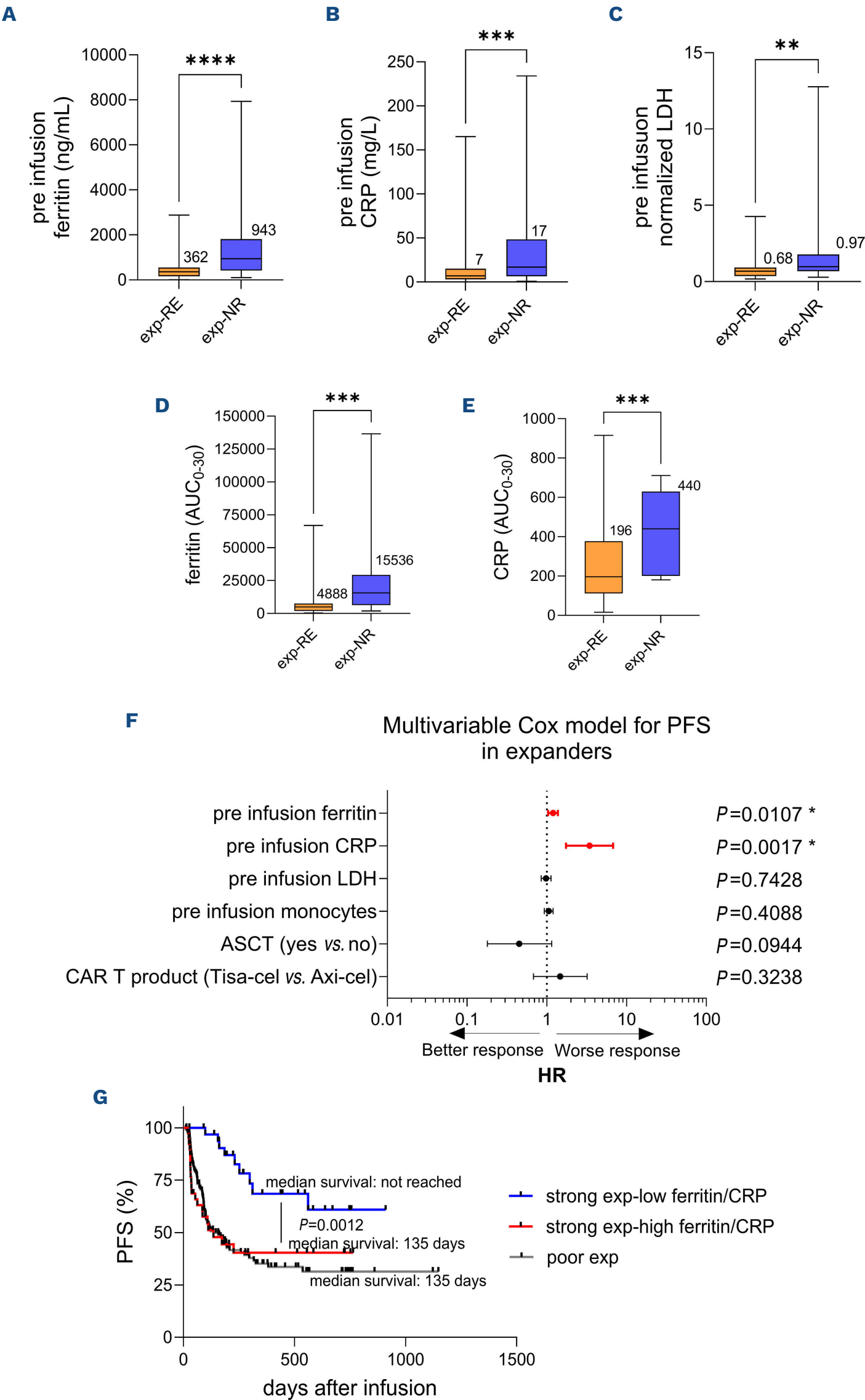
(Spearman $r=0.3270$ and $r=0.3452$, respectively; $P<0.01$). Notably, higher CAR⁺CD8⁺ levels and lower CAR⁺CD4/CD8 ratios in infusion products were linked to better day 90 response and PFS (*Online Supplementary Figure S19*). These findings underscore the role of infusion product composition in driving clinical outcomes and expansion. Lastly, we investigated T-cell composition in leukapheresis products. While no significant differences in CD4⁺ or CD8⁺ levels between strong and poor expanders were detected (*data not shown*), CAR⁺CD8⁺ T-cell levels and CAR⁺CD4/CD8 ratios in infusion products correlated with CD8⁺ T-cell levels and CD4/CD8 ratios in leukapheresis products (Spearman $r=0.3829$ and $r=0.3733$; $P<0.01$). Higher CD8⁺ levels and lower CD4/CD8 ratios in leukapheresis were associated with enhanced day 90 response but not PFS (*Online Supplementary Figure S20*).

Together, these findings suggest that the phenotype of T cells in leukapheresis and infusion products has a more direct link to clinical response compared to their role in driving expansion, and highlight the importance of T-cell functional quality over mere expansion.

Patients expanding CAR T cells but not experiencing a disease response are characterized by high levels of inflammatory markers

While CAR T expansion is generally associated with favorable clinical outcomes, it cannot be considered a definitive predictive biomarker at the individual patient level. In fact, some patients exhibit robust CAR T expansion without achieving a disease response, while others show limited expansion but experience durable responses (Figure 3). To investigate these discrepancies, we analyzed clinical and biological features at infusion, categorizing patients into expanders who responded by day 90 (exp-RE, N=77, 29.4%) and expanders who did not respond (defined as expanders-non responders [exp-NR], N=42, 16%). No significant differences were observed between the two groups in terms of lymphocyte and monocyte counts in PB, disease status at infusion, the occurrence of CRS, or the percentage of CD8⁺ central memory CAR T cells in infusion products^{5,21} and at peak expansion (*Online Supplementary Figure S21*).

Nonetheless, compared to exp-RE patients, exp-NR patients displayed significantly higher pre-infusion levels of inflammatory markers, including ferritin (median 943 ng/mL [exp-NR] vs. 362 ng/mL [exp-RE]; $P<0.0001$) and C-reactive protein (CRP) (median 17 mg/L [exp-NR] vs. 7 mg/L [exp-RE]; $P=0.0002$), as well as LDH (median normalized LDH 0.97 [exp-NR] vs. 0.68 [exp-RE]; $P=0.0022$) (Figure 4A-C). Notably, ferritin, CRP, and LDH levels were comparable among responding patients, regardless of differences in CAR T expansion (*Online Supplementary Figure S22*). Elevated ferritin and CRP levels persisted in exp-NR patients during the first month post infusion with significantly higher cumulative levels median AUC₀₋



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Figure 4. Patients expanding CAR T cells but not responding to therapy are characterized by high levels of inflammatory markers. Graphs showing pre infusion values of (A) ferritin (N=107), (B) C-reactive protein (CRP) (N=110) and (C) normalized lactate dehydrogenase (LDH) (N=94) in expanders who responded (exp-RE) and expanders who did not respond by day 90 (exp-NR). LDH levels were divided by the respective upper limit of normal (ULN), to generate normalized ratios. Ratios >1 correspond to LDH levels higher than ULN. Graphs showing cumulative levels of (D) ferritin and (E) CRP within the first months after infusion (AUC₀₋₃₀, N=52). In (A-E), exact median values are reported. *P* values were calculated applying the Mann-Whitney test; ***P*<0.01; ****P*<0.001; *****P*<0.0001. (F) Forest plot reporting the results of a multivariable Cox model assessing potential risk factors for progression-free survival (PFS) in expander patients (N=72). (G) Kaplan-Meier curve showing PFS according to chimeric antigen receptor (CAR) T expansion and pre infusion levels of ferritin and CRP (N=205). Patients were dichotomized into strong and poor expanders based on the median AUC₀₋₃₀. High ferritin/CRP: patients with both ferritin and CRP levels higher than the median (406 ng/mL and 9 mg/L, respectively); low ferritin/CRP: patients with both ferritin and CRP levels lower than the median. Comparisons were made applying the log-rank test. HR: hazard ratio.

ferritin: 4,888 versus 15,536 for exp-RE and exp-NR (*P*=0.0002); median AUC₀₋₃₀ CRP: 196.3 versus 439.8 for exp-RE and exp-NR (*P*=0.0008) (Figure 4D, E).

Univariable models fitted in the expanders group confirmed that LDH, ferritin and CRP levels significantly impacted day 90 response (LDH: OR=0.44; 95% confidence interval [CI]: 0.22-0.88; *P*=0.0483; ferritin: OR=0.37; 95% CI: 0.15-0.9; *P*=0.0007; CRP: OR=0.36; 95% CI: 0.16-0.81; *P*=0.00053) and PFS (LDH: HR=1.56; 95% CI: 1-2.43; *P*=0.0262; ferritin: HR=1.3; 95% CI: 0.77-2.21; *P*=0.00001; CRP: HR=2; 95% CI: 1.25-3.2; *P*=0.0006). Bivariable models revealed that ferritin had a more pronounced negative impact on survival in strong expanders than in poor expanders (PFS interaction *P*=0.0342; OS interaction *P*=0.0454), while CRP effect differed among strong and poor expanders primarily on disease response (interaction *P*=0.0685). LDH levels did not show significant differences between strong and poor expanders (*Online Supplementary Tables S14-16*). Multivariable analyses in the strong expanders subgroup confirmed the roles of ferritin and CRP as independent predictors of outcomes, including both day 90 response (ferritin: OR=0.47; 95% CI: 0.30-0.76; *P*=0.002; CRP: OR=0.66; 95% CI: 0.45-0.96; *P*=0.0302) (*Online Supplementary Figure S23*) and PFS (ferritin: HR=1.2, 95% CI: 1.04-1.38; *P*=0.0107; CRP: HR=3.44; 95% CI: 1.74-6.79; *P*=0.0017) (Figure 4F).

The negative impact of inflammation on the outcome of expander patients was further investigated by assessing the pre-infusion levels of different cytokines. Significantly higher levels of interleukin (IL)-6, IL-10 and IL-18 were detected in exp-NR while no differences were observed in IL-11, IL-1β, TNF-α, IFN-γ and TGF-β levels (*Online Supplementary Figure S24*). Of note, a moderate but significant correlation was found among IL-6, IL-10, IL-18 and pre-infusion ferritin and CRP levels (*Online Supplementary Table S17*).

Finally, when pre-infusion ferritin and CRP levels were combined with expansion data, the shortest PFS was observed in expanders with both markers above the median (median PFS: not reached for exp-low ferritin/low CRP vs. 135 days for exp-high ferritin/high CRP; *P*=0.0012). Notably, this subgroup displayed a median PFS equivalent to poor expanders (Figure 4G), indicating that expansion alone does not implicate a lymphoma response.

Persistent inflammation from leukapheresis to infusion correlates with more differentiated T-cell subsets and inferior outcomes

We next sought to examine the association of inflammatory markers with the phenotypic features of T-cell and CAR T-cell subtypes. Patients whose ferritin and CRP levels exceeded the median at the time of infusion (406 ng/mL and 9 mg/L, respectively), exhibited reduced percentages of CAR⁺CD8⁺ T_{CM}, in both, infusion products and at peak expansion (*P*<0.05) (Figure 5A, B). Notably, ferritin and CRP levels at leukapheresis were correlated with those measured prior to infusion in our patient cohort (ferritin: Spearman *r*=0.7163; *P*<0.0001; CRP: Spearman *r*=0.5135; *P*<0.0001; *Online Supplementary Figure S25A, B*). Consistent with this observation, we identified lower frequencies of immature T-cell populations (T-stem cell memory (T_{SCM}), CD45RO-CD197⁺CD62L⁺CD95⁺; *P*<0.05) also in leukapheresis products (Figure 5C). A similar pattern emerged when specifically analyzing patients with ferritin levels above the median (437 ng/L) measured around the time of leukapheresis (+/-7 days; Figure 5D), but not with high CRP levels. These discrepancies might reflect the less robust correlation between CRP values at LK and infusion (*Online Supplementary Figure S25B*) as CRP is a more dynamic acute-phase reactant susceptible to short-term changes. In contrast, ferritin remains more stable and tends to mirror persistent inflammatory states and disease burden, potentially accounting for its more consistent association with T-cell phenotypes across all time points.

The correlation of high ferritin and CRP across time points in our study indicates that bridging therapy could not reverse the inflammatory state in a significant subset of patients. Additionally, given that immature T-cell phenotypes have been shown to augment the therapeutic potential of CAR T cells,²² the consistent presence of more mature T-cell populations during CAR T-cell therapy, supports the notion that inflammation may contribute to suboptimal responses, potentially skewing T cells into less effective states.

This is further substantiated by the observation that a median PFS as short as 83 days was observed in patients displaying high ferritin/CRP and CAR⁺CD8⁺ cells depleted infusion products (percentages below the median) prior to therapy.

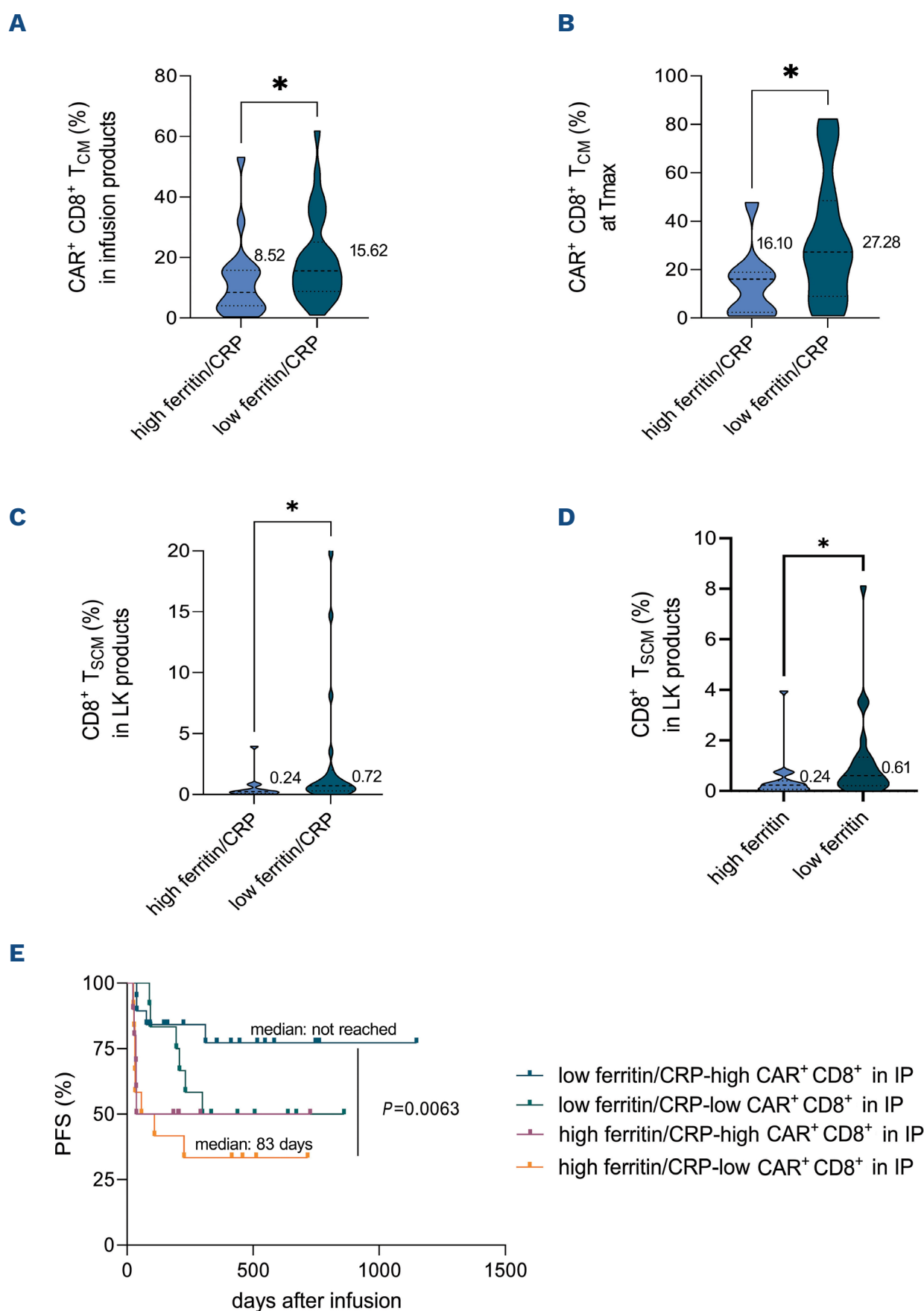


Figure 5. High levels of inflammatory markers impact T-cell composition in infusion products, at peak expansion and in leukapheresis. Levels of chimeric antigen receptor-positive (CAR⁺)CD8⁺ T central memory (T_{CM}) cells in infusion products (IP) (N=49) based on inflammatory markers at infusion (A), CAR⁺CD8⁺ T_{CM} cells at peak expansion (C_{max}, N=25) based on inflammatory markers at infusion (B), CD8⁺ T-stem cell memory (T_{SCM}) cells in leukapheresis (LK) products (N=40) based on inflammatory markers at infusion (C), CD8⁺ T_{SCM} cells in LK products (N=40) based on ferritin levels at leukapheresis (D). Patients were dichotomized based on ferritin and C-reactive protein (CRP) values before infusion. High ferritin/CRP: patients with both ferritin and CRP levels higher than the median (406 ng/mL and 9 mg/L, respectively); low ferritin/CRP: patients with both ferritin and CRP levels lower than the median for (A-C). In (D) patients were dichotomized based on ferritin values (437 ng/L) measured around the time of leukapheresis (+/-7 days). In (A-D) exact median values are reported. *P* values were calculated applying the Mann-Whitney test; **P*<0.05. (E) Kaplan-Meier curve showing progression-free survival (PFS) according to the levels of CAR⁺CD8⁺ in infusion products (IP) and pre-infusion levels of ferritin and CRP (N=205). High ferritin/CRP: patients with both ferritin and CRP levels higher than the median; low ferritin/CRP: patients with both ferritin and CRP levels lower than the median. High CAR⁺CD8⁺ in IP: CAR⁺CD8⁺ levels in IP higher than the median; low CAR⁺CD8⁺ in IP: CAR⁺CD8⁺ levels in IP lower than the median. Comparisons were made applying the log-rank test.

In contrast, the median PFS was not reached for patients with low ferritin/CRP and CAR⁺CD8⁺ percentages above the median (*P*=0.0063) (Figure 5E). Interestingly, although inflammation and CAR T phenotypes at infusion influenced survival probabilities, when patients were stratified by inflammatory-marker levels, responders (RE) and non-responders (NR) showed similar frequencies of both CD8⁺ and CD4⁺ T cells in their infusion product and likewise in their leukapheresis products (*Online Supplementary Figures S26-27*). These collective findings underscore that systemic inflammation often persists after bridging therapy and may compromise T-cell quality and, consequently, CAR T-cell efficacy.

Discussion

This multicenter, real-world study represents the largest cohort published to date analyzing *in vivo* CAR T expansion, providing a comprehensive evaluation of CAR T-cell monitoring, immunophenotypic characterization, and the impact of systemic inflammation on clinical outcomes in patients with second or subsequent relapsed/refractory LBCL. Consistent with prior clinical trial results, our findings confirm in a large real-world cohort that CAR T expansion strongly correlated with day-90 response and PFS^{1-7,23} with no major differences between axi-cel and tisa-cel treated

patients. Although strong expanders exhibited lower CD4/CD8 ratios, higher frequencies of cytotoxic CAR⁺CD8⁺ T cells with central-memory features, and fewer exhausted CAR⁺CD8⁺ T cells (PD1, LAG3, TIM3) post-infusion, none of these subpopulations alone determined outcome. Rather, in line with recently published findings,^{18,22,24-27} the phenotypic composition of the infusion and leukapheresis products, characterized by higher CD8⁺CAR⁺ T-cell and T-cell frequencies, respectively and lower CAR T and T CD4/CD8 ratios, respectively, were both found to be correlated with favorable responses.

Our findings underscore the importance of integrating complementary monitoring methodologies in clinical practice. Of note, while ddPCR which is commonly used to assess CAR T expansion in clinical studies,^{2,4,28-30} providing robust quantification of CAR transgene copies, only flow cytometry enables detailed immunophenotyping, identifying viable subpopulations and phenotypic markers.^{31,32} Remarkably, our study reveals that product-specific differences in vector copy numbers (VCN) complicate direct expansion metric comparisons. However, ddPCR can be helpful for studying CAR T cells in low-cellularity samples or unconventional tissues, sample types such as bone marrow, tumor biopsies, and cell-free DNA thus providing information on CAR T trafficking, demonstrating the distinct yet synergistic roles of MFC and molecular approaches in CAR T-cell monitoring. A key novel finding of this study is the detrimental impact of systemic inflammation on CAR T-cell efficacy. In particular, we show that elevated ferritin and CRP levels, persisting post-infusion, were associated with poor outcomes despite robust CAR T expansion. Moreover, these inflammatory biomarkers emerged as independent predictors of outcomes after adjusting for disease-related risk factors in multivariable models. Importantly, median PFS of patients expanding CAR T cells with high baseline CRP and ferritin levels was short and comparable to that of poor expanders. Overall, these results suggest that systemic inflammation as a factor complements, rather than merely reflecting traditional disease-risk features.

Prior studies, including those by Locke et al.,¹⁸ have linked elevated ferritin levels to reduced expansion normalized to tumor burden and non-response. Our findings extend this by revealing that systemic inflammation impairs CAR T-cell phenotypic quality, as evidenced by lower cytotoxic CD8⁺ T cells with T central memory characteristics. The elevated levels of IL-6, IL-10 and IL-18 along with ferritin and CRP, in patients expanding CAR T but not responding, and the significant correlations among IL-6, IL-10, IL-18 and pre-infusion ferritin and CRP levels, support the notion that this inflammation-driven suppression may, in part, be mediated by myeloid-derived cytokines whose role has been recently highlighted by us and others.^{21,33} In addition to creating a hostile immune environment, these soluble mediators compromise the phenotypic quality of T cells, including those expressing CAR, thereby impairing

CAR T-cell functionality. Although our results are based on correlative analysis and functional validations are still required, *in vitro* and *in vivo* studies reporting the role of IL-6, IL-10 and IL-18 in suppressing T-cell functions³⁴⁻⁴¹ further support these findings.

Understanding how systemic and local inflammation shape T-cell states is critical for improving long-term therapeutic outcomes of CAR T-cell therapy. Addressing systemic inflammation offers a promising avenue for enhancing CAR T-cell therapy outcomes. Strategies such as IL-6 blockade, ferritin-lowering therapies, or CRP modulation could mitigate inflammation's negative effects. Although limited, current evidence does not suggest a benefit on CAR T expansion and efficacy from prophylactic IL-6 blockade;⁴² however, an optimized timing of these interventions - before leukapheresis to improve the quality of collected T cells or post-infusion to protect expanding CAR T cells - may further enhance therapeutic efficacy.

In conclusion, CAR T-cell therapy efficacy depends not only on robust expansion but also on the phenotypic quality of CAR T cells and the systemic inflammatory state. Addressing inflammation and optimizing T-cell quality represent actionable strategies for improving clinical outcomes. Future studies should explore the mechanistic links between inflammation, T-cell cytotoxicity, and persistence, while testing targeted anti-inflammatory interventions to refine treatment strategies.

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Contributions

Conception and design by CC and PC. Provision of study materials or patients by all authors. Collection and assembly of data by MMag, SJ, GZ, CC and PC. Data analysis and interpretation by MMag, SJ, SL, CC and PC. Funding acquisition by PC. All authors wrote the manuscript and approved the final version of the manuscript.

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Data-sharing statement

For data-sharing and any further information please contact the corresponding author.

References

1. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377(26):2531-2544.
2. Locke FL, Neelapu SS, Bartlett NL, et al. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. *Mol Ther*. 2017;25(1):285-295.
3. Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med*. 2017;377(26):2545-2554.
4. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380(1):45-56.
5. Monfrini C, Stella F, Aragona V, et al. Phenotypic composition of commercial anti-CD19 CAR T cells affects in vivo expansion and disease response in patients with large B-cell lymphoma. *Clin Cancer Res*. 2022;28(15):3378-3386.
6. Ayuk FA, Berger C, Badbaran A, et al. Axicabtagene ciloleucel in vivo expansion and treatment outcome in aggressive B-cell lymphoma in a real-world setting. *Blood Adv*. 2021;5(11):2523-2527.
7. Wittibschlager V, Bacher U, Seipel K, et al. CAR T-cell persistence correlates with improved outcome in patients with B-cell lymphoma. *Int J Mol Sci*. 2023;24(6):5688.
8. Nastoupil LJ, Jain MD, Feng L, et al. Standard-of-care axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma: Results from the US lymphoma CAR T consortium. *J Clin Oncol*. 2020;38(27):3119-3128.
9. Jacobson CA, Hunter BD, Redd R, et al. Axicabtagene ciloleucel in the non-trial setting: outcomes and correlates of response, resistance, and toxicity. *J Clin Oncol*. 2020;38(27):3095-3106.
10. Vercellino L, Di Blasi R, Kanoun S, et al. Predictive factors of early progression after CAR T-cell therapy in relapsed/refractory diffuse large B-cell lymphoma. *Blood Adv*. 2020;4(22):5607-5615.
11. Jacobson CA, Locke FL, Ma L, et al. Real-world evidence of axicabtagene ciloleucel for the treatment of large B cell lymphoma in the United States. *Transplant Cell Ther*. 2022;28(9):581.e1-581.e8.
12. Riedell PA, Hwang WT, Nastoupil LJ, et al. Patterns of use, outcomes, and resource utilization among recipients of commercial axicabtagene ciloleucel and tisagenlecleucel for relapsed/refractory aggressive B cell lymphomas. *Transplant Cell Ther*. 2022;28(10):669-676.
13. Bachy E, Le Gouill S, Di Blasi R, et al. A real-world comparison of tisagenlecleucel and axicabtagene ciloleucel CAR T cells in relapsed or refractory diffuse large B cell lymphoma. *Nat Med*. 2022;28(10):2145-2154.
14. Bethge WA, Martus P, Schmitt M, et al. GLA/DRST real-world outcome analysis of CAR T-cell therapies for large B-cell lymphoma in Germany. *Blood*. 2022;140(4):349-358.
15. Kuhn A, Roddie C, Kirkwood AA, et al. A national service for delivering CD19 CAR-Tin large B-cell lymphoma - the UK real-world experience. *Br J Haematol*. 2022;198(3):492-502.
16. Kwon M, Iacoboni G, Reguera JL, et al. Axicabtagene ciloleucel compared to tisagenlecleucel for the treatment of aggressive B-cell lymphoma. *Haematologica*. 2023;108(1):110-121.
17. Turicek DP, Giordani VM, Moraly J, Taylor N, Shah NN. CAR T-cell detection scoping review: an essential biomarker in critical need of standardization. *J Immunother Cancer*. 2023;11(5):e006596.
18. Locke FL, Rossi JM, Neelapu SS, et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2020;4(19):4898-4911.
19. Ruella M, Korell F, Porazzi P, Maus MV. Mechanisms of resistance to chimeric antigen receptor-T cells in haematological malignancies. *Nat Rev Drug Discov*. 2023;22(12):976-995.

20. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of hodgkin and non-hodgkin lymphoma: the Lugano classification. *J Clin Oncol.* 2014;32(27):3059-3068.
21. Carniti C, Caldarelli NM, Agnelli L, et al. Monocytes in leukapheresis products affect the outcome of CD19-targeted CAR T-cell therapy in lymphoma patients. *Blood Adv.* 2024;8(8):1968-1980.
22. Haradhvala NJ, Leick MB, Maurer K, et al. Distinct cellular dynamics associated with response to CAR-T therapy for refractory B cell lymphoma. *Nat Med.* 2022;28(9):1848-1859.
23. Cappell KM, Sherry RM, Yang JC, et al. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. *J Clin Oncol.* 2020;38(32):3805-3815.
24. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med.* 2018;24(5):563-571.
25. López-Cantillo G, Urueña C, Camacho BA, Ramírez-Segura C. CAR-T cell performance: how to improve their persistence? *Front Immunol.* 2022;13:878209.
26. García-Calderón CB, Sierro-Martínez B, García-Guerrero E, et al. Monitoring of kinetics and exhaustion markers of circulating CAR-T cells as early predictive factors in patients with B-cell malignancies. *Front Immunol.* 2023;14:1152498.
27. Maurer K, Grabski IN, Houot R, et al. Baseline immune state and T-cell clonal kinetics are associated with durable response to CAR-T therapy in large B-cell lymphoma. *Blood.* 2024;144(24):2490-2502.
28. Badbaran A, Berger C, Riecken K, et al. Accurate in-vivo quantification of cd19 car-t cells after treatment with axicabtagene ciloleucel (Axi-cel) and tisagenlecleucel (tisa-cel) using digital pcr. *Cancers (Basel).* 2020;12(7):1970.
29. Mika T, Maghnouj A, Klein-Scory S, et al. Digital-droplet PCR for quantification of CD19-directed CAR T-cells. *Front Mol Biosci.* 2020;7:84.
30. Gkazi SA, Gravett E, Bautista C, Bartram J, Ghorashian S, Adams SP. Clinically applicable assessment of tisagenlecleucel CAR T cell treatment by digital droplet PCR for copy number variant assessment. *Int J Mol Sci.* 2022;23(14):7573.
31. Blache U, Weiss R, Boldt A, et al. Advanced flow cytometry assays for immune monitoring of CAR-T cell applications. *Front Immunol.* 2021;12:658314.
32. Peinelt A, Bremm M, Kreyenberg H, et al. Monitoring of circulating CAR T cells: validation of a flow cytometric assay, cellular kinetics, and phenotype analysis following tisagenlecleucel. *Front Immunol.* 2022;13:830773.
33. Jain MD, Zhao H, Wang X, et al. Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. *Blood.* 2021;137(19):2621-2633.
34. Huseni MA, Wang L, Klementowicz JE, et al. CD8+ T cell-intrinsic IL-6 signaling promotes resistance to anti-PD-L1 immunotherapy. *Cell Rep Mod.* 2023;4(1):100878.
35. Vilgelm AE. Illuminating the mechanism of IL-6-mediated immunotherapy resistance. *Cell Rep Med.* 2023;4(1):100901.
36. Tsukamoto H, Fujieda K, Senju S, Ikeda T, Oshiumi H, Nishimura Y. Immune-suppressive effects of interleukin-6 on T-cell-mediated anti-tumor immunity. *Cancer Sci.* 2018;109(3):523-530.
37. Yu T, Jiang W, Wang Y, et al. Chimeric antigen receptor T cells in the treatment of osteosarcoma (Review). *Int J Oncol.* 2024;64(4):40.
38. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor- β : the role of T regulatory cells. *Immunology.* 2006;117(4):433-442.
39. Groux H, Bigler M, de Vries JE, Roncarolo MG. Inhibitory and stimulatory effects of IL-10 on human CD8+ T cells. *J Immunol.* 1998;160(7):3188-3193.
40. Nakamura K, Kassem S, Cleynen A, et al. Dysregulated IL-18 is a key driver of immunosuppression and a possible therapeutic target in the multiple myeloma microenvironment. *Cancer Cell.* 2018;33(4):634-648.e5.
41. Lim HX, Hong H-J, Cho D, Kim TS. IL-18 enhances immunosuppressive responses by promoting differentiation into monocytic myeloid-derived suppressor cells. *J Immunol.* 2014;193(11):5453-5460.
42. Locke FL, Neelapu SS, Bartlett NL, et al. Tocilizumab prophylaxis following axicabtagene ciloleucel in relapsed or refractory large B-cell lymphoma. *Transplant Cell Ther.* 2024;30(11):1065-1079.