Morphologic and immunophenotypic characterization of lymphocytosis following BCMA-targeted CAR-T cell therapy in relapsed/refractory multiple myeloma

B-cell maturation antigen (BCMA)-targeting chimeric antigen receptor T-cell (CAR-T) therapy has emerged as a standard treatment for relapsed/refractory multiple myeloma (R/R MM).[1] The U.S. Food and Drug Administration has approved two autologous BCMA-targeted CAR-T products: ciltacabtagene autoleucel (cilta-cel) and idecabtagene vicleucel (ide-cel). These approvals were based on robust clinical trial data showing overall response rates exceeding 70% in heavily pretreated patients.[2,3]

A well-established phenomenon following CAR-T therapy is the *in vivo* expansion of infused T cells, which correlates with both therapeutic efficacy and immune-related toxicities such as cytokine release syndrome (CRS).[4-6] Transient post-infusion lymphocytosis has been observed in R/R MM following BCMA CAR-T infusion. In this context, the absolute lymphocyte count (ALC) serves as a surrogate marker for CAR-T expansion, typically peaking around days +11 to +12. Higher maximum ALC (ALC_{max}) has been associated with improved depth of response, longer progression-free survival, and more durable remissions.[7–9]

The cytomorphology and immunophenotype of the expanded CAR-T cells in BCMA-directed therapies remain poorly characterized, posing diagnostic challenges in the post-infusion period. Additionally, BCMA CAR-T therapy is associated with neurotoxicities beyond CRS. These include immune effector cell-associated neurotoxicity syndrome (ICANS) and a distinct delayed non-ICANS neurotoxicity, the latter of which is not typically observed in CD19 CAR-T therapy. A recent study demonstrated that higher ALC_{max} correlates with the timing of CRS and ICANS onset.[9] However, the relationships between CAR-T expansion kinetics, subset composition, and these toxicities remain unclear.

Here, we present a combined retrospective and prospective analysis aimed at characterizing the morphologic and immunophenotypic features of lymphocytosis following BCMA CAR-T infusion in R/R MM patients. To our knowledge, this is the first study utilizing standard-of-care flow cytometry to measure BCMA CAR-T kinetics in the peripheral blood following infusion. We specifically addressed the diagnostic challenges encountered during routine clinical follow-up, including the morphologic features of expanded CAR-T cells and their immunophenotypic profiles. Additionally, we monitored the longitudinal dynamics of CAR-T cells, including ALC and CAR-T expansion kinetics and subset composition, providing further insight into the cellular dynamics underlying therapeutic response and their potential association with neurotoxicity. The Institutional Review Board of Weill Cornell Medicine approved this study.

We initially conducted a retrospective analysis of six patients with R/R MM who received BCMA CAR-T therapy between 2023 and 2024 and underwent peripheral blood flow cytometry due to marked post-infusion lymphocytosis. A total of seven peripheral blood specimens from these patients were included. The patients' characteristics, lymphocyte counts, and flow cytometric immunophenotyping data were collected and summarized (*Online Supplementary Table S1*). Corresponding peripheral blood smear slides were reviewed by board-certified hematopathologists to characterize the morphology of the lymphocytes. Flow cytometry used standard T-cell markers and fluorophore-conjugated BCMA protein to detect CAR-T cells specifically.

All of the seven post-infusion specimens (days 10–28) demonstrated elevated lymphocyte counts (range, 1.7–26 $\times 10^9$/L; median 2.7$\times 10^9$/L). Atypical lymphocytes constituted 6%–82% (median 53%) of total lymphocytes. Morphologically, smears revealed large, atypical lymphocytes with abundant pale blue cytoplasm and occasional cytoplasmic granules (Figure 1A).

Flow cytometry showed a predominance of $CD3^+$ T cells (range, 16–99%; median 98%), with frequent partial loss of CD7 (range, 10.2–54.4%; median 24.1%) and CD5 (range, 7–17.2%; median 9.2%) (Figure 1B). These aberrant populations included both $CD4^+$ and $CD8^+$ T cells, although $CD8^+$ cells were more frequently predominant. All samples exhibited a decreased CD4:CD8 ratio. In one patient (day +10), CD45 downregulation resulted in a significant proportion of T cells being classified within the "blast" gate in the CD45 *versus* side scatter plot (33.6%) (Figure 1C). Fluorophore-conjugated BCMA protein confirmed the presence of expanded BCMA CAR-T cells in all tested patients (N=4) (Figure 1D).

Following this observation, we collected samples from 21 R/R MM patients receiving BCMA CAR-T therapy for longitudinal monitoring of CAR-T kinetics through flow cytometry. The patients' characteristics for this cohort are summarized in *Online Supplementary Table S2*. Peripheral blood samples were collected at intervals from day 0 to week 30. ALC and BCMA CAR-T cell frequencies among total $CD3^+$ T cells were quantified (*Online Supplementary Table S3*). Consistent with our previous report,[7] ALC_{max} was observed around 2 weeks after infusion (days +11 to +12) (Figure 2A). Flow cytometry demonstrated a parallel expansion of BCMA CAR-T cells during this period (Figure 2B). At the peak of expansion, CAR-T cells accounted for 6.6% to 86.5% of the

CD3⁺ T cells in individual patients, with a median of 56.6%. By week 6, CAR-T cells were undetectable in most patients, although three patients exhibited persistent CAR-T cells through weeks 7 to 9.

Although a predominance of CD8⁺ BCMA CAR-T cell expansion was reported in a prior cohort,[10] in our study, the proportion of CD4⁺ or CD8⁺ CAR-T cells varied among individual patients. Specifically, ten of 21 patients (47.6%) demonstrated predominant CD4⁺ CAR-T expansion, whereas 11 of 21 patients (52.4%) had a predominance of CD8⁺

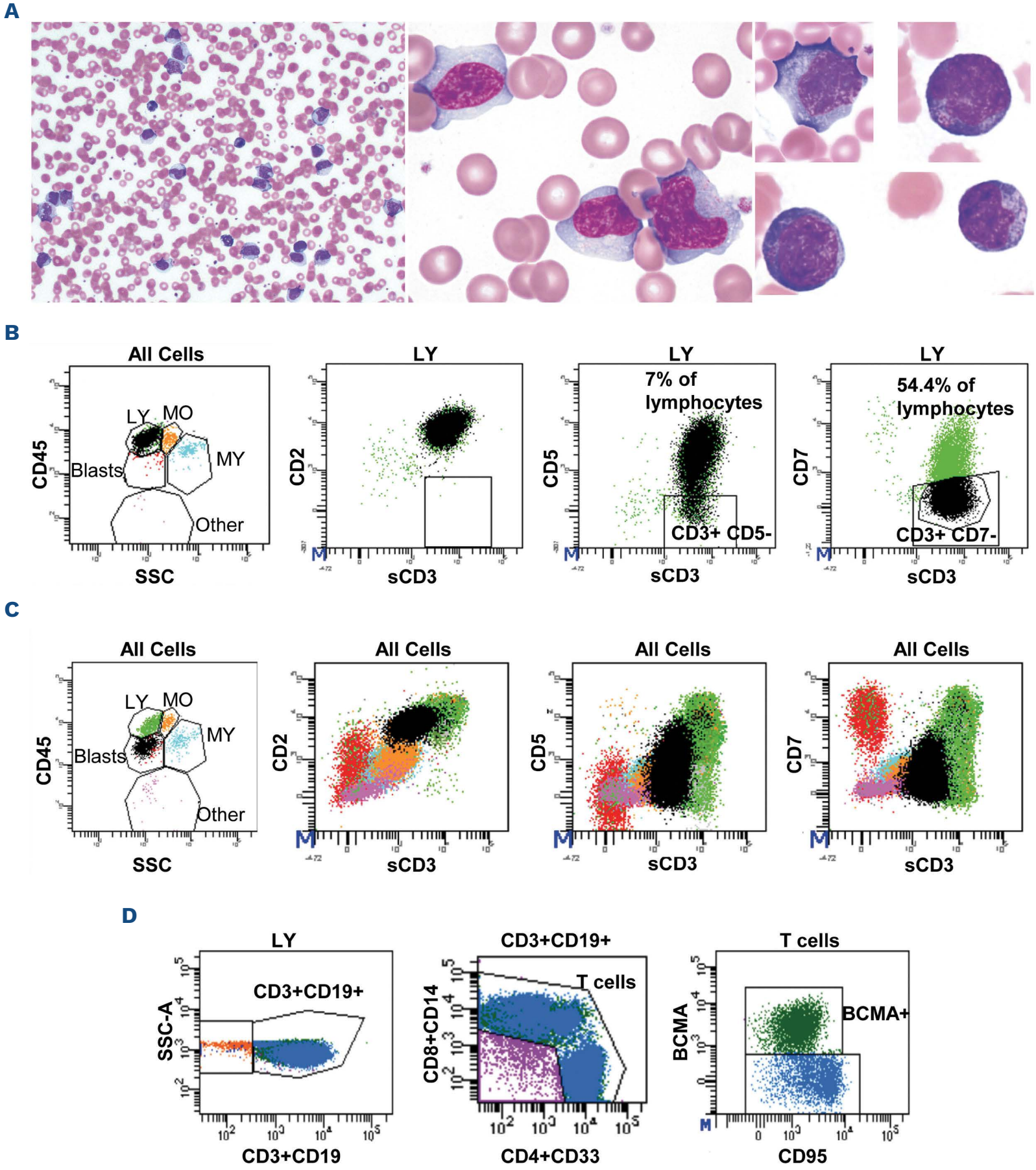


Figure 1. Morphologic and immunophenotypic features of lymphocytosis following BCMA CAR-T cell therapy. (A) Atypical lymphocytes identified on peripheral blood smears following BCMA CAR-T cell infusion. Wright–Giemsa stain. Left: 200x; top right: 1,000x; bottom right: 1,000x. (B) A representative flow cytometry plot demonstrating immunophenotypic features of T-lymphocytosis after BCMA CAR-T infusion (day +11). (C) A flow cytometry plot showing marked downregulation of CD45 in T cells from one patient (day +10). (D) Detection of expanded CAR-T cells using fluorophore-labeled BCMA protein, confirming substantial *in vivo* proliferation (day +10). BCMA: B-cell maturation antigen; CAR-T: chimeric antigen receptor T-cell; LY: lymphocytes; MO: monocytes; MY: myeloid cells.

CAR-T cells (Figure 2C, D). This inter-individual variability in CD4/CD8 predominance within the CAR-T cell populations suggests heterogeneity in the composition of the infusion products or differences in *in vivo* expansion dynamics. As

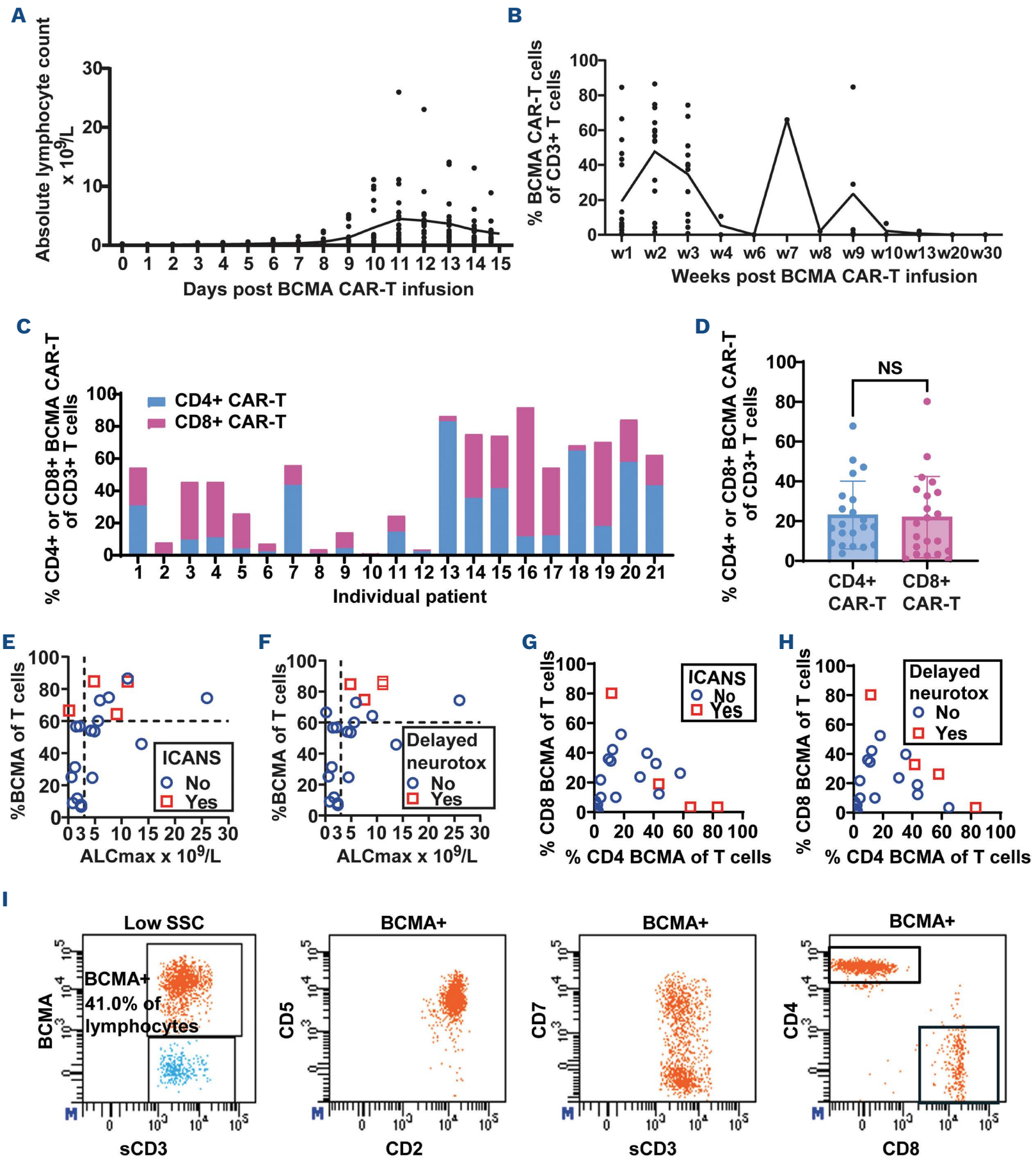


Figure 2. Kinetics of lymphocytosis, BCMA CAR-T cell expansion and association with neurotoxicity. (A) The kinetics of the ALC post-infusion across 21 patients (data derived from complete blood counts). (B) The expansion kinetics of BCMA CAR-T cells post-infusion, as detected by flow cytometry using fluorophore-labeled BCMA protein. (C) Individual percentages of $CD4^+$ and $CD8^+$ CAR-T cells. (D) A cohort-level summary of $CD4^+/CD8^+$ CAR-T cell percentages. (E-H) Scatter plots showing relationships between CAR-T expansion parameters and neurotoxicity outcomes. Each point represents an individual patient (N=21). (E) Association of ALC_{max}/BCMA CAR-T percentage with ICANS. (F) Association of ALC_{max}/BCMA CAR-T percentage with delayed neurotoxicity. (G) Association of $CD4^+/CD8^+$ CAR-T percentage with ICANS. (H) Association of $CD4^+/CD8^+$ CAR-T percentage with delayed neurotoxicity. (I) Detection of expanded CAR T cells in the cerebrospinal fluid of a patient with ICANS using fluorophore-conjugated BCMA protein. ALC: absolute lymphocyte count; BCMA: B-cell maturation antigen; CAR-T: chimeric antigen receptor T-cell; ALC_{max}: maximum ALC: ICANS: immune effector cell-associated neurotoxicity syndrome; NS: non-significant.

reported for CD19 CAR-T cells, the $CD4^+/CD8^+$ ratio varied among the infusion products.[11]

CRS and neurotoxicities, including ICANS and delayed neurotoxicity, are significant toxicities linked to BCMA CAR-T cell therapy. While CRS and ICANS are driven by cytokine release following the activation of CAR T cells, delayed

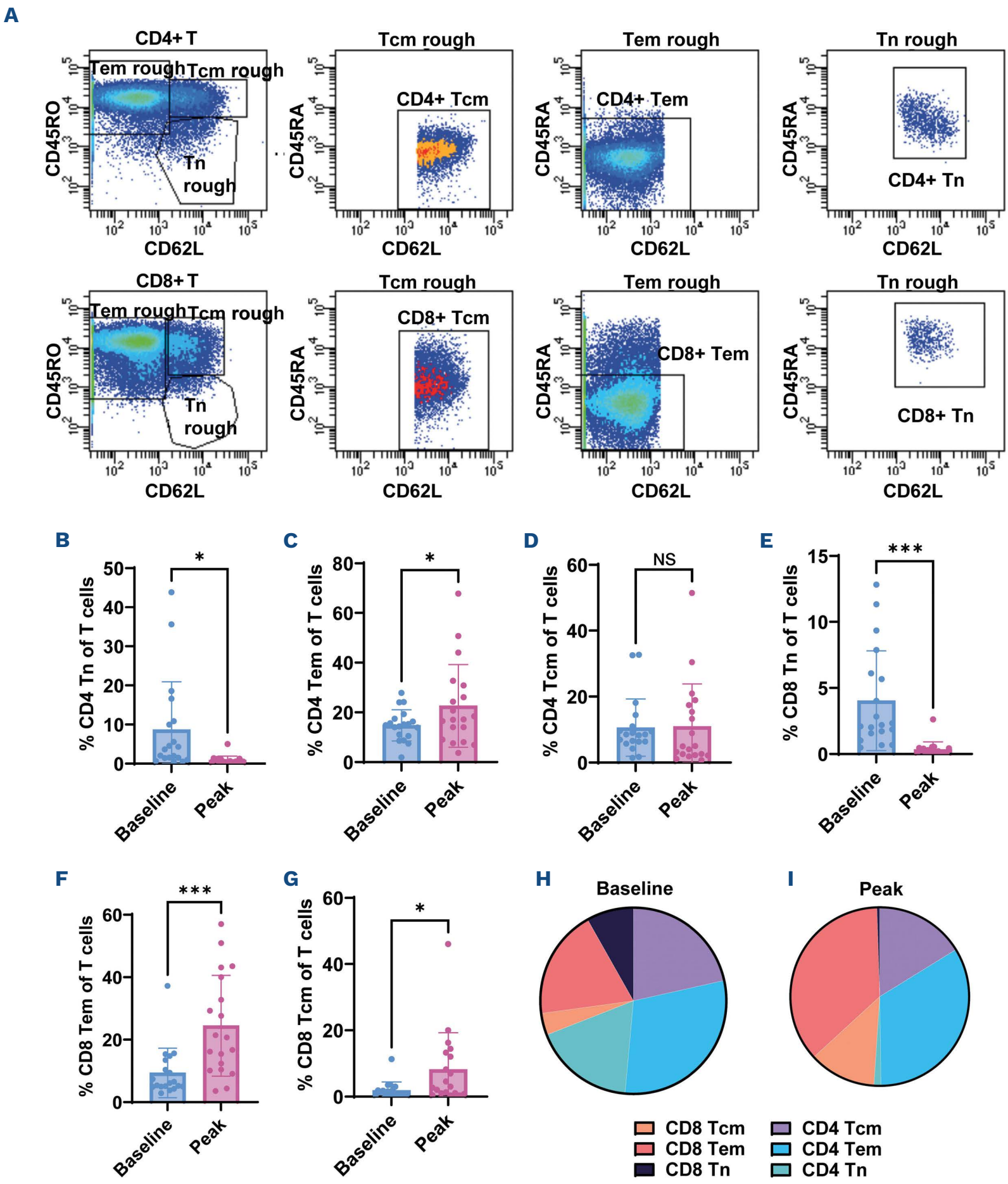


Figure 3. Alterations in T-cell subset composition following BCMA CAR-T cell infusion. (A) A representative flow cytometry plot illustrating the gating strategy for the delineation of T-cell subsets: naïve T cells (Tn; $CD62L^+CD45RA^+CD45RO^-$), effector memory T cells (Tem; $CD62L^-CD45RA^-CD45RO^{+/-}$), and central memory T cells (Tcm; $CD62L^+CD45RA^-CD45RO^+$). (B-G) Comparative analysis of T-cell subsets at baseline (day –5 or 0) and at peak CAR-T expansion: $CD4^+$ naïve T cells (B); $CD4^+$ effector memory T cells (C); $CD4^+$ central memory T cells (D); $CD8^+$ naïve T cells (E); $CD8^+$ effector memory T cells (F); $CD8^+$ central memory T cells (G). (H, I) Summary plots showing the overall distribution of T-cell subsets at baseline (H) and peak CAR-T expansion (I). BCMA: B-cell maturation antigen; CAR-T: chimeric antigen receptor T-cell. NS: non-significant. *$P \leq 0.05$; ***$P \leq 0.001$.

neurotoxicity is thought to result from CAR-T cell infiltration into the central nervous system. In our prospective cohort, the majority of patients (19/21, 90.5%) developed CRS of varying severity. ICANS was observed in four of 21 patients (19.0%), and delayed neurotoxicity occurred in a similar proportion (4/21, 19.0%). Patients who developed ICANS or delayed neurotoxicity generally had higher ALC_{max} values compared with those without neurotoxicity. Among patients with ALC_{max} $\geq 3\times10^9$/L,[12] 25.0% (3/12) developed ICANS and 33.3% (4/12) developed delayed neurotoxicity, compared to 11.1% (1/9) and 0% in those with ALC_{max} $<3\times10^9$/L. Neurotoxicity was also strongly associated with the degree of CAR-T expansion. Patients with ≥60% $BCMA^+$ CAR-T cells among total T cells showed higher rates of neurotoxicity. In this group, 44.4% (4/9) developed ICANS and 44.4% (4/9) developed delayed neurotoxicity. In contrast, no cases of either type of toxicity were observed in patients with <60% $BCMA^+$ CAR-T cells (Fisher exact test, P=0.0211 for both comparisons) (Figure 2E, F; *Online Supplementary Tables S2* and *S3*). In addition, ICANS was more frequent in patients with predominant $CD4^+$ CAR-T expansion (3/10, 30%) than in those with predominant $CD8^+$ expansion (1/11, 9.1%). Likewise, delayed neurotoxicity was more common in the $CD4^+$ predominant group (3/10, 30%) than the $CD8^+$ predominant group (1/11, 9.1%) (Figure 2G, H; *Online Supplementary Tables S2* and *S3*). Flow cytometric analysis of the cerebrospinal fluid was performed in one patient with delayed neurotoxicity, revealing a substantial population of BCMA CAR-T cells, predominantly of the $CD4^+$ phenotype (Figure 2I). Although limited by a small sample size, these findings suggest that both the expansion kinetics and the predominance of $CD4^+$ CAR-T cells may contribute to neurotoxicity. In this limited cohort, the factors associated with higher risk include higher ALC_{max} ($\geq 3\times10^9$/L), greater proportion of $BCMA^+$ CAR-T cells (≥60% of total T cells), and $CD4^+$ predominant CAR-T expansion. Larger cohort studies will be necessary to investigate this relationship further, and although BCMA CAR-T cells have been detected in the cerebrospinal fluid of patients with delayed neurotoxicity,[13] cerebrospinal fluid data from patients without neurotoxicity are lacking, so it is unclear if traffic of CAR-T is routinely seen or truly associated with neurotoxicity.

Lastly, we examined T-cell subset composition following CAR-T infusion. Previous studies in CD19-directed CAR-T therapies have shown that specific memory T-cell phenotypes are associated with improved clinical responses.[14,15] T-cell subset characterization included naïve T-cell (Tn; $CD62L^+CD45RA^+CD45RO^-$), effector memory T-cell (Tem; $CD62L^-CD45RA^-CD45RO^{+/-}$), and central memory T-cell (Tcm; $CD62L^+CD45RA^-CD45RO^+$) populations (Figure 3A). Similar to prior observations,[10] we found a marked increase in $CD4^+$ and $CD8^+$ Tem and $CD8^+$ Tcm subsets at the time of peak CAR-T expansion compared to baseline (collected on day -5 or day 0). Conversely, $CD4^+$ and $CD8^+$ naïve T-cell subsets were significantly decreased at peak expansion relative to baseline (Figure 3B-I). The shift from naïve to memory phenotype suggests robust memory differentiation during CAR-T expansion.

In summary, our study provides a detailed morphologic and immunophenotypic analysis of lymphocytosis following BCMA-targeted CAR-T cell therapy in patients with R/R MM. We demonstrate that the post-infusion lymphocytosis observed in these patients is primarily driven by CAR-T cell expansion, which is accompanied by distinct morphologic features and immunophenotypic changes. The ALC_{max} and CAR-T expansion typically occurred approximately 2 weeks after infusion, and both the magnitude and phenotype of expansion may contribute to the risk of neurotoxicity. Subset analysis revealed a preferential expansion of Tem and Tcm cells, alongside a reduction in Tn populations. The use of fluorophore-conjugated BCMA protein enabled the reliable detection of CAR-T cells using conventional flow cytometry. These findings highlight the importance of integrating clinical context with flow cytometric and morphologic data to differentiate reactive lymphocytosis from disease progression or secondary lymphoproliferative disorders. The results have practical implications for the clinical interpretation of post-CAR-T lymphocytosis and for future studies that aim to explore the relationships between CAR-T expansion dynamics, cell composition at apheresis/infusion, efficacy, and toxicity.

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MMS has participated in a speaker's bureau for Johnson & Johnson.

The other authors have no conflicts of interest to disclose.

Contributions

JNC contributed to the review of peripheral blood smears, data analysis, and manuscript drafting. PDS contributed to flow cytometric data analysis and manuscript review. KG and FC contributed to flow cytometric sample processing, data acquisition, and data analysis. DSJ contributed to the collection of patients' clinical data and samples. MB contributed to study design, patients' clinical data collection, and manuscript review. GGI contributed to study design, data analysis, flow cytometric sample processing, and manuscript review. JTG contributed to study design, peripheral blood smear review, data analysis, and manuscript review. MMS contributed to study design, collection of patients' clinical data, data analysis, and manuscript review.

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Data-sharing statement

The datasets generated and analyzed in this study are available from the corresponding author on reasonable request.

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