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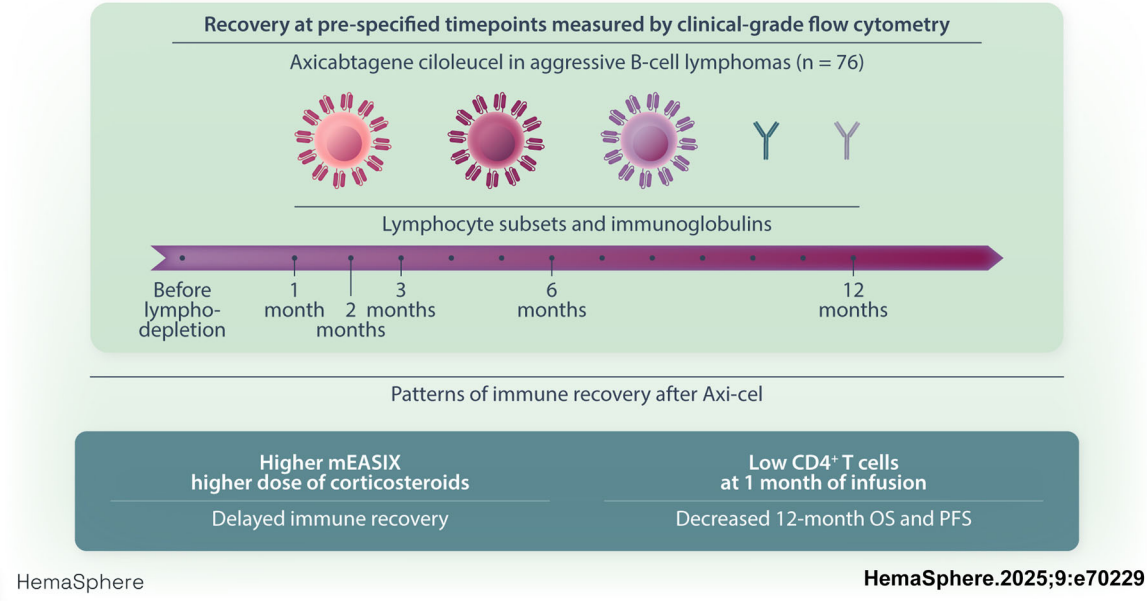
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ARTICLE

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Graphical Abstract



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Abstract

Hematotoxicity after anti-CD19 chimeric antigen receptor T-cell therapy has drawn attention for potential long-term effects, and yet, recovery of lymphocyte subsets and immunoglobulin levels beyond B-cell aplasia remains poorly understood. We retrospectively analyzed 76 consecutive axicabtagene ciloleucel (axi-cel) recipients with relapsed/refractory aggressive B-cell lymphoma between 2020 and 2024, focusing on basic immune recovery patterns and their impact on outcomes. Median $CD8^+$ T and NK cells increased to >300/mm^3 and >100/mm^3, respectively, within 2 months after infusion. $CD4^+$ T-cell recovery was slower, with 42% cumulative incidence of >200/mm^3 cell count at 12 months. B-cells were detectable in 18% at 12 months. Immunoglobulin levels initially declined and then plateaued, with 51% incidence of immunoglobulin G (IgG) levels > 400 mg/dL at 12 months. Modified EASIX > 2.00 was associated with delayed kinetics of $CD3^+$ T-cells, $CD4^+$ T-cells, and $CD8^+$ T-cells, and cumulative dexamethasone dose > 120 mg with delayed recovery of $CD4^+$ T-cells, NK cells, and IgG. At 1 month post-infusion, low $CD4^+$ T-cell count and high CAR-HEMATOTOX predicted worse 12-month progression-free survival (hazard ratio [HR] 2.81 and 3.34) and overall survival (HR 3.21 and 4.41), respectively. After axi-cel, $CD4^+$ T-cells and IgG recovered slowly during the first year, while $CD8^+$ T and NK cells increased rapidly. A higher modified EASIX score may predict slower cellular recovery, while corticosteroids may delay both cellular and humoral recovery. Lower $CD4^+$ T-cell count was associated with worse outcomes.

INTRODUCTION

Chimeric antigen receptor T-cells (CAR-T) have shown curative potential in aggressive B-cell lymphomas, but some concerning toxicities have nonetheless been observed.[1,2] Hematotoxicity, especially late-onset anemia, thrombocytopenia, neutropenia, and cellular and humoral defects, is of particular interest during long-term follow-up.[3–5] Anemia and thrombocytopenia have been extensively studied and reviewed in meta-analyses,[6] and the CAR-HEMATOTOX score, which includes bone marrow function (hemoglobin, neutrophil, and platelet counts) and inflammatory markers (ferritin and C-reactive protein), has been proven to be useful for predicting the incidence and duration of CAR-T-related hematotoxicity, as well as infectious complications and overall mortality.[5] Rejeski et al. found that biphasic neutrophil recovery was associated with improved survival and that prolonged neutropenia predisposed to severe infections, possibly increasing non-relapse mortality (NRM).[7] Regarding cellular immune recovery, recent data have shown that low $CD4^+$ T-cell and B-cell

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lymphocyte counts persist in 25%–60% of recipients at 12 months after CAR-T therapy,[4,8] although with subtle differences between anti-CD19 products.[9] Persistent $CD4^+$ T-cell count < 200 cells/mm^3 in older patients may increase the risk of late opportunistic infections, even beyond the first year after CAR-T therapy.[10] Persistent hypogammaglobulinemia, usually defined as immunoglobulin G (IgG) < 400 mg/dL, has also been reported in almost half the patients at 3 months[11] and 12 months.[4] With the exception of B-cell aplasia, which has been linked to CAR-T activity and disease relapse,[10] immune recovery profiles by lymphocyte subtype and gammaglobulin levels remain poorly characterized after anti-CD19 CAR-T therapy in aggressive B-cell lymphoma recipients.

This study aims to analyze basic cellular and humoral immune recovery patterns and their impact on CAR-T outcomes in a homogeneous cohort of axicabtagene ciloleucel (axi-cel) recipients for relapsed/refractory aggressive B-cell lymphomas.

METHODS

Patients and procedures

This retrospective analysis included all consecutive adult patients ≥ 18 years old with a diagnosis of aggressive B-cell lymphoma undergoing CAR-T-cell infusion with axi-cel at Hospital Universitari i Politècnic La Fe (HUPLF) and Hospital Clinic Universitari de València (HCUV) between January 2020 and July 2024. Inclusion criteria included patients with at least two available lymphocyte subpopulation and Ig determinations at different time points (including pre-infusion). Exclusion criteria included early death (≤28 days after CAR-T infusion). All patients provided informed consent for data collection. The study was approved by the institutional review board of both centers and was in accordance with the Declaration of Helsinki (reference 2025/588/Axi-RecIm for HUPLF and 2024/252 for HCUV).

Patients were enrolled to receive axi-cel as second-line therapy or beyond for refractory or relapsed disease. Bridging therapy while waiting for production was at the discretion of the referring physician and cell therapy unit team. Patients received lymphodepletion with fludarabine 30 mg/m^2 and cyclophosphamide 500 mg/m^2, at Days −5, −4, and −3 prior to CAR-T infusion (Day 0). All patients received anti-infectious prophylaxis with levofloxacin 500 mg and fluconazole 100 mg or posaconazole 300 mg per day (from admission or neutropenia till neutrophil recovery above 500/μL or 2–4 weeks after corticosteroid withdrawal in the case of posaconazole), trimethoprim-sulfamethoxazole 800/160 mg thrice weekly, and acyclovir 800 mg twice daily until 6–12 months after CAR-T and according to CD4 T-cell levels. Patients who developed cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were treated according to international practice recommendations.[12] Briefly, in HUPLF, tocilizumab 8 mg/kg/day up to 3 days was used as first line for Grade 1 CRS in the first 24 h after infusion, persistent Grade 1 fever (>72 h), or CRS Grade ≥2, and in case of deterioration or no response, dexamethasone was prescribed as second line. In HCUV, tocilizumab 8 mg/kg/day up to 3 days along with three doses of 10 mg dexamethasone every 12 h was initiated for CRS ≥ 2. For ICANS in HUPLF, dexamethasone 10 mg every 6 h was prescribed for Grade 2, dexamethasone 20 mg every 6 h or methylprednisone 1000 mg/day for 1–3 days for Grade 3, and in case of deterioration, anakinra at a maximum dose of 10 mg/kg every 8 h was used as second line. In HCUV, dexamethasone 10 mg every 6 h was started for ICANS Grade ≥2. In case of progression in the first 24 h or no response in 48–72 h, anakinra 8 mg/kg/day was prescribed until resolution. Prophylactic Ig replacement was not routinely performed, except in cases with severe infections (requiring additional hospitalization for intravenous antimicrobials) and IgG levels below 400 mg/mL.

Patients were closely monitored in the outpatient clinic after hospital discharge. They were instructed to stay at a 1-h distance from the hospital within the first month, and regular visits were scheduled during the first 3 months after CAR-T-cell infusion. Baseline disease status was assessed through PET-CT before lymphodepletion, at 3, 6, and 12 months after CAR-T for all patients, and when clinically indicated. After patient discharge from the ambulatory setting to the referral hospitals, follow-up was performed in person and/or by telephone, and electronic medical records were consulted for a minimum of 18 months after infusion.

Immune reconstitution monitoring

Immunophenotyping was performed using flow cytometry (FC) on fresh whole-blood samples collected in ethylenediaminetetraacetic acid to monitor the absolute lymphocyte count and lymphocyte subsets. The following lymphoid populations were quantified: T-cells ($CD3^+$), subdivided into $CD3^+$ $CD4^+$ T-cells and $CD3^+$ $CD8^+$ T-cells; B-cells ($CD19^+$); and NK cells ($CD3^-$ $CD16^+$ $CD56^+$). BD Multitest 6-color TBNK reagents (containing a combination of CD3, CD4, CD8, $CD16^+$ CD56, CD19, and CD45 monoclonal antibodies conjugated to fluorochromes) were used for this purpose. Samples were analyzed on a FACSCanto-II cytometer using FACSCanto software (Becton Dickinson, San Jose, CA, USA) at both centers. Automated cell gating was reviewed, and percentages and absolute counts were compared with the manufacturer's reference values. Lymphocyte counts and Ig levels were assessed at Days 30, 60, 90, 180, and 365 after CAR-T in both centers.

Definitions

Patients who relapsed after prior response to immunochemotherapy (at least partial remission [PR]) were classified as chemosensitive, while patients not achieving this response were categorized as refractory. B-cell aplasia was considered as indetectable $CD19^+$ cell count through FC analysis, as described above. Overall survival (OS) was defined as survival from the time of infusion, with death from any cause as an event. Progression-free survival (PFS) was defined as survival without lymphoma relapse/progression from the time of CAR-T-cell infusion. Adverse events were graded following the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. CRS and ICANS were graded based on retrospective review, following the American Society for Transplantation and Cellular Therapy consensus criteria.[13] CAR-HEMATOTOX[5] and modified EASIX (mEASIX: serum creatinine, lactate dehydrogenase, and platelet count)[14] were calculated prior to lymphodepletion. $CD4^+$ T-cell recovery was defined as a count >200/mm^3 and IgG recovery as levels >400 mg/dL.

Endpoints and statistical analyses

The primary endpoint was to characterize the kinetic pattern of lymphocyte subsets and Ig recovery from the time of infusion up to 12 months, using time-point analyses. The secondary outcomes were to estimate the incidence of immune recovery and identify factors that influenced immune recovery over time while exploring the impact of immune markers at 1 month after CAR-T-cell infusion in PFS and OS at 12 months.

Categorical variables were presented as percentages and compared using Pearson's chi-square test. Continuous variables were expressed as

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mean and median values, along with their respective ranges, and compared using the Mann–Whitney *U* or Kruskal–Wallis test.

Immune recovery kinetics were represented using absolute values along time, using the exact date of sample extractions. Locally estimated scatterplot smoothing curves were constructed to visually illustrate data trends. The 1-year cumulative incidence of $CD4^+$ T-cell, B-cell, and IgG recovery was estimated using a competing risks framework, where NRM and disease relapse were treated as competing events. For 1-year NRM cumulative incidence estimates, disease relapse was treated as the competing event. To analyze factors that impacted on kinetics for each lymphocyte subset or Ig subtype, we used a logarithmic transformation to construct linear regression models and interpret coefficients. Linear mixed-effect models were created to estimate slopes and 95% confidence intervals (CIs), with random intercepts for each patient and fixed effects for patient, disease, and CAR-T-related variables. Covariates with an adverse effect on immune recovery kinetics were represented by negative slope values.

Unadjusted time-to-event analyses were performed using Kaplan–Meier estimates and log-rank tests. To estimate the effect of lymphocyte subsets or Ig subtypes levels on OS and PFS at 12 months, we developed landmark Cox regression models, controlling for significant baseline and CAR-T-related covariates. Landmarks were established from the date of lymphocyte and Ig assessment at 1 month (±15 days) after CAR-T-cell infusion. To determine optimal thresholds for lymphocyte and Ig values, the *maxstat* R package was used,[15] along with a 10-fold cross-validation to prevent overfitting. A bootstrap procedure with 1000 iterations calculated the concordance index (c-index), and the cutoff with the highest c-index stratified patients into low- and high-count groups in Cox models.

For mixed-effects linear models, variable selection began with a full model approach, followed by stepwise backward selection based on the Akaike Information Criteria. In Cox models, the LASSO penalty was used, with 10-fold cross-validation determining the optimal lambda value while maintaining a 10:1 sample–variable ratio. In both analyses, bootstrapping procedures for model fitting were performed 1000 times on resampled data sets, retaining variables selected in at least 50% of iterations. Key covariates included patient characteristics, diagnosis, treatment responses, and immune recovery metrics. Statistical significance was set at $P < 0.05$, with analyses conducted using R version 4.1.3. For detailed statistical methods, see the Supplementary Material.

TABLE 1 Patient and disease characteristics.

Characteristics	
Number of patients, *n* (%)	76
Recipient age, years, median (range)	58 (21–78)
≥60 years, *n* (%)	36 (47)
Recipient sex, female, *n* (%)	32 (42)
B-cell lymphoma subtype, *n* (%)	
Diffuse large	58 (76)
High-grade	8 (11)
Primary mediastinal	10 (13)
Cell of origin, Hans algorithm, *n* (%)	
Germinal center	43 (57)
Non-germinal center	28 (37)
Not available	5 (6)
Previous therapy lines, median (range)	2 (1–4)
One, *n* (%)	1 (1)
Two, *n* (%)	60 (79)
Three, *n* (%)	9 (12)
Four or more, *n* (%)	6 (8)
Last response group, *n* (%)	
Refractory	32 (42)
Chemosensitive	44 (58)
CAR-line, third or more, *n* (%)	75 (99)
Bridging, *n* (%)	36 (47)
Response at lymphodepletion, *n* (%)	
Complete/partial remission	19 (25)
Stable/progressive disease	57 (75)
CAR-HEMATOTOX at lymphodepletion, *n* (%)	
Low	36 (47)
High	40 (53)
mEASIX at lymphodepletion, median (range)	1.98 (0.26–276)

RESULTS

Patient, disease, and CAR-T characteristics

We included 76 of 80 consecutive axi-cel recipients who fulfilled the inclusion criteria (two were excluded due to early death and another two due to missing data). Clinical characteristics and CAR-T outcomes for the whole cohort are reported in Tables S1 and S2. For longitudinal immune monitoring, patients' main clinical characteristics are summarized in Table 1. The median age was 58 years (range, 21–78), and the most common diagnosis was diffuse large B-cell lymphoma (76%), with 99% receiving CAR-Ts as a third or higher line of treatment. At lymphodepletion, 75% of recipients had stable or progressive disease and 53% presented high CAR-HEMATOTOX; the median mEASIX score was 1.98 (range, 0.26–276).

CAR-T complications and outcomes

The main complications after CAR-T are summarized in Table 2. CRS and ICANS were documented in 95% and 67% of patients, with 32% and 43% Grade ≥2, respectively. The median time until CRS and ICANS onset was 1 day (range, 0–9) and 7 days (range, 1–15), respectively. Overall, 26% of patients required tocilizumab for CRS Grade 1 and 32% for Grade ≥2, whereas 73% of patients received dexamethasone for CRS/ICANS Grade ≥2 and 17% anakinra for steroid-refractory ICANS. Eighty percent of patients achieved complete remission (CR) or PR as the best disease response after CAR-T.

Overall, 32 patients deceased at a median of 163 days after CAR-T infusion (range, 36–1680 days). Twenty-three out of 76 (30%) deceased from disease relapse/progression: 7 before Day +100, 10 from Day +100 to 12 months, and 6 beyond 12 months. Of the remaining nine patients (12%) who deceased from NRM causes, seven succumbed to infections at a median of 52 days after CAR-T infusion (range 37–830). Four patients deceased from bloodstream infection (one from *Candida krusei*, one from *Escherichia coli*, one from *both Pseudomonas aeruginosa* and *Stenotrophomonas maltophila*, and the last one from *Salmonella*), whereas two deceased patients from COVID-19. Finally, one patient deceased from microbiologically undocumented septic shock. Additionally, one patient deceased from late recurrent ICANS at 6 months (confirmed in autopsy by the presence of CAR-T-cells in cerebral tissue),

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TABLE 2 Chimeric antigen receptor T-cells (CAR-T)-related complications and outcomes.

Characteristics	
Number of patients, *n*	76
CRS, grade, *n* (%)	
1	48 (63)
2	20 (26)
3	4 (5)
4	1 (1)
CRS onset, days, median (range)	1 (0–9)
ICANS, grade, *n* (%)	
1	18 (24)
2	10 (13)
3	12 (16)
4	10 (13)
5	1 (1)
ICANS onset, days, median (range)	7 (1–15)
Tocilizumab doses, *n*, median (range)	1 (0–4)
No tocilizumab, *n* (%)	32 (58)
Cumulative dexamethasone (mg), median (range)	128 (0–3720)
No dexamethasone, *n* (%)	21 (28)
Anakinra doses, *n*, median (range)	0 (0–20)
No anakinra, *n* (%)	64 (12)
Best response after CAR-T, *n* (%)	
CR/PR	61 (80)
SD/PD	12 (16)
Not evaluated	3 (4)
Deaths, *n* (%)	32 (42)
Median follow-up for survivors, months (range)	19 (3–53)

Abbreviations: CR, complete response; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; PD, progressive disease; PR, partial response; SD, stable disease.

and one patient deceased from an unknown cause at 55 months. Twelve-month OS and PFS were 69% (95% CI 59–80) and 54% (95% CI 44–67), respectively. Twelve-month NRM was 8% (95% CI 3–16).

Lymphocyte and immunoglobulin measurements

Table 3 presents the lymphocyte subset counts and immunoglobulin measurements at various time points for evaluable patients, along with the missing data and reasons for study withdrawal over time. Overall, 256 data points for lymphocyte subsets and 230 for Ig were analyzed.

The median $CD4^+$ T-cell count recovery was slower than other lymphocyte subtypes (Figure 1A). The cumulative incidence of the first $CD4^+$ T-cell count > 200/mm^3 was 11% (95% CI 5–19) at 1 month, 24% (95% CI 15–34) at 2 months, 28% (95% CI 18–38) at 3 months, 34% (95% CI 24–35) at 6 months, and 42% (95% CI 30–53) at 12 months. The median $CD8^+$ T-cell counts increased steadily to >300/mm^3 and median NK cells increased to >100/mm^3 after 2 months of axi-cel infusion (Table 3). Finally, the cumulative incidence of detectable B-cells was 2.6% (95% CI 0.5–8.3) at 1 month, 5.3% (95% CI 1.7–12) at 2 months, 7.9% (95% CI 3.2–15) at 3 months, 13% (95% CI 6.7–22) at 6 months, and 18% (95% CI 9.8–27) at 12 months.

A total of five patients received intravenous Ig (IVIG) support within the first year of CAR-T therapy, administered at Months +2 (*n* = 1), +6 (*n* = 1), +7 (*n* = 1), and +9 (*n* = 2). Immunoglobulin levels were censored for those receiving IVIG support at the time of first supplementation (six time points; Table 3). The median immunoglobulin levels decreased in the first month after infusion and plateaued, recovering only at 12 months (Figure 1B). The cumulative incidence of IgG levels > 400 mg/dL was 33% (95% CI 23–44) at 1 month, 47% (95% CI 35–58) at 2 and 3 months, 50% (95% CI 38–61) at 6 months, and 51% (95% CI 39–62) at 12 months.

Factors influencing immune kinetics profile

Stepwise multivariate model analyses of factors influencing immune recovery kinetics of lymphocyte subsets and immunoglobulins are presented in Table 4. An elevated mEASIX score (>2.00) negatively impacted the recovery kinetics of $CD3^+$ T-cells, $CD4^+$ T-cells, and $CD8^+$ T-cells. Higher cumulative doses of dexamethasone used in the context of CRS and ICANS had a negative impact on $CD4^+$ T-cell, NK cell, and IgG recovery. Finally, anakinra negatively impacted $CD3^+$ T-cell recovery.

Factors associated with 1-year PFS and OS

Using landmark analysis at 1 month (±15 days) after CAR-T-cell infusion, 74 patients had available data points for lymphocyte subsets and 65 patients for Ig. After group stratification using the threshold maximizing differences in PFS, the factors negatively impacting 12-month PFS in univariate analysis were low $CD4^+$ T-cell count (≤43/mm^3, 38% of patients), low $CD8^+$ T-cell count (≤258/mm^3, 58% of patients), low NK cell count (≤21/mm^3, 27% of patients), and low IgG (≤498 mg/dL, 25% of patients). In multivariate analysis, we included the $CD4^+$ T-cell count, response to treatment, CAR-HEMATOTOX, tocilizumab, and dexamethasone cumulative doses. Significant variables for 12-month PFS were low $CD4^+$ T-cell count (hazard ratio [HR] 2.81, 95% CI 1.17–6.73, P = 0.020), disease refractoriness before axi-cel (HR 2.96, 95% CI 1.27–6.93, P = 0.012), and high CAR-HEMATOTOX (HR 3.34, 95% CI 1.32–8.42, P = 0.011). Using the 65 patients with lymphocyte subset and available Ig values, in multivariate analysis IgG levels, response to treatment, mEASIX, and dexamethasone cumulative dose were included. No immune recovery variable showed statistical significance. Figure 2A presents the PFS curves according to the $CD4^+$ T-cell count groups at 1 month post-infusion. Other significant variables in multivariate PFS analysis are available in Figure S1A,B.

For 12-month OS and again using landmark analysis at 1 month (±15 days), low $CD4^+$ T-cell count (≤43/mm^3, 38% of patients), low $CD8^+$ T-cell count (≤258/mm^3, 58% of patients), low NK cell count (≤21/mm^3, 27% of patients), and low IgG (≤302 mg/dL, 25% of patients) showed a negative impact in univariate analysis. In multivariate analysis, we included $CD4^+$ T-cell count, NK cell count, response to treatment, mEASIX, CAR-HEMATOTOX, and dexamethasone cumulative doses. Significant variables for 12-month OS were low $CD4^+$ T-cell count (HR 3.21, 95% CI 1.21–8.53, P = 0.019) and high CAR-HEMATOTOX (HR 4.41, 95% CI 1.51–12.85, P = 0.007). Using the 65 patients with lymphocyte subset and available Ig values, multivariate analysis included $CD4^+$ T-cell count, IgG levels, response to treatment, mEASIX, CAR-HEMATOTOX, and dexamethasone cumulative doses. IgG did not impact OS. In contrast, $CD4^+$ T-cell count (HR 2.79,

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TABLE 3 Lymphocyte subsets and immunoglobulins before and after chimeric antigen receptor T-cells (CAR-T).

Time points	Before apheresis	1 month (±15 days)	2 months (±15 days)	3 months (±15 days)	6 months (±30 days)	12 months (±30 days)
Patients	76	76	66	55	49	38
Lymphocyte subset, /mm^3, median (interquartile range)						
T-cells	800 (482–1176)	351 (138–575)	533 (363–906)	469 (334–956)	503 (356–1008)	724 (469–1152)
CD4$^+$ T-cells	300 (179–500)	80 (29–129)	112 (81–217)	158 (84–226)	175 (100–200)	212 (128–300)
CD8$^+$ T-cells	448 (192–705)	215 (71–442)	366 (207–671)	330 (152–653)	409 (210–753)	456 (188–1040)
B-cells	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–14)
NK cells	110 (52–200)	91 (20–170)	150 (78–262)	120 (70–236)	158 (88–267)	163 (108–309)
Missing, *n*	-	2	12	7	16	10
Immunoglobulins, mg/dL, median (interquartile range)						
G	535 (421–754)	405 (304–539)	382 (295–521)	371 (264–492)	326 (238–449)	325 (243–560)
A	100 (55–143)	34 (20–53)	27 (17–38)	25 (12–43)	31 (10–43)	36 (21–54)
M	36 (24–48)	15 (11–26)	14 (9–26)	16 (8–26)	13 (7–24)	26 (6–37)
Missing, *n*	-	11	20	6	15	11
Excluded for IVIG, *n*	-	-	1	1	2	2
Excluded patients, *n*						
NRM	-	-	4	-	-	2
Progression	-	-	6	11	6	4
No follow-up	-	-	-	-	-	5

Abbreviations: IVIG, intravenous immunoglobulin; NRM, non-relapse mortality.

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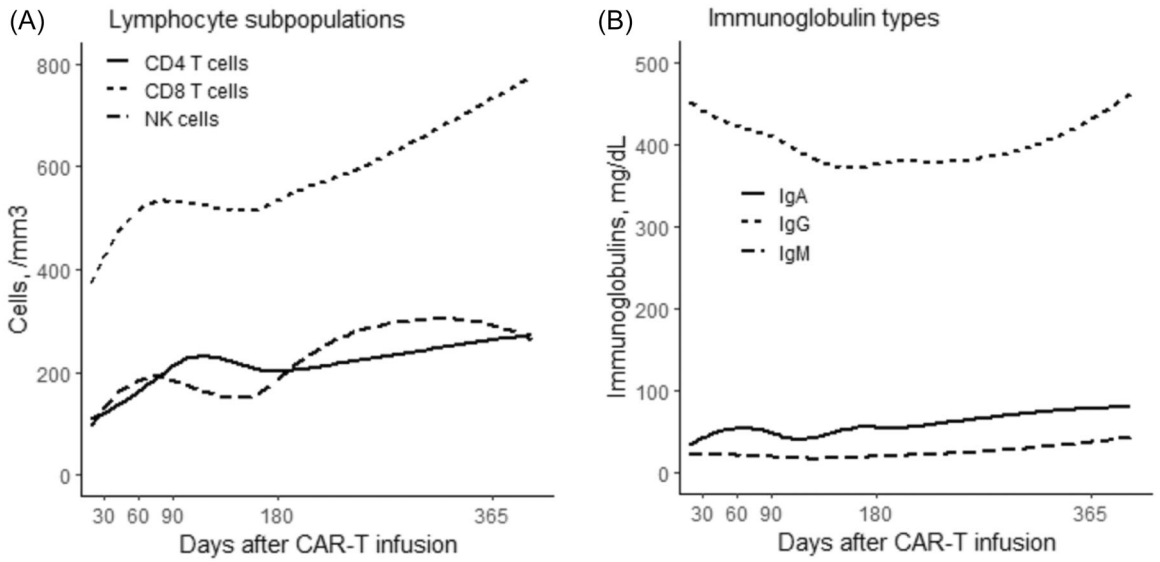


FIGURE 1 **Loess-smoothed curves of recovery in (A) lymphocyte subpopulations and (B) immunoglobulin (Ig) types.** CAR-T, chimeric antigen receptor T-cells.

95% CI 1.07–7.26, P = 0.036), chemorefractoriness (HR 3.25, 95% CI 1.27–8.60, P = 0.018), and high CAR-HEMATOTOX (HR 4.08, 95% CI 1.27–13.1, P = 0.018) were associated with worse OS. Figure 2B presents the OS curves according to $CD4^+$ T-cell count groups. Other significant variables in multivariate OS analysis are available in Figure S2A,B.

DISCUSSION

This study describes the immune recovery patterns of lymphocytes and immunoglobulins after axi-cel and their potential impact on outcomes. While $CD8^+$ T-cells and NK cells showed a steady increase within the first months post-infusion, $CD4^+$ T-cells' and IgG recovery occurred more gradually, but the $CD4^+$ T-cell count remained below 200 cells/mm^3 and IgG levels below 400 mg/dL in a substantial proportion of patients at 12 months after CAR-T. Our analyses revealed that a high pre-infusion mEASIX was associated with slower lymphocyte subset recovery, whereas higher cumulative doses of corticosteroids predicted slower $CD4^+$ T and NK cell and humoral recovery. Notably, lower $CD4^+$ T-cell count at 1 month post-infusion and high CAR-HEMATOTOX were both associated with worse PFS and OS. Our results depict the immune reconstitution kinetics and highlight the clinical implications of faster immune recovery in terms of outcomes in axi-cel recipients with aggressive B-cell lymphoma.

Our findings on $CD4^+$ T-cell recovery following CAR-T therapy are in line with previous reports,[16,17] as only 42% of patients were able to recover $CD4^+$ above 200 cells/mm^3 at 12 months. In contrast, we confirmed that NK cells and $CD8^+$ T-cells have faster recovery patterns, as previously reported.[4] This phenomenon may be partly attributed to the intrinsic higher proliferative capability of $CD8^+$ T-cells and/or higher proliferation in response to infections.[4,18] Intriguingly, this immune recovery pattern is very similar in the allogeneic stem cell transplant setting.[19] We also found that the median immunoglobulin levels show an early decline after infusion, reaching a nadir at 6 months and recovering IgG values above 400 mg/dL in 51% of patients at 12 months, similar to the percentage observed (47%) in a prior study.[4] Our observation is also consistent with a prior study that reported an IgG nadir at 6 months and 53% low IgG levels at 12 months after CAR-T.[16] Overall, our results support an association between axi-cel and hypogammaglobulinemia,[20] which may last over 12 months. B-cell aplasia persisted in more than 90% at 6 months and in over 70% at 12 months in our cohort. The rate of B-cell aplasia in our series is higher than previously reported rates, which range from 25% to 60%.[10,16,21,22] Given the high B-cell aplasia rate in our series, we were not able to detect any associated conditions. However, the higher steroid and anakinra use in our study, despite similar levels of CRS and neurological complications, may partly explain this finding. Likewise, differences between B-cell aplasia definitions may also account for these discrepancies.[4]

Interestingly, we found two conditions associated with delayed immune recovery: higher mEASIX predicted slower $CD3^+$, $CD4^+$, and $CD8^+$ T-cell recovery, while higher cumulative corticosteroid doses delayed $CD4^+$ T-cell, NK cell, and humoral recovery. This is in contrast with a prior study in which delayed $CD4^+$ T-cell recovery was correlated with older age but not with previous lines of therapy, bridging regimen, CRS severity, or use of corticosteroids.[10] The mEASIX has recently attracted interest for predicting complications and lower OS after CAR-T.[14,23] In our study, a high mEASIX score was associated with delayed cellular recovery (including $CD4^+$ T-cells) at 1 month post CAR-T. Low $CD4^+$ T-cell count, in turn, predicted lower OS. This relationship may explain why mEASIX can predict OS, thereby supporting the consistency of our findings. Nonetheless, this observation should be confirmed in future studies. The negative effect of corticosteroids on immune recovery is not unexpected, given that they suppress NK cell activity, reduce the function of phagocytic and antigen-presenting cells, and decrease $CD4^+$ and $CD8^+$ T lymphocyte levels.[24] In contrast to a previous large retrospective study,[9] the CAR-HEMATOTOX score was not useful in predicting slow immune recovery in our series. This difference may be partly attributable to the weight in our cohort of post-infusion variables not included in this score, such as the use of corticosteroids and lower rates of tocilizumab.

Our study found that slower $CD4^+$ T-cell recovery negatively impacted both 1-year PFS and OS, consistent with a previous publication.[9] Although infectious complications were not specifically addressed in this work, it is conceivable that impaired immune recovery confers greater infectious risk, which consequently contributes to higher NRM.[25] Besides lower infectious risk, immune recovery is not typically regarded as impacting durable disease remission to the same extent as other

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TABLE 4 Stepwise backward multivariate analyses of patient- and chimeric antigen receptor T-cells (CAR-T)-related variables in immune recovery after CAR-T.

Variables*	$CD3^+$ T-cells		$CD4^+$ T-cells		$CD8^+$ T-cells		NK cells		IgG		IgA		IgM	
	Slope (95% CI)	P	Slope (95% CI)	P	Slope (95% CI)	P	Slope (95% CI)	P	Slope (95% CI)	P	Slope (95% CI)	P	Slope (95% CI)	P
Age (years), >60	#		#		#		#		#		#		#	
Sex, male	#		#		#		#		#		0.50 (0.06, 0.94)	0.026	#	
B-cell lymphoma by histology	#		#		#		#		#		#		#	
Response group, refractory	#		#		#		#		#		#		#	
Previous lines, continuous	#		#		#		#		#		#		#	
Bridge therapy, yes	#		#		#		#		#		#		#	
Response at LD, SD/PD	#		#		#		#		#		#		#	
CARHEMATOTOX at LD, high	#		#		#		#		#		#		−0.36 (−0.70, −0.02)	0.036
mEASIX at LD, >2.00	−0.53 (−0.98, −0.08)	0.021	−0.59 (−0.99, −0.19)	0.005	−0.65 (−1.20, −0.09)	0.022	#		#		#		#	
Tocilizumab, yes	#		#		#		#		#		#		#	
Dexamethasone, >120 mg	#		−0.73 (−1.12, −0.33)	0.001	#		−0.67 (−1.11, −0.23)	0.004	−0.32 (−0.55, −0.10)	0.005	#		#	
Anakinra, yes	−0.86 (−1.50, −0.22)	0.009	#		#		#		#		#		#	

Note: *, all variables were included in multivariable models; #, excluded in the stepwise model.

Abbreviations: Ig, immunoglobulin; LD, lymphodepletion; PD, progressive disease; SD, stable disease.

factors such as initial response, baseline tumor volume, extramedullary disease, or lymphodepletion regimen.[26] However, our study found that a lower $CD4^+$ T-cell count negatively impacted PFS and OS, as did the CAR-HEMATOTOX score. One could hypothesize that a dysregulated immune system early after CAR-T infusion hinders its effectiveness, coinciding with observations in a tumor microenvironment (TME) study, where an immunosuppressive TME inhibited CAR-T-cell function.[27] However, our findings contrast with those of a recent study,[28] in which NK cell recovery, but not $CD4^+$ T-cell recovery, was associated with improved PFS and OS in patients treated with axi-cel, tisagenlecleucel, or lisocabtagene maraleucel. Several differences between our study and that of Luan et al. may partly explain this discrepancy. The most relevant include the lack of fixed time points for assessing immune cell recovery in that study, which may have increased analytical variability. Furthermore, this study did not account for cumulative steroid exposure, a factor known to impact lymphocyte recovery. Additionally, Luan et al. reported an inverse correlation between inflammatory markers, including lower levels of CRP, ferritin, TNF-α, and IL-10, and NK cell recovery. This suggests that NK cell recovery may serve as a surrogate marker of more effective disease control at the time of CAR-T infusion. Further research is needed to clarify the specific contributions of individual lymphocyte subsets to CAR-T-cell therapy outcomes.

Finally, our findings support the utility of immune recovery monitoring after cellular therapy. The EBMT published a multicenter study on immune monitoring, in which 61% of centers acknowledged the impact of monitoring on clinical decisions, though only 24% used this information to guide prophylactic or preemptive CRS/ICANS treatment and 37% to guide anti-infectious prophylactic strategies.[29] There is a clear need for immune monitoring guidelines, including management actions to be taken, so that CAR-T centers can standardize practices and foster international collaboration. Prospective studies on immune reconstitution are also warranted.

Our study has several limitations that warrant consideration. First, the lymphocyte subpopulation analysis was restricted to $CD4^+$, $CD8^+$, NK, and B-cell lymphocyte subsets, which limited a detailed characterization, such as cell activation and exhaustion, and outcome correlation. Axi-cel CAR-T-cell expansion and/or persistence were not analyzed, thus precluding correlation with immune recovery. The retrospective nature and a somewhat small sample size could be seen as further drawbacks. The limited number of observations may have reduced the statistical power of our tests, and variables that may have subtle but clinically relevant impact on outcomes may have not been detected. Additionally, the potential for overfitting in the value cut-offs and predictive models for immune recovery and outcomes should be considered. Therefore, it is essential that these findings be validated in larger cohorts to enhance their reliability and generalizability. Finally, as most patients received axi-cel in third or later lines of treatment, our findings may not be generalizable to second-line therapy. However, our study also has notable strengths. By focusing on a single CAR-T product and specific B-cell histological subtypes, we ensured a uniform evaluation, enhancing the consistency of results. Moreover, we comprehensively evaluated patient and disease characteristics, along with CAR-T management variables, enabling a robust analysis of immune recovery factors. Finally, the use of an accessible lymphocyte subset and Ig panels in daily clinical practice supports the real-world applicability of our findings.

In conclusion, $CD4^+$ T-cells and IgG show slow recovery through the first year after axi-cel, while $CD8^+$ T-cell and NK cell counts increase steeply during the first months. Higher mEASIX may predict slower cellular recovery, while corticosteroids may slow both cellular and humoral recovery. Lower $CD4^+$ T-cells are associated with worse outcomes. Harmonization and prospective studies are needed to establish the role of immune monitoring after CAR-T in aggressive B-cell lymphoma.

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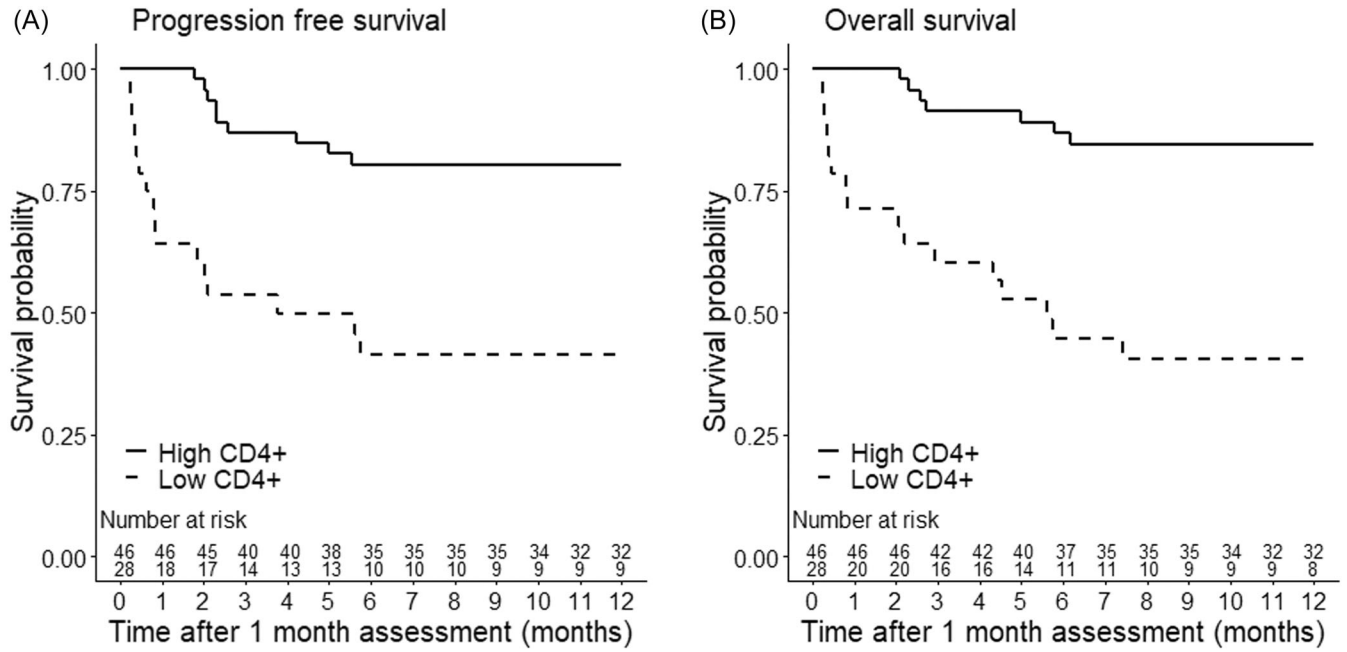


FIGURE 2 CD4⁺ T-cell count group impact on (A) progression-free survival and (B) overall survival.

AUTHOR CONTRIBUTIONS

André Airosa Pardal: Conceptualization; data curation; formal analysis; writing—original draft; writing—review and editing. **Ana Benzaquén**: Conceptualization; data curation; writing—original draft; writing—review and editing. **Pablo Granados**: Writing—review and editing. **Paula Amat**: Writing—review and editing. **Rafael Hernani**: Writing—review and editing. **Aitana Balaguer-Roselló**: Writing—review and editing. **Ariadna Pérez**: Writing—review and editing. **Marta Villalba**: Writing—review and editing. **Pedro Asensi**: Writing—review and editing. **Blanca Ferrer**: Writing—review and editing. **Lourdes Cordón**: Writing—review and editing. **Irene Pastor-Galán**: Writing—review and editing. **Juan Montoro**: Writing—review and editing. **María José Remigia**: Writing—review and editing. **Juan Carlos Hernández-Boluda**: Writing—review and editing. **Amparo Sempere**: Writing—review and editing. **Jaime Sanz**: Writing—review and editing. **María José Terol**: Writing—review and editing. **Carlos Solano**: Writing—review and editing. **Pedro Chorão**: Writing—review and editing; writing—original draft; conceptualization; data curation; formal analysis. **José Luis Piñana**: Conceptualization; writing—original draft; writing—review and editing; formal analysis.

CLINICAL TRIAL REGISTRATION

Does not apply.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The study was approved by the institutional review board of both centers and was in accordance with the Declaration of Helsinki (reference 2025/588/Axi-RecIm for HUPLF and 2024/252 for HCUV).

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PATIENT CONSENT

All patients provided informed consent for data collection.

SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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REFERENCES

1. Hines MR, Knight TE, McNerney KO, et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. *Transplant Cell Ther*. 2023;29(7):438.e1-438.e16.
2. Perna F, Parekh S, Diorio C, et al. CAR T-cell toxicities: from bedside to bench, how novel toxicities inform laboratory investigations. *Blood Adv*. 2024;8(16):4348-4358.
3. Zhuang TZ, Rupji M, Santapuram PR, Graiser M, Romancik JT, Cohen JB. Real-world analysis of cytopenias after chimeric antigen receptor T-cell therapy: a comparison of BCMA and CD19 based outcomes. *Blood*. 2022;140(suppl 1):12133-12134.
4. Galli E, Fresa A, Bellesi S, et al. Hematopoiesis and immune reconstitution after CD19 directed chimeric antigen receptor T-cells (CAR-T): a comprehensive review on incidence, risk factors and current management. *Eur J Haematol*. 2024;112(2):184-196.
5. Rejeski K, Perez A, Sesques P, et al. CAR-HEMATOTOX: a model for CAR T-cell–related hematologic toxicity in relapsed/refractory large B-cell lymphoma. *Blood*. 2021;138(24):2499-2513.

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6. Gagelmann N, Bishop M, Ayuk F, et al. Axicabtagene ciloleucel versus tisagenlecleucel for relapsed or refractory large B cell lymphoma: a systematic review and meta-analysis. *Transplant Cell Ther*. 2024;30(6):584.e1-584.e13.
7. Rejeski K, Perez A, Iacoboni G, et al. Severe hematotoxicity after CD19 CAR-T therapy is associated with suppressive immune dysregulation and limited CAR-T expansion. *Sci Adv*. 2023;9(38):eadg3919.
8. Wudhikarn K, Perales MA. Infectious complications, immune reconstitution, and infection prophylaxis after CD19 chimeric antigen receptor T-cell therapy. *Bone Marrow Transplant*. 2022;57(10):1477-1488.
9. Stock S, Bücklein VL, Blumenberg V, et al. Prognostic significance of immune reconstitution following CD19 CAR T-cell therapy for relapsed/refractory B-cell lymphoma. *HemaSphere*. 2025;9(1):e70062.
10. Baird JH, Epstein DJ, Tamaresis JS, et al. Immune reconstitution and infectious complications following axicabtagene ciloleucel therapy for large B-cell lymphoma. *Blood Adv*. 2021;5(1):143-155.
11. Hill JA, Li D, Hay KA, et al. Infectious complications of CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy. *Blood*. 2018;131(1):121-130.
12. Hayden PJ, Roddie C, Bader P, et al. Management of adults and children receiving CAR T-cell therapy: 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). *Ann Oncol*. 2022;33(3):259-275.
13. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant*. 2019;25(4):625-638.
14. Pennisi M, Sanchez-Escamilla M, Flynn JR, et al. Modified EASIX predicts severe cytokine release syndrome and neurotoxicity after chimeric antigen receptor T cells. *Blood Adv*. 2021;5(17):3397-3406.
15. Lausen B, Schumacher M. Maximally selected rank statistics. *Biometrics*. 1992;48(1):73.
16. Jennifer M. Logue L, Elisa Zucchetti Z, Christina A. Bachmeier B, et al. Immune reconstitution and associated infections following axicabtagene ciloleucel in relapsed or refractory large B-cell lymphoma. *Haematologica*. 2020;106(4):978-986.
17. Paolo Strati S, Ankur Varma V, Sherry Adkins A, et al. Hematopoietic recovery and immune reconstitution after axicabtagene ciloleucel in patients with large B-cell lymphoma. *Haematologica*. 2020;106(10):2667-2672.
18. Seder RA, Ahmed R. Similarities and differences in $CD4^+$ and $CD8^+$ effector and memory T cell generation. *Nat Immunol*. 2003;4(9):835-842.
19. Storek J. Immunological reconstitution after hematopoietic cell transplantation – its relation to the contents of the graft. *Expert Opin Biol Ther*. 2008;8(5):583-597.
20. Wat J, Barmettler S. Hypogammaglobulinemia after chimeric antigen receptor (CAR) T-cell therapy: characteristics, management, and future directions. *J Allergy Clin Immunol In Pract*. 2022;10(2):460-466.
21. Deyà-Martínez A, Alonso-Saladrigues A, García AP, et al. Kinetics of humoral deficiency in CART19-treated children and young adults with acute lymphoblastic leukaemia. *Bone Marrow Transplant*. 2021;56(2):376-386.
22. 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.
23. Gavriilaki E, Tzannou I, Batsis I, et al. EASIX and m-EASIX predict severe cytokine release syndrome and overall survival after CAR T-cell therapy. *Blood Vessel Thromb Hemost*. 2024;1(4):100025.
24. SCHACKE H. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther*. 2002;96(1):23-43.
25. Cordas Dos Santos DM, Tix T, Shouval R, et al. A systematic review and meta-analysis of nonrelapse mortality after CAR T cell therapy. *Nat Med*. 2024;30(9):2667-2678.
26. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat Rev Clin Oncol*. 2023;20(6):359-371.
27. Liu Z, Lei W, Wang H, Liu X, Fu R. Challenges and strategies associated with CAR-T cell therapy in blood malignancies. *Exp Hematol Oncol*. 2024;13(1):22.
28. Luan D, DeWolf S, Fei T, et al. Dynamics of immune reconstitution and impact on outcomes across CAR-T cell products in large B-cell lymphoma. *Blood Cancer Discov*. 2025;6(2):119-130.
29. Pagliuca S, Malard F, Mooyaart JE, et al. The landscape of immune monitoring in CAR-T cell therapy: a comprehensive review and survey study by the Cellular Therapy and Immunobiology Working Party of the EBMT. *Blood Rev*. 2025;71:101272.

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