

Differential Immune Dynamics in Large B-Cell Lymphoma Patients Treated With Axi-Cel and Tisa-Cel Car-T Cell Products

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Abstract

CD19-directed chimeric antigen receptor (CAR)-T-cell therapy has improved the outcome of patients with relapsed/refractory (R/R) large B-cell lymphoma (LBCL), yet the biologic determinants underlying differences among CAR-T products remain incompletely understood. We prospectively studied 67 patients treated with tisagenlecleucel (tisa-cel; n = 28) or axicabtagene ciloleucel (axi-cel; n = 39), integrating baseline inflammatory variables with longitudinal flow cytometry, stimulated intracellular cytokine production, plasma cytokines, and infusion-product phenotyping. A lower pre-infusion inflammatory profile, reflected by reduced ferritin, LDH levels and CAR-HEMATOTOX score, was associated with early complete response and improved hematologic recovery, supporting baseline immune fitness as a key determinant of treatment outcome. Following infusion, immune kinetics were shaped by both tumor burden and CAR construct. In patients with low tumor burden, tisa-cel induced a greater expansion of circulating T cells without translating into superior tumor control, whereas axi-cel achieved more durable responses despite lower peripheral lymphocyte counts, suggesting a qualitative immune advantage. Axi-cel induced a rapid *in vivo* CAR⁺ expansion, a more balanced CD4⁺/CD8⁺ CAR-T ratio, a differentiation towards effector and exhausted phenotypes, and a pronounced bystander activation of endogenous CAR⁻ T cells. Cytokine profiling identified IL-8 as an important mediator, associated with neurotoxicity, reduced circulating lymphocytes and inferior clinical outcomes. Collectively, these findings define distinct biologic programs associated with axi-cel and tisa-cel administration and support a model in which CAR-T-cell efficacy in R/R LBCL is driven by qualitative immune remodeling integrating host inflammatory fitness with construct-specific immune reprogramming.

INTRODUCTION

Chimeric antigen receptor (CAR)-T-cell therapy produces durable and potentially curative remissions in patients with relapsed/refractory (R/R) large B-cell lymphoma (LBCL) [1–3]. The approved CD19-directed products tisagenlecleucel (tisa-cel) and axicabtagene ciloleucel (axi-cel) incorporate 4-1BB and CD28 costimulatory domains, respectively, generating distinct expansion, persistence, differentiation, metabolic, efficacy, and toxicity profiles [4]. CD28 signaling favors rapid expansion and glycolytic effector differentiation, whereas 4-1BB signaling tends to preserve mitochondrial fitness and memory-like states [5].

Treatment outcome, however, is not determined solely by the magnitude or duration of peripheral CAR-T-cell expansion. Host immune status, tumor burden, systemic inflammation, and infusion-product composition and fitness interact in determining response and toxicity [6, 7]. High-dimensional studies in LBCL have associated response with activated, memory-like, and multifunctional CD8⁺ CAR-T-cell populations, whereas exhausted CD8⁺ states and regulatory CAR-T-cell subsets have been linked to resistance [8–10]. These findings underscore the importance of evaluating CAR-T-cell differentiation and functional heterogeneity in addition to their overall kinetics.

CAR-T-cell therapy also induces remodeling of the non-engineered host immune compartment. CAR⁺ and CAR⁻ T cells can undergo partially shared differentiation trajectories after infusion, and longitudinal immune profiling has associated durable responses with the activation and clonotypic expansion of native cytotoxic and effector-memory T cells, together with preserved NK-cell function [11, 12]. Consistently, our group previously showed that tisa-cel can induce prolonged bystander activation of endogenous lymphoid populations and enhance their cytokine-producing capacity [13]. While such immune-mediated mechanisms are central to the therapeutic efficacy, they also contribute to cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which remain important determinants of treatment safety [14].

Despite growing clinical experience, the biologic determinants of both efficacy and toxicity, particularly the relative contributions of CAR⁺ vs non-CAR-T cells, as well as the dynamic evolution of lymphocyte maturation states following infusion, remain insufficiently understood. We therefore compared the composition of tisa-cel and axi-cel infusion products and longitudinally characterized circulating CAR⁺ and CAR⁻ lymphocyte populations, their cytokine-producing capacity, plasma cytokine profiles, and patterns of maturation, exhaustion, and senescence in patients with R/R LBCL.

MATERIALS AND METHODS

Patients and treatment characteristics

Sixty-seven patients with R/R LBCL were prospectively enrolled at the Sapienza Hematology Center of Rome and treated with commercial tisa-cel (n = 28) or axi-cel (n = 39). After leukapheresis and optional bridging therapy, patients received fludarabine/cyclophosphamide or bendamustine lymphodepletion followed by inpatient CAR-T-cell infusion. Adverse events were graded according to ASTCT criteria [15] and responses were assessed by PET at months 1, 3, and 6 using the Lugano classification [16]. Samples were collected after written informed consent in accordance with the Declaration of Helsinki and local Ethic Committee approval (CE 5629, 11/21/2019).

Immunophenotyping and intracellular cytokine production

Multiparametric flow cytometry was performed on peripheral blood (PB) to evaluate major lymphocyte populations at predefined timepoints: at T0 (post-lymphodepletion, pre-infusion), and at days 3 (T3d), 7 (T7d), 14 (T14d), 28 (T28d) and months 3 (T3M) and 6 (T6M). IFN γ and TNF α production was assessed by intracellular staining after stimulation. In a subset of patients, we also evaluated the CAR-T infusion products and the patients' peripheral blood mononuclear cells (PBMCs) before lymphodepletion (Pre-LD) and at days 10 (T10d) and 28 (T28d) to assess CAR⁺ cells; naïve, central-memory, effector-memory, terminal effector memory (TEMRA) subsets; and exhaustion and senescence markers within CAR⁺ and CAR⁻ compartments. All staining procedures were performed using fluorochrome-conjugated monoclonal antibodies panels as detailed in the Supplemental Methods and Supplemental Table S1A-B.

Stained cells were acquired using the FACS Celesta (Becton Dickinson, BD, San Jose, CA) flow cytometer, collecting at least 30000 events.

Plasma cytokine determination

For plasma collection, PB samples supernatants were harvested and stored at -80°C until cytokine analysis. Plasma concentrations of IL-2, IL-4, IL-6, IL-8, IL-10, IL-17a, IFN γ , TNF α , GM-CSF and MIP-1 α were quantitatively determined using the Cytometric Bead Array (CBA) technique (BD) according to the manufacturer's instructions. Following the acquisition of samples on the FACS Celesta flow cytometer, the results were generated using the CBA Software FCAP 3.0.1 (BD).

RESULTS

Patients' toxicities and responses

A total of 67 patients received CAR-T-cell therapy; 39 (58.2%) were treated with axi-cel and 28 (41.8%) with tisa-cel. The median age at infusion was 51.7 years (range 20–71), and most patients were male (41/67, 61.2%). Baseline characteristics showed several descriptive differences between treatment groups. Tisa-cel-treated patients were older than axi-cel-treated patients, with a median age of 59.4 years (34–71) versus 49.2 years (20–70), respectively. Consistent with the product-specific indications approved at the time of treatment, all patients receiving tisa-cel had undergone at least two previous lines of therapy, compared to 74.4% of those receiving axi-cel; the remaining 25.6% of the axi-cel cohort received only one previous line of therapy.

The tisa-cel cohort also presented more frequently with adverse disease-related features, including an IPI ≥ 2 (60.7% vs 33.3%), stage III–IV disease (64.3% vs 33.3%), extranodal involvement (53.6% vs 20.5%) and elevated LDH (≥ 2.5 ULN; 50.0% vs 30.8%). Elevated ferritin (≥ 650 ng/mL) was slightly more frequent among tisa-cel-treated patients (42.9% vs 35.9%). Elevated LDH and ferritin were each detected in 26 patients (38.8%). CAR-HEMATOTOX [17] was low (score 0–1) in 47 patients (70.1%) and high (≥ 2) in 20 (29.9%), with a comparable distribution between products (high risk: 32.1% with tisa-cel vs 28.2% with axi-cel). Baseline characteristics are summarized in Supplemental Table S2A-C.

Following CAR-T infusion, patients were stratified according to the infused product and monitored for treatment-related toxicities and response. Among patients treated with tisa-cel, CRS occurred in 19 out of 28 cases (67.8%), with events mainly of low to moderate severity (grades 1–2) and only 2 with grade 3 CRS. ICANS was infrequent in this cohort, occurring in 3 patients (10.7%), all presenting with grade 1 symptoms.

In the axi-cel cohort, CRS was observed in 38 patients (97.4%), spanning the full spectrum of severity from grade 1 to grade 4. ICANS was more commonly reported in axi-cel-treated patients, affecting 22 individuals (56.4%), with grades ranging from 1 to 4.

Treatment response was assessed by PET imaging at 1, 3, and 6 months following infusion and defined as complete response (CR), partial response (PR), progressive disease (PD) or relapse (R). In the tisa-cel cohort, overall response rates were 85.7% at month 1, 60.7% at month 3, and 50.0% at month 6, with a relapse rate of 41.6% within 6 months of monitoring. Axi-cel treatment resulted in more durable responses over time, with overall response rates of 84.2% at month 1, 68.4% at month 3, and 65.8% at month 6, and a markedly lower relapse rate of 15.6%. Noteworthy, in this cohort of treatment, 3 patients (7.7%) died due to severe toxicity. A comprehensive summary of toxicities and treatment responses in both cohorts is provided in Supplemental Table S3.

Longitudinal lymphocyte monitoring

Following infusion, total and CAR⁺ lymphocyte subsets were longitudinally monitored in the PB of treated patients (tisa-cel n = 21; axi-cel n = 24). A sustained increase in total lymphocyte counts ($\times 10^9/L$), as well as in CD4⁺, CD8⁺ and CD56⁺ NK cell subsets, was observed. This increase became statistically significant within the first days after CAR-T cell infusion and persisted for up to 6 months across all lymphoid populations. CD3⁺ T lymphocytes showed a progressive and sustained increase at all post-infusion timepoints compared with baseline (T0): T3d ($p < 0.01$), T7d ($p < 0.01$), T14d ($p < 0.001$), T28d ($p < 0.001$), T3M ($p < 0.001$) and T6M ($p < 0.001$) (Fig. 1A, Supplemental Table S4). This trend resulted from a significant expansion of both CD3⁺CD4⁺ and CD3⁺CD8⁺ T-cell subsets. A similar kinetic pattern was observed for circulating CD3⁺CD56⁺ NK cells, which consistently increased after infusion (T3d $p < 0.05$; T7d, T14d and T28d $p < 0.001$; T3M and T6M $p < 0.001$) (Fig. 1A). When comparing data from the different CAR-T products, a significantly higher increase in CD3⁺CD4⁺ was observed from T3d to T3M in tisa-cel- compared to axi-cel-treated patients (T3d $p < 0.05$; T7d, T14d, T28d $p < 0.01$; T3M $p < 0.05$).

CAR⁺ T cells expanded *in vivo* following both tisa-cel and axi-cel infusion, reaching their highest absolute levels at the T10d timepoint and subsequently declining through day 28 (Fig. 1B). Early expansion was more pronounced with axi-cel, consistent with the more rapid early expansion typically associated with the CD28-based construct [18] (Fig. 1C). By day 28, the difference between products was attenuated. Analysis of CAR⁺ T-cell composition at day 10 showed an almost balanced CD8⁺/CD4⁺ distribution in the axi-cel cohort (CD8⁺ and CD4⁺ CAR-T cells: 56.4% and 43.6%, respectively), approaching a 1:1 ratio. Conversely, tisa-cel remained markedly CD8⁺-dominant (83.1% CD8⁺ and 16.9% CD4⁺) (Fig. 1D). CD8⁺ CAR-T cells continued to predominate with both products at day 28, particularly in the tisa-cel cohort.

Immunomodulatory cytokine production by stimulated lymphocytes

Absolute counts ($\times 10^9/L$) of the major IFN γ - and TNF α -producing lymphocyte subsets were assessed after *in vitro* stimulation in 45 patients (tisa-cel, n = 21; axi-cel, n = 24). Compared with the post-lymphodepletion baseline (T0), IFN γ -producing CD3⁺, CD4⁺ and CD8⁺ T-cell counts increased significantly from day 7 and remained elevated through month 6 (T7d, $p = 0.001$; T14d, T28d, T3M and T6M, $p < 0.001$ for all subsets). IFN γ -producing NK cells increased earlier, reaching significance at day 3 and remaining elevated at all subsequent timepoints (T3d, $p < 0.05$; T7d, $p = 0.01$; T14d, T28d, T3M and T6M, $p < 0.001$).

(Fig. 2A, B; Supplemental Table S4). A similar pattern was observed for TNF α -producing populations. TNF α -producing CD3⁺ T-cell and NK-cell counts increased significantly at every post-infusion timepoint (T3d and T7d, $p = 0.01$; T14d, $p = 0.001$; T28d, T3M and T6M, $p < 0.001$). TNF α -producing CD4⁺ and CD8⁺ T cells increased significantly from day 7 through month 6 (T7d, $p < 0.01$; T14d, T28d, T3M and T6M, $p < 0.001$) (Fig. 2A, B).

Product-stratified analyses showed higher counts of several cytokine-producing lymphocyte populations in tisa-cel-treated patients. IFN γ -producing total lymphocytes were higher in the tisa-cel cohort at days 7 and 14 ($p < 0.05$ for both). IFN γ -producing CD4⁺ T cells were higher from day 3 through day 28 (T3d, T7d and T14d, $p < 0.05$; T28d, $p < 0.01$), whereas a difference in IFN γ -producing CD8⁺ T cells was observed only at day 14 ($p < 0.05$; Supplemental Fig. S1A–C; Supplemental Table S4).

Similarly, TNF α -producing CD3⁺ T-cell counts were higher in the tisa-cel cohort at days 7, 14 and 28 ($p < 0.01$) and at month 6 ($p < 0.05$). TNF α -producing CD4⁺ T cells were higher from day 7 through month 6 (T7d and T3M, $p < 0.05$; T14d, T28d and T6M, $p < 0.01$), whereas TNF α -producing CD8⁺ T cells differed only at day 14 ($p < 0.01$; Supplemental Fig. S1D–F, Supplemental Table S4). No significant product-associated differences were observed in cytokine-producing NK cells.

Factors influencing lymphocyte expansion and treatment response

Lymphocyte expansion and response following CAR-T-cell infusion were influenced by multiple pre- and post-infusion factors. Baseline markers of inflammation and disease burden were associated with both treatment response and hematologic recovery. Elevated ferritin and LDH levels were associated with a lower response rate at month 1 after CAR-T cells (Supplemental Table S5). CAR-HEMATOTOX risk was also associated with post-infusion WBC count recovery, with more favorable reconstitution observed in low-risk patients.

Longitudinal lymphocyte counts were also associated with treatment-related toxicities. Lower absolute counts of CD3⁺ and CD4⁺ T cells were associated with a higher risk of CRS ($p < 0.05$ for CD3⁺; $p < 0.01$ for CD4⁺), while lower CD3⁺, CD4⁺, and CD8⁺ T-cell counts were associated with an increased risk of ICANS ($p < 0.001$ for all parameters). Notably, the occurrence of ICANS, regardless of severity, was also associated with a reduction in total WBC counts ($p = 0.001$).

In analyses stratified by baseline tumor burden, no significant differences in lymphocyte kinetics or cytokine-producing populations were detected between products in patients with high tumor burden. Conversely, within the low-tumor-burden subgroup, tisa-cel-treated patients showed a greater CD3⁺ T-cell expansion at days 7 and 14 ($p < 0.05$ for both) and a greater CD4⁺ T-cell expansion at day 7 ($p < 0.05$), day 14 ($p = 0.01$), day 28 and month 3 ($p < 0.01$ for both) than axi-cel-treated patients (Fig. 3A). Tisa-cel was also associated with greater expansion of IFN γ - and TNF α -producing CD3⁺, CD4⁺ and CD8⁺ T cells at several timepoints in this subgroup (Fig. 3B, C). Thus, product-associated differences in numerical and functional T-cell expansion were most evident in patients with a lower baseline tumor burden.

Immunophenotypic analysis of the infusion products

The cellular composition of residual infusion-bag material was analyzed in 10 tisa-cel and 18 axi-cel products. As previously reported [19], tisa-cel products were markedly enriched in CD4⁺ T cells, which represented $85.9\% \pm 7.0\%$ of total T cells, with $13.9\% \pm 6.7\%$ CD8⁺ T cells. By contrast, axi-cel products showed a more balanced CD4⁺/CD8⁺ composition, approaching a 1:1 ratio ($53.3\% \pm 15.2\%$ and $46.8\% \pm 15.5\%$, respectively) (Supplemental Figure S2A). The proportion of CAR⁺ cells also differed substantially between products. CAR⁺ cells represented $37.2\% \pm 4.6\%$ of total T cells in tisa-cel products, comprising $29.5\% \pm 4.0\%$ CD4⁺CAR⁺ and $7.7\% \pm 2.5\%$ CD8⁺CAR⁺ cells. In axi-cel products, the CAR⁺ fraction was approximately twice as high, accounting for $72.5\% \pm 9.4\%$ of total T cells and comprising $42.0\% \pm 13.0\%$ CD4⁺CAR⁺ and $30.5\% \pm 10.4\%$ CD8⁺CAR⁺ cells (Supplemental Fig. S2A).

Differentiation profiling within the CAR⁺ compartment revealed distinct product-associated patterns. Compared to axi-cel, tisa-cel products contained significantly higher proportions of naïve ($p < 0.01$) and TEMRA ($p = 0.001$) CD4⁺CAR⁺ T cells. Conversely, axi-cel products contained higher proportions of central-memory ($p < 0.05$) and effector-memory ($p = 0.01$) CD8⁺CAR⁺ T cells (Supplemental Fig. S2B). This CD8⁺ memory-enriched composition may have contributed to the more potent expansion observed in vivo with axi-cel [19]. Exhaustion and senescence phenotypes accounted for only small fractions of CAR⁺ T cells in both products. Descriptively, exhausted CAR⁺ T cells were more frequent in tisa-cel than in axi-cel products ($5.3\% \pm 4.2\%$ vs $1.9\% \pm 2.3\%$), whereas senescent CAR⁺ T cells were more frequent in axi-cel products ($4.1\% \pm 3.9\%$ vs $0.5\% \pm 0.4\%$; Supplemental Fig. S2C).

Maturation, exhaustion and senescence programs in CAR⁺ and CAR⁻ compartments

In the same cohort of 28 patients (tisa-cel $n = 10$; axi-cel $n = 18$), we performed an in-depth analysis of T-cell differentiation patterns and the eventual emergence of exhaustion or senescence phenotypes, within both CAR⁺ and CAR⁻ compartments.

At day 10, axi-cel-treated patients had higher proportions of CD4⁺CAR⁺ T cells than tisa-cel-treated patients across the naïve ($p = 0.01$), effector-memory ($p < 0.05$) and TEMRA ($p < 0.05$) subsets (Fig. 4A). Exhausted CAR⁺ T cells were also numerically more frequent following axi-cel, while senescent cells were significantly increased in both the CD4⁺CAR⁺ and CD8⁺CAR⁺ compartments ($p < 0.05$ for both).

At day 28, product-associated differences were mainly observed within the CAR⁻ compartment. Axi-cel-treated patients had higher proportions of naïve CD4⁺CAR⁻ T cells ($p < 0.05$) and effector-memory CD4⁺CAR⁻ ($p = 0.01$) and CD8⁺CAR⁻ T cells ($p < 0.05$) than tisa-cel-treated patients (Fig. 4B). Biologically, this temporal shift from early changes within the CAR⁺ compartment to later enrichment of CAR⁻ memory populations is consistent with a rapid axi-cel-associated effector response followed by broader remodeling of endogenous T-cell immunity, although the accumulation of senescent CAR⁺ cells may indicate reduced long-term proliferative reserve.

Plasma cytokine monitoring

Plasma cytokine concentrations were longitudinally measured in 38 patients treated with tisa-cel (n = 19) or axi-cel (n = 19) before and at multiple timepoints after CAR-T-cell infusion. No significant variations were observed for IL-2, IL-4, IL-17A, TNF- α , GM-CSF, or MIP-1 α . In contrast, a clear modulation of IL-6, IL-8, IL-10 and IFN γ was detected, with all cytokines displaying an early expansion peak after infusion, although clinical interventions to manage cytokine related toxicities influenced the timing and magnitude of cytokine expansion.

Product-stratified analyses showed a more pronounced inflammatory cytokine profile following axi-cel. IL-8 concentrations were higher in axi-cel- than in tisa-cel-treated patients at days 7 ($p < 0.05$) and 28 ($p < 0.01$), while IL-10 was higher at day 7 ($p < 0.05$).

Importantly, IL-8 levels were associated with both treatment related toxicities and clinical response. Patients who developed grade ≥ 2 ICANS showed an early increase in IL-8, peaking around day 14. Furthermore, early IL-8 elevation, characterized by a rapid increase shortly after infusion and a peak at day 3, was associated with inferior response at month 1, as patients achieving PR or experiencing PD exhibited higher IL-8 levels compared with those in CR (Fig. 5A). Similarly, patients with PD or relapse at month 3 had persistently higher IL-8 concentrations than patients in CR (Fig. 5B).

Finally, IL-10 also appeared to be associated with disease progression. At month 3, patients with PD or PR displayed detectable IL-10 levels, whereas patients achieving CR showed lower or undetectable levels at this timepoint (Fig. 5C).

DISCUSSION

In this study, we integrated baseline inflammatory and disease-related variables with longitudinal monitoring of circulating lymphocyte populations, functional cytokine-producing cells, plasma cytokines, and the cellular composition of the infused CAR-T products. Our findings indicate that immune dynamics after CAR-T-cell therapy are multidimensional and cannot be described solely by the magnitude or persistence of circulating CAR $^{+}$ cells. Rather, axi-cel and tisa-cel were associated with distinct, compartment-specific immune trajectories that were further influenced by the patient's baseline inflammatory and tumor context. Tisa-cel was characterized by greater peripheral CD4 $^{+}$ T-cell expansion and higher numbers of IFN γ - and TNF α -producing T cells, whereas axi-cel showed more rapid early CAR $^{+}$ expansion, broader remodeling of both CAR $^{+}$ and endogenous CAR $^{-}$ T-cell compartments, and a more pronounced plasma inflammatory response. These findings suggest that peripheral lymphoid reconstitution, cellular functional capacity, and soluble inflammatory mediators represent related but non-equivalent components of the response to CAR-T-cell therapy.

In our cohort, lower pre-infusion ferritin and LDH levels were associated with early CR, and a low CAR-HEMATOTOX score was associated with more favorable hematologic recovery. These findings are consistent with previous reports linking baseline inflammation and impaired hematopoietic reserve to

post-CAR-T cytopenias, infectious complications, and clinical outcome [6, 7, 20, 21]. In particular, recent studies have shown that robust CAR-T-cell expansion may not overcome the adverse effect of a highly inflammatory baseline state, emphasizing that the quality of the host environment may be as relevant as the magnitude of cellular expansion [6, 7]. Ferritin and LDH, however, are not specific immune biomarkers and may reflect systemic inflammation, disease burden, tissue damage, or a combination of these processes. Our findings, therefore, support an interaction between baseline inflammatory and disease-related conditions and subsequent CAR-T-cell activity, rather than identifying an isolated mechanism.

Following infusion, we overall observed an expansion of CD3⁺, CD4⁺, CD8⁺ and NK cells with sustained IFN γ and TNF α production. However, immune kinetics were strongly modulated by tumor burden and CAR construct. In patients with a high tumor burden, lymphocyte dynamics were comparable between axi-cel and tisa-cel cohorts, suggesting that the strong antigenic stimulation may attenuate product-specific differences, consistent with prior observations [22]. In contrast, in patients with low tumor burden: tisa-cel induced a greater CD3⁺ and CD4⁺ T-cell expansion without improved tumor control, whereas axi-cel achieved superior responses despite lower circulating lymphocyte counts. This finding may underscore a qualitative, rather than quantitative, immune advantage of axi-cel.

The analysis of residual infusion-bag material provided a possible basis for the divergent *in vivo* trajectories. Axi-cel products contained a higher proportion of CAR⁺ cells, displayed an approximately balanced CD4⁺CD8⁺ composition, and were enriched in CD8⁺ central- and effector-memory CAR⁺ subsets, capable of a rapid cytotoxic activation. By contrast, tisa-cel products had a lower CAR⁺ fraction and were markedly enriched in CD4⁺ cells. Recent evidence [23] suggests that product composition, including CD4/CD8 ratio, CAR density and memory differentiation state, critically influence expansion kinetics, effector polarization and clinical outcome, supporting the concept that construct design and manufacturing features precondition subsequent immune remodeling. Consistently, axi-cel showed rapid *in vivo* CAR⁺ expansion, a broad differentiation towards effector and exhausted phenotypes, and a marked bystander activation of endogenous CAR⁻ T cells, consistent with a CD28 driven inflammatory program favoring immune trafficking and redistribution.

The circulating compartment did not simply reproduce the composition of the infused product. Whereas axi-cel maintained an approximately balanced CD4⁺/CD8⁺ distribution at day 10, circulating tisa-cel CAR⁺ cells became markedly CD8⁺ predominant despite the strong CD4⁺ predominance of the corresponding infusion products. This transition suggests that infusion-product composition is substantially reshaped after administration through differential expansion, survival, or redistribution of individual subsets.

Cytokine profiling revealed a partially distinct inflammatory axis. Although tisa-cel-treated patients had higher numbers of IFN γ - and TNF α -producing lymphocytes after *in vitro* stimulation, axi-cel was associated with higher circulating IL-8 and IL-10 concentrations at selected timepoints and with substantially more frequent CRS and ICANS. Moreover, plasma TNF α did not vary significantly despite the sustained increase in TNF α -producing lymphocyte counts. These apparently discordant findings

emphasize that the abundance of cytokine-producing lymphocytes and the concentration of soluble cytokines in plasma provide different biological information. Plasma cytokine concentrations integrate cytokine release from CAR-T cells and endogenous immune populations—particularly activated monocytes and macrophages—as well as endothelial activation, tissue distribution, clearance, and the effects of anti-inflammatory treatment.

Among the evaluated soluble mediators, IL-8 emerged as the most informative candidate biomarker. IL-8 concentrations were higher after axi-cel at days 7 and 28, and an early IL-8 increase was observed in patients who developed grade ≥ 2 ICANS, as already reported [24]. Higher early or persistent IL-8 levels were also associated with inferior responses at months 1 and 3. IL-10 showed a more limited pattern: it did not vary significantly in the overall cohort but was higher after axi-cel at day 7 and remained detectable at month 3 in patients with PR or PD. These cytokines should therefore be regarded as candidate markers of an inflammatory state associated with toxicity and unfavorable response rather than as established causal mediators.

Collectively, these findings define distinct biologic programs underlying axi-cel and tisa-cel. Our data support a model in which CAR-T cell efficacy in R/R LBCL is not exclusively driven by a cellular expansion or long-term persistence, but rather by the integration of baseline host inflammatory fitness with construct specific immune reprogramming. Beyond direct CAR⁺ T-cell activity, this therapy profoundly reshapes the host immune system by modulating endogenous CAR⁻ immune populations, highlighting the contribution of bystander immune activation to anti-tumor efficacy.

In this context, our findings are relevant for the interpretation of emerging CAR-T cell products, such as lisocabtagene maraleucel (liso-cel), which combine a defined 1:1 CD4:CD8 composition, shared with axi-cel, with a 4-1BB costimulatory domain, analogous to tisa-cel. The favorable balance between efficacy and reduced toxicity reported with liso-cel [25] suggests that a controlled cellular composition may preserve the qualitative immune advantages observed with axi-cel while mitigating excessive inflammatory signaling. Our comparative analysis therefore provides a biologic framework to understand how construct-design and immune programming jointly shape clinical outcomes in next-generation CAR-T therapies increasingly adopted in R/R LBCL. Larger prospective studies incorporating balanced product comparisons, longer clinical follow-up, tissue-based analyses, and single-cell and clonotypic profiling will be required to determine whether these distinct immune trajectories can be translated into clinically useful biomarkers or individualized therapeutic strategies.

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Competing interests

The authors have no conflicting financial interests.

Author Contributions

GR NP and MA carried out the lab work, analyzed the data and wrote the paper; AliceDR critically revised the manuscript, processed the clinical data and managed patients; LD processed the clinical data and managed the patients; AriannaDR carried out the statistical analysis; AG and RF designed the research study, discussed the results and critically revised the manuscript.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

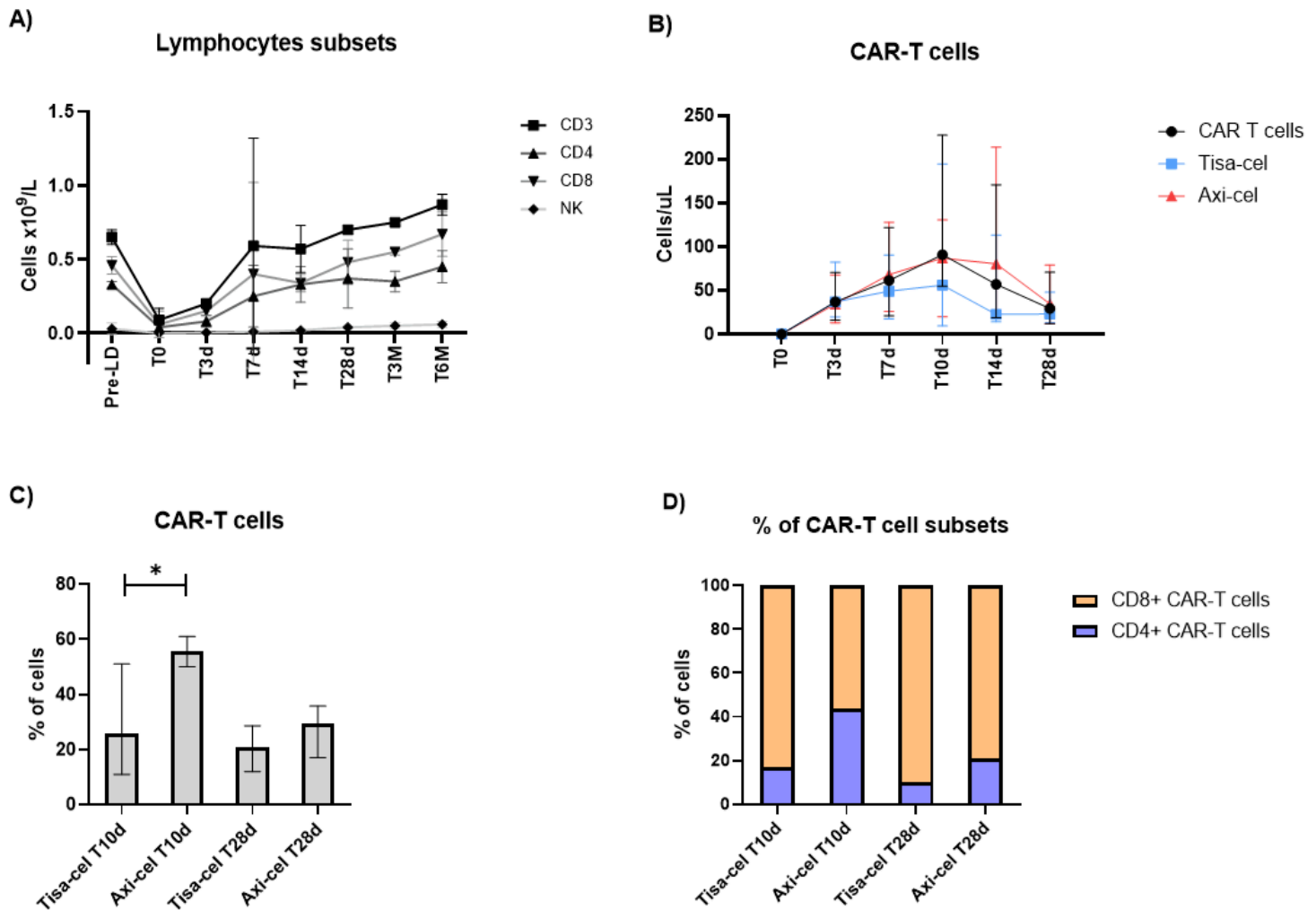


Figure 1

In vivo longitudinal monitoring of CAR- and CAR+ compartments (A) Absolute counts of peripheral CD3, CD3⁺CD4⁺, CD3⁺CD8⁺ and NK (CD3⁺CD56⁺) cells at different timepoints (B) Absolute circulating CAR-T-cell counts from T0 to T28d, overall and according to the infused product (C) Comparison of CAR-T-cell frequencies between tisa-cel- and axi-cel-treated patients at days 10 and 28 (D) Relative distribution of CD4⁺ and CD8⁺ CAR-T-cell subsets at the same timepoints. Data are reported as means ± standard deviations (SD) of the absolute number of the different populations studied. Significant differences are indicated as ****p* < 0.001, ***p* < 0.01, **p* < 0.05

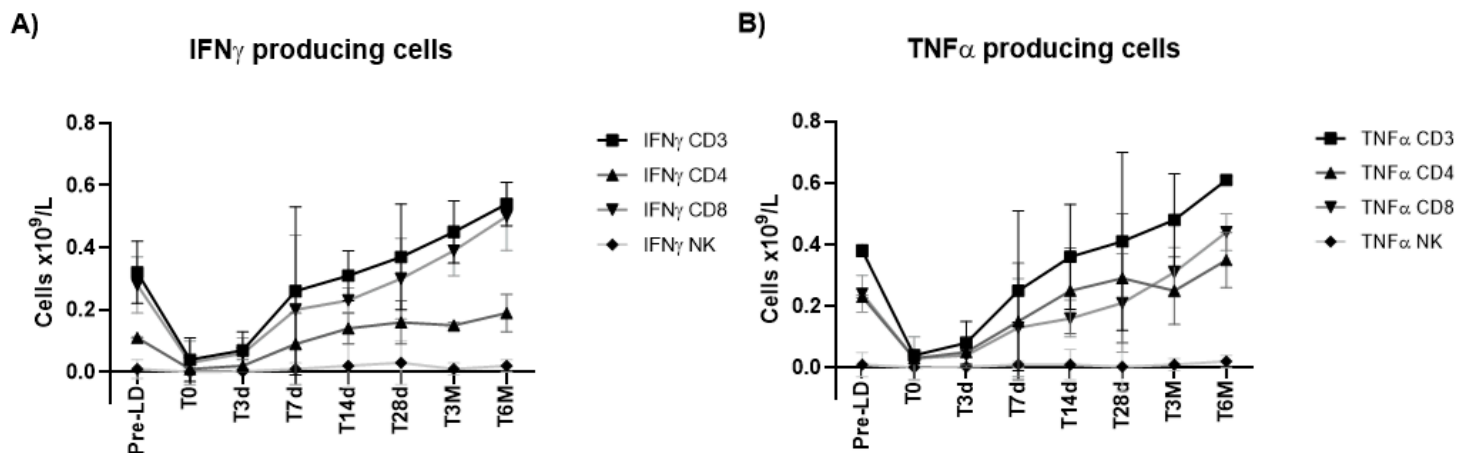


Figure 2

Longitudinal kinetics of IFN γ - and TNF α -producing lymphocyte subsets following CAR-T-cell infusion **(A)** Absolute counts ($\times 10^9/L$) of IFN γ -producing CD3 $^+$, CD4 $^+$, CD8 $^+$ and NK cells were measured after in vitro stimulation at the post-lymphodepletion pre-infusion baseline (T0), days 3, 7, 14 and 28, and months 3 and 6 after infusion **(B)** TNF α -producing CD3 $^+$, CD4 $^+$, CD8 $^+$ and NK cells were measured after in vitro stimulation at the post-lymphodepletion pre-infusion baseline (T0), days 3, 7, 14 and 28, and months 3 and 6 after infusion. Data are presented as mean $\pm$ SD. Statistical significance was calculated relative to T0: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Significant differences are reported in Supplemental Table S3

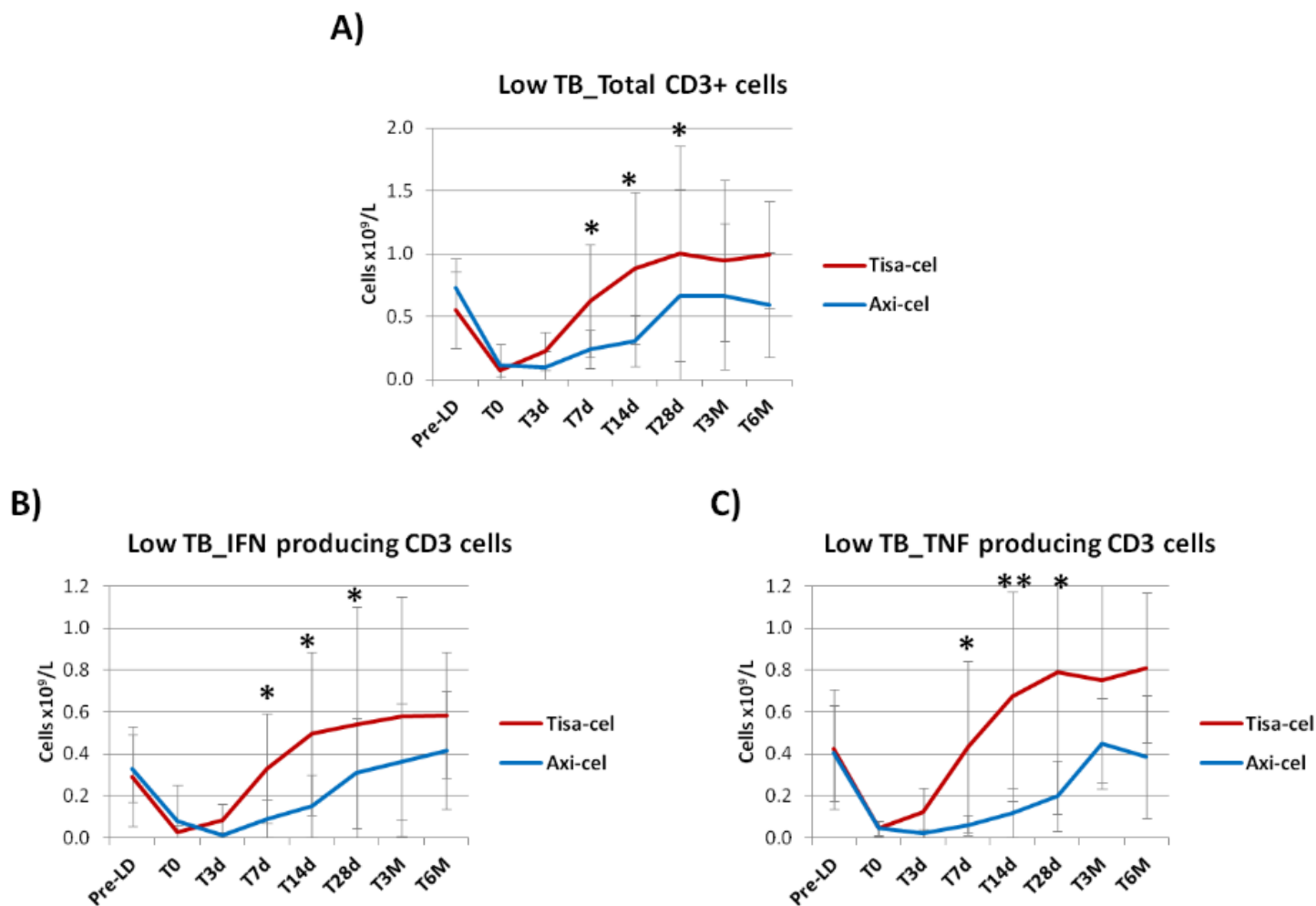


Figure 3

In vivo longitudinal immune monitoring stratified according to the infused CAR-T product, in the basal low tumor burden cohorts **(A)** Absolute number ($\times 10^9/L$) of CD3⁺ total lymphocytes at different timepoints before and after CAR-T cell infusion **(B)** IFN γ -producing CD3⁺ lymphocytes populations at different timepoints before and after CAR-T cell infusion **(C)** TNF α -producing CD3⁺ lymphocytes populations at different timepoints before and after CAR-T cell infusion. Data are reported as means $\pm$ standard deviations (SD) of the absolute number of the different populations studied. Relative significant differences are reported in Supplemental Table S3

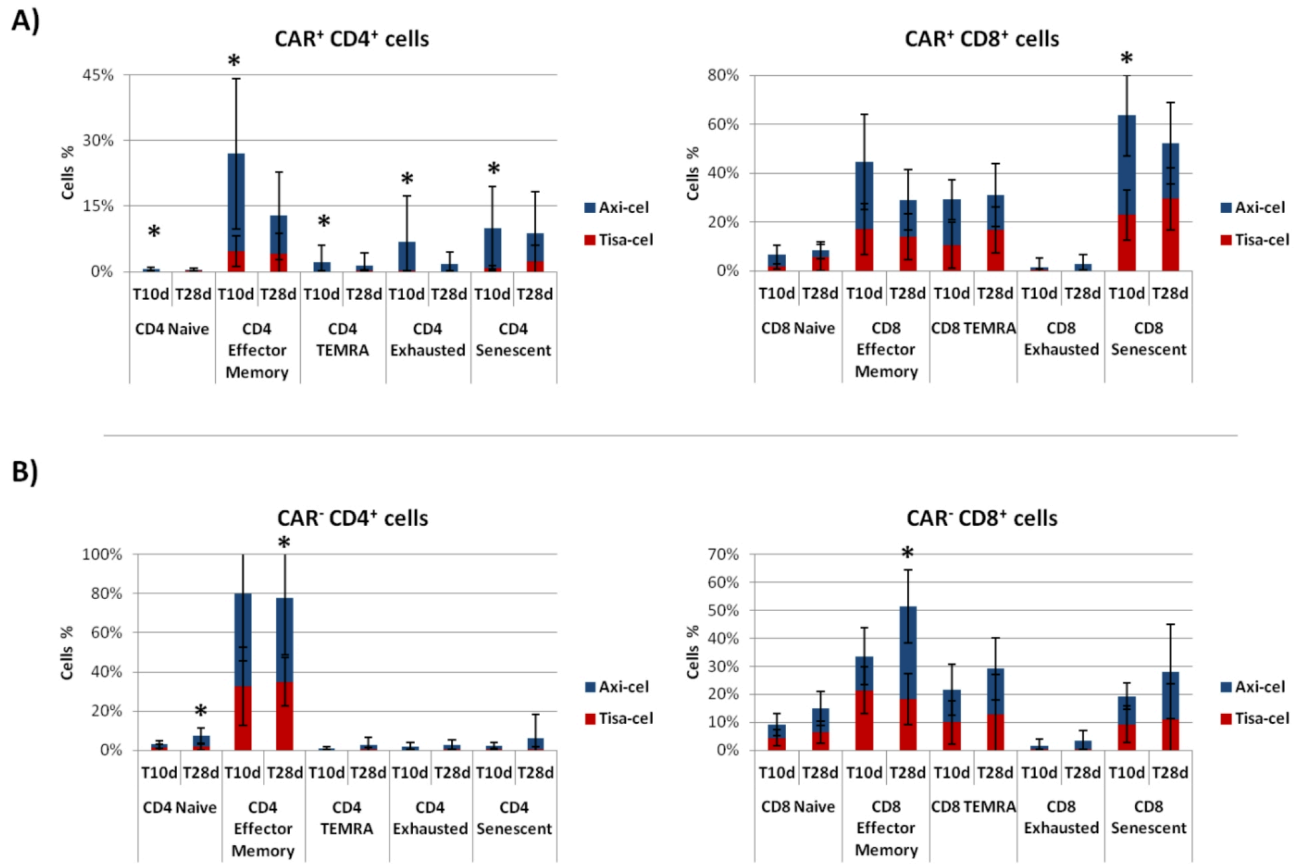


Figure 4

In vivo longitudinal phenotypic profiling of peripheral CD4⁺ and CD8⁺ lymphocytes in CAR⁺ and CAR⁻ compartments, at day 10 and day 28 after infusion (**A**) Bar plots show the percentage of CAR⁺ CD4 and CD8 T-cells stratified according to maturation status (naïve, effector memory, and terminal effector memory –TEMRA–) as well as exhaustion and senescence phenotypes (**B**) Bar plots show the percentage of CAR⁻ CD4 and CD8 T-cells stratified according to maturation status (naïve, effector memory, and terminal effector memory –TEMRA–) and exhaustion and senescence phenotypes. Data are reported as means $\pm$ SD. Significant differences were calculated comparing populations percentages in the axi-cel versus tisa-cel cohorts and indicated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

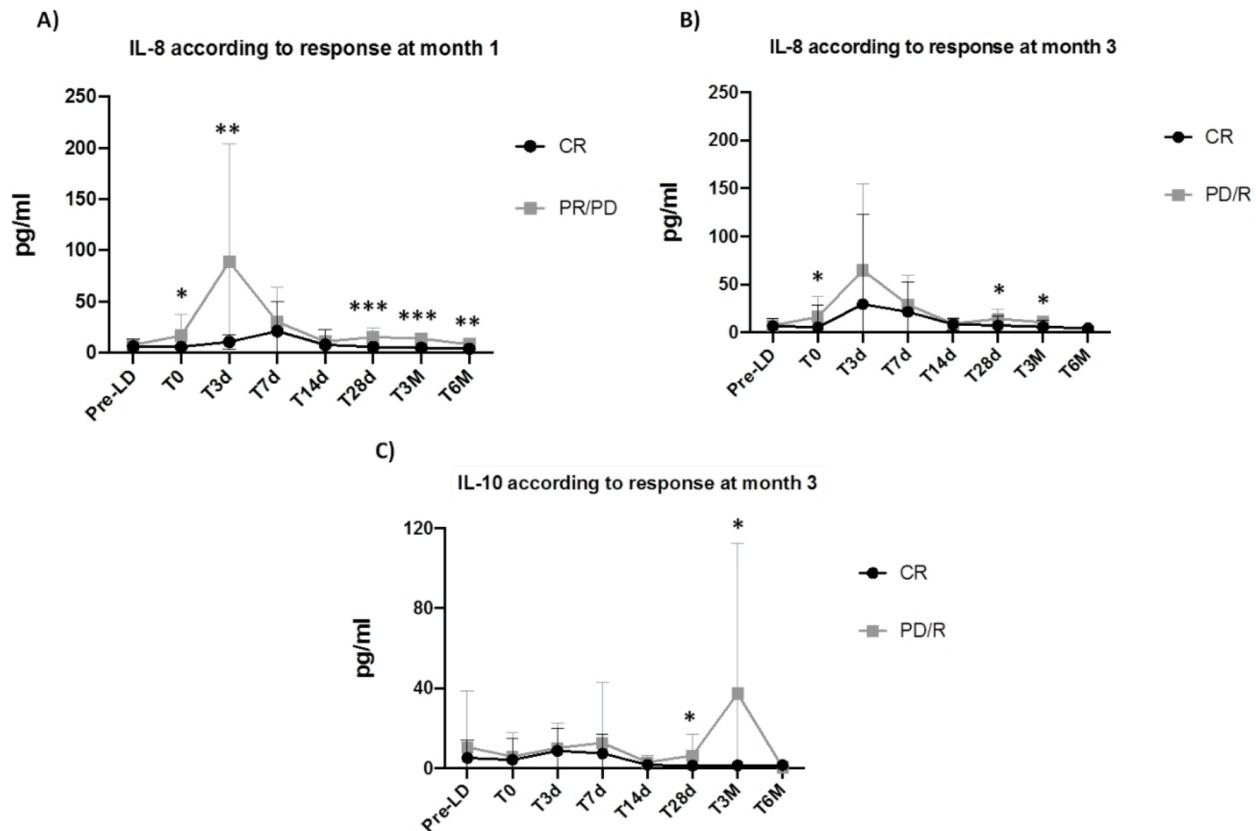


Figure 5

In vivo longitudinal monitoring of circulating IL-8 and IL-10 levels at different timepoints before and after CAR-T cell infusion according to the response evaluated at month 1 or month 3. Plasma cytokines were detected before and up to 6 months after the infusion and divided in patients who obtained a CR and in patients who achieved a PR, did not respond or relapsed. **(A)** Concentration of plasmatic IL-8 according to response at month 1 and **(B)** Concentration of plasmatic IL-8 according to response at month 3 and **(C)** Concentration of IL-10 according to response at month 3. Data are reported as means $\pm$ SD of the concentration (pg/ml). Significant differences were calculated compared to the CR group and indicated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarydata209.docx](#)