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The role of CHIP and pre-treatment neutropenia in patients treated with chimeric antigen receptor T cells for hematological malignancies

Running Title: CHIP and Outcomes after CART

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Abstract

Chimeric antigen receptor T-cell (CAR-T) therapies are effective in relapsed and refractory B-cell neoplasia but can cause immune-effector cell (IEC)-related toxicities, including cytokine release syndrome (CRS), immune effector cell–associated neurotoxicity syndrome (ICANS), hematologic toxicity (ICAHT), and hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS). We hypothesized that clonal hematopoiesis of indeterminate potential (CHIP) may influence outcomes by modulating inflammatory responses. We retrospectively analyzed 113 patients treated with CAR-T-cells for B-cell neoplasia, evaluating the impact of CHIP and pre-treatment neutropenia on patient outcomes. CHIP was detected in 30.8% of patients with multiple myeloma (MM); 13.7% of patients with large B-cell lymphoma (LBCL) and 28.6% in follicular and mantle cell lymphoma, with DNMT3A being the most frequently detected CHIP-associated mutation among 62 sequenced patients. CHIP remained stable after CAR T-cell therapy, with comparable variant allele frequencies (VAFs) and mutational burden in paired pre- and post-treatment samples (n=33). Pre-treatment neutropenia was present in approximately 30%. Neither CHIP nor pre-treatment neutropenia influenced overall survival (OS), progression-free survival (PFS), or the incidence of CRS and ICANS. However, CHIP was associated with increased IEC-HS and higher ferritin levels in MM, while pre-treatment neutropenia was associated with severe ICAHT in the overall cohort and CD19-directed cohort. These findings suggest that CHIP and pre-treatment neutropenia are not major determinants of outcome after CAR-T cell therapy but may identify patients at increased risk for specific toxicities. This study is limited by its small sample size, heterogeneous cohort, and retrospective design. Prospective studies in larger cohorts are warranted to further investigate these effects.

Introduction

CAR-T-cell therapies have been established for various hematological malignancies since the first FDA approval of tisagenlecleucel (tisa-cel) in 2017¹. Current indications include relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL), aggressive B-cell lymphomas, low-grade B cell lymphomas (e.g., follicular lymphoma (FL), mantle cell lymphoma (MCL) and marginal zone lymphoma (MZL)), and multiple myeloma (MM). Moreover, their impact continues to expand as CAR-T-cell therapies are increasingly used in earlier lines of treatment^{2,3}, and their application is emerging in the field of autoimmune diseases (AID)^{4,5}. Besides their impressive efficacy, CAR T-cell therapies can also induce several immune effector cell (IEC) -related adverse events, including cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), immune effector cell-associated hematotoxicity (ICAHT), and immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS). Patient- and therapy-related risk factors for these adverse events have been identified, including older age, higher tumor burden, elevated inflammatory markers, the nature of the targeted antigen and the CAR-T-cell construct, and higher CAR T-cell doses⁶⁻⁸. However, reliable prediction of CRS, ICANS and IEC-HS remains challenging.

A potential mediator of CAR-T-cell efficacy and IEC-related adverse events is clonal hematopoiesis of indeterminate potential (CHIP). However, current evidence remains inconclusive, with some studies reporting no association between CHIP and CRS or ICANS⁹⁻¹¹, and others describing links to one of the two^{12,13}, potentially in an age-dependent manner¹⁴.

CHIP is defined by the presence of somatic mutations with variant allele frequency (VAF) >2%, in the absence of diagnostic criteria for a myeloid neoplasm. Preclinical data suggest that CHIP promotes a proinflammatory state and may, in turn, be accelerated by inflammatory conditions¹⁵⁻¹⁸. In patients undergoing autologous transplantation (ASCT) for MM¹⁹, CHIP detection is associated with poorer patient outcomes. Conversely, experimental modulation of the CHIP-associated genes DNMT3A or TET2 enhanced the efficacy of CAR-T-cells in

preclinical studies, by promoting a less exhausted phenotype and altering T-cell differentiation^{20, 21}. We thus hypothesized that preexisting CHIP mutations in patients undergoing CAR T-therapy might influence treatment response, perhaps enhancing efficacy but perhaps also the risk of IEC associated adverse events. Furthermore, we investigated the impact of pre-treatment neutropenia, as a potential result of CHIP, on survival and IEC-related adverse events in patients undergoing CAR T-therapy.

Materials and Methods

Patients and data collection

In this single-center analysis, we included all adult patients who underwent CAR T-therapy with commercially available products for non-Hodgkin B-cell lymphoma, and MM at our center between May 2019 and April 2025 (n=113). The study was approved by the ethics committee of the University of Tuebingen (277/2020BO2 and 771/2023BO2). Lymphodepletion was performed in all patients using fludarabine and cyclophosphamide according to the manufacturer's recommendations.

CRS and ICANS were graded according to ASTCT (American Society for Transplantation and Cellular Therapy), and ICAHT according to EHA/EBMT (European Hematology Association/ European Society for Blood and Marrow Transplantation) guidelines^{22, 23}. IEC-HS diagnosis followed Hines et al. describing a pathological condition associated with cytopenia, hyperferritinemia, coagulopathy, and transaminitis^{24, 25}. The CAR-HEMATOTOX scoring was calculated as previously described by Rejeski et al.⁶. If toxicity grading had not been established at the time of treatment, it was assessed retrospectively. For the purpose of the analysis, we categorized neutropenia as lower than an absolute neutrophil count (ANC) of $1.5 \times 10^9/L$.

Flow cytometry (FC) analysis

CAR T-cell detection was performed by FC on whole-blood samples using CAR detection reagent (Miltenyi Biotec, Germany). CAR and Non-CAR cells were analyzed using the antibody panel listed in Supplementary Table 1. Data acquisition was performed on a BD FACS

Lyric flow cytometer using BD FACSuite software v1.5 (BD Biosciences, USA). The gating strategy is shown in Supplementary Figure 1. Samples were collected on days 7 ± 4 , 15 ± 4 , 30 ± 10 , 60 ± 10 and 180 ± 30 (Figure 1A).

Sequencing of peripheral blood

Sequencing analyses were preferentially conducted with targeted next generation sequencing (NGS) using the Ion Torrent™ Genexus™ Integrated Sequencer with the Oncomine™ Myeloid Assay GX v2 using samples obtained at the time of apheresis or before start of the lymphodepletion and at day +30 or at day +100 after CAR-T cell infusion ²⁶. Variants were called for single nucleotide variants and insertion/deletions and were classified according to the American College of Medical Genetics and Genomics (ACMG) criteria. Only Tier 1 and 2 variants were included ^{27, 28}. However, due to limited availability of sufficient sample material, analyses for some patients had to be performed using samples collected at later time points. In addition, one patient was included who had previously undergone sequencing as part of a case report. Sequencing in this case was performed as described by Riedel et al ²⁵.

CHIP was defined as the presence of a somatic variant of defined genes with a VAF $>1.9\%$, in accordance with the definition proposed by Steensma et al. ²⁹. As no matched germline samples were available, variants with a VAF of 48-52% were considered potentially germline and not analyzed as CHIP-associated.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 10 (San Diego, CA, USA) and R (version 4.4.1). Group comparisons, longitudinal analyses, survival analyses, and regression models were performed using standard statistical methods as described in the Supplementary Methods.

Results

Patient characteristics

The 113 included patients are divided into three subgroups: patients with large B-cell lymphomas (LBCL) (n=64), patients with MM (n=39), follicular lymphoma (FL) or mantle cell lymphoma (MCL) (n=10). The median age among individual groups was between 63 and 68

years, 30-46.2% were female and 53.8-70% had a progressive disease (PD) at the start of the lymphodepletion (Table 1). Over 60% (n=74) of the patients were treated with CD19-targeted CAR T-cells, 31% (n=39) with anti-BCMA CAR T-cells. The exact and relative distribution of the CAR T-products used and detailed patient characteristics are shown in Table 1.

Pre-treatment neutropenia

We investigated the associations of pre-treatment neutropenia with treatment response and survival, as well as with IEC-related adverse events, including CRS and ICANS, and with quality-of-life indicators such as duration of hospitalization.

Pre-treatment neutropenia (ANC <1500/ μ l) was observed in approximately 30% of the cohort. The subgroup including MM exhibited highest rates of pre-treatment neutropenia (30.8%). Numerical differences in causes of death were observed in both LBCL (Figure 1B) and MM (Figure 1C), with higher rates of non-relapse mortality (NRM) and infection-related deaths in particular among patients with pre-treatment neutropenia (lymphoma: 22.22% vs. 4.17% and MM: 66.67% vs. 0%). However, these differences were not statistically significant by Fisher's exact test, likely due to the small sample size.

Pre-treatment neutropenia in patients with LBCL was not significantly associated with overall survival (log-rank test; median OS (ANC <1500/ μ l): 951 days, median OS (ANC >1500/ μ l): 603 days; p=0.91) (Figure 2A). Likewise, pre-treatment neutropenia did not significantly affect progression-free survival (PFS) in patients with LBCL treated with anti-CD19-directed CAR T-cell therapy (log-rank test; p=0.56) (Figure 2 B) and the cumulative incidence of NRM did not differ between groups (Fine-Gray model, p=0.30) (Figure 2 C). In line with these findings, pre-treatment neutropenia in MM patients undergoing anti-BCMA CAR T-cell therapy was not associated with inferior OS (log-rank test; median OS (ANC <1500/ μ l): not reached, median OS (ANC >1500/ μ l): not reached; p=0.56) (Figure 2 A), nor with a higher PFS (log-rank test; p=0.73) (Figure 2 B). Furthermore, the cumulative incidence of NRM did not differ significantly between groups (Fine-Gray model, p=0.73) (Figure 2 C).

Analysis of early ($\leq$ 30 days post-infusion) and late (>30 days post-infusion) infection rates revealed no significant differences between patients with and without pre-treatment

neutropenia in the LBCL cohort (Fisher's exact test: early $p=0.53$; late $p=0.43$) or the MM cohort (Fisher's exact test: early $p=0.08$; late $p=0.71$) (Supplementary Figure 2A). Bacterial infections predominated in the early post-CAR T-cell treatment period, accounting for up to 81.25% of infections in LBCL patients with ANC $>1500/\mu\text{L}$ and 87.50% in MM patients with ANC $>1500/\mu\text{L}$ (Supplementary Figure 2B). In contrast, the proportion of viral infections increased after day 30 post-infusion in both groups (53.85% and 22.22%) (Supplementary Figure 2C).

Univariable risk analysis identified pretreatment neutropenia as a risk factor for severe early and late ICAHT in the overall patient population (OR, 95-CI; early: 3.20; 1.29-7.99; late: 2.96, 1.19-7.41), as well as in the anti-CD19 cohort for early severe ICAHT (OR, 95-CI; early: 9.20, 2.90-32.49) (Table 2). Notably, this association was not observed in the anti-BCMA cohort (Table 2). Exploratory multivariable analysis including CHIP, pre-treatment neutropenia ($<1500/\mu\text{L}$) and normalized LDH as covariates confirmed pre-treatment neutropenia as an independent risk factor for early severe ICAHT (before day +30 and $>\text{grade } 2$) in the overall cohort (OR, 95-CI; early: 3.73, 1.13-14.13) and the anti-CD19 subcohort (OR, 95-CI; early: 11.04, 1.46-114.1) (Table 3).

To verify isolated pre-treatment neutropenia as a predictive marker for IEC-related adverse events, we compared its performance with the CAR-HEMATOTOX score. The results were largely comparable. In the univariable analysis, severe late-onset ICAHT was similarly predicted by pre-treatment neutropenia or the CAR-HEMATOTOX score (OR, 95% CI: 1.93, 1.32–3.03), whereas pretreatment neutropenia showed superior predictive performance for early-onset severe ICAHT (OR, 95% CI: 3.20, 1.29–7.99 vs. 1.43, 0.98–2.11) (Table 2).

Use of G-CSF after discharge was necessary in 69.4% of MM patients, whereas only 44.3% of LBCL patients required G-CSF within the first 180 days post-infusion. Comparison of G-CSF use between patients with pre-treatment neutropenia and those with normal ANCs showed no significant differences in either the LBCL or MM subgroups (Fisher's exact test; LBCL: 64.29% vs. 38.30%, $p = 0.13$; MM: 83.33% vs. 62.50%, $p = 0.27$). Only one MM patient received an autologous hematopoietic cell boost, and this patient had no pre-treatment neutropenia.

Lastly, hospitalization duration was analyzed according to pre-treatment ANC. Notably, pre-treatment neutropenia had no impact on early (Supplementary Figure 2D) or late hospitalization after CAR T-cell therapy, e.g. due to infection (Supplementary Figure 2E).

CHIP

NGS was performed in 62 patients based on sample availability. A comparison of sequenced and non-sequenced patients is provided in the Supplementary Table 2. Of these, 33 patients were analyzed longitudinally and samples collected both, before (between day -62 and -4) and after CAR T-cell therapy (between day +17 and +240) were sequenced. Patients were classified as CHIP-positive if at least one sequencing at any time point detected a typical CHIP-associated mutation, as defined in the method section. CHIP mutations were identified in 13.8% of LBCL patients (n=4/29), and 30.8% of MM patients (n=8/36). The most common mutations were in *DNMT3A* (50%), followed by *TP53* (0-25%) and *PPM1D* (12.5-50%) (Table 1).

CHIP and CAR-T-cell expansion

To investigate the role of CHIP in CAR T-cell patients, we analyzed CAR T-cell expansion patterns in CHIP-positive and CHIP-negative patients. Moreover, changes in VAF and in the absolute number of mutations were compared pre- and post-therapy to investigate whether CAR T-cell therapy and subsequent CAR T-cell activation influence the expansion of clones harboring CHIP-typical mutations.

We analyzed the number of CHIP mutations before and after therapy (Figure 3A top) as well as changes in VAF in each patient (Figure 3A bottom). The absolute number of CHIP mutations remained largely stable in the overall cohort, only three patients acquired one new mutation, and one patient has lost a CHIP-defining mutation. In line with these findings, we observed balanced changes in VAF among the 10 patients with CHIP mutations. Exactly 50% showed higher VAFs after therapy ($\Delta+1.40$ to $+3.25\%$), whereas the remaining 50% showed lower VAFs ($\Delta-2.00$ to -7.80%).

The expansion of relative anti-CD19 and anti-BCMA CAR T-cells (Figure 3B) in relation to the total lymphocyte count did not show significant differences between CHIP-positive and CHIP-negative patients at days 7 (Median, Mann-Whitney-U test; LBCL: 43.10% (n=3) vs. 24.70% (n=22), $p=0.91$; MM: 43.50% (n=8) vs. 37.17% (n=14), $p=0.76$) and 15 (LBCL: 12.50% (n=3) vs. 5.29% (n=17), $p=0.25$; MM: 52.90% (n=5) vs. 48.45% (n=7), $p=0.53$). Moreover, CD4/CD8 ratios did not differ between CHIP-positive and CHIP-negative patients on day 7 (median, Mann–Whitney U test; LBCL: 8.1 (n=2) vs. 0.70 (n=22), effect size too low; MM: 0.81 (n = 8) vs. 0.36 (n=13), $p=0.97$) or day 15 (LBCL: 0.27 (n=3) vs. 0.34 (n = 17), $p = 0.62$; MM: 0.14 (n=5) vs. 0.15 (n=7), $p = 0.64$), indicating no preferential expansion of either T-cell subset (Figure 3C).

Furthermore, CD4+ and CD8+ non-CAR T-cell counts did not differ between CHIP-positive and CHIP-negative patients in the mixed-effects model (CD4+ LBCL: $p = 0.71$; CD4+ MM: $p = 0.90$; CD8+ LBCL: $p = 0.55$; CD8+ MM: $p = 0.23$) (Supplementary Figure 3).

Interestingly, while CRP levels did not differ significantly between CHIP-positive and CHIP-negative patients, ferritin levels were significantly higher in CHIP-positive patients treated with anti-BCMA CAR T-cell therapy (median, Mann–Whitney U test: 130.5 $\mu\text{g/l}$ [n = 6] vs. 34.0 $\mu\text{g/l}$ [n = 15]; $p = 0.04$) (3D).

CHIP and post-CAR T-cell therapy outcome

Here, we analyzed the impact of CHIP mutations on overall survival, event-free survival (EFS) and IEC-related adverse events in patients treated with CAR T-cells for aggressive B-cell lymphoma or MM.

We observed no significant differences between CHIP-positive and -negative patients with LBCL regarding median age at treatment, sex, proportion of PD before lymphodepletion, median Karnofsky performance status, or median normalized LDH levels (Supplementary Table 3). In contrast, among MM patients, those with CHIP were significantly older than CHIP-negative patients (72 vs. 62.5 years; Mann-Whitney U-test, $p=0.02$) (Supplementary Table 3). CHIP did not have a significant impact on overall survival rates in either cohort. In patients with LBCL median survival was not reached in both groups (log-rank test, $p=0.64$) (Figure 4A top).

Similarly, in the MM cohort median survival was not reached in either group (log-rank test, $p=0.10$) (Figure 4A bottom).

We further compared the PFS in both cohorts. PFS was defined as the time from CAR T-cell infusion to relapse or death from any cause (including relapse-related mortality and NRM). No statistically significant difference in PFS was observed between CHIP-positive and CHIP-negative patients in the LBCL cohort (log-rank test, median time to event: CHIP-positive – 313.5 days and CHIP-negative 182.0 days, $p=0.79$) (Figure 4B top). Likewise, in MM patients, PFS did not differ between groups (log-rank test, median time to event: 455 days for CHIP-positive vs. not reached for CHIP-negative, $p=0.11$) (Figure 4B bottom).

We next performed a risk analysis of IEC-associated adverse events in the anti-BCMA cohort, the anti-CD19 cohort, and in the overall patient population, respectively. Interestingly, in the univariable analysis, a significant association between CHIP and late-onset ICAHT was observed in the overall cohort (OR: 4.84; 95% CI: 1.14–33.53) and, specifically in the anti-BCMA cohort, for IEC-HS (OR: 10.20; 95% CI: 1.05–233.9) (Table 2 and Figure 5). In an exploratory multivariable analysis, we could not confirm this finding due to perfect separation resulting from small sample size (Table 3).

In total, 10 patients developed a secondary myeloid neoplasm during follow-up, including eight cases of myelodysplastic syndromes (MDS) and two cases of acute myeloid leukemia (AML). The incidence was comparable between patients with LBCL and MM, at approximately 10%. Four of these patients underwent sequencing; two were analyzed at the predefined time points before and after CAR T-cell therapy, whereas the other two were analyzed only after CAR T-cell therapy. However, no CHIP was detected at any of the analyzed time points prior to the diagnosis of the secondary malignancies. Moreover, five patients had pre-treatment neutropenia, and all but one subsequently developed severe late ICAHT. Insertion analyses were not performed as part of this study.

Discussion

CAR T-cell therapies have revolutionized the treatment of relapsed and refractory MM, lymphoma and B-ALL. Nevertheless, IEC-associated inflammation-driven adverse events, such as CRS, ICANS and IEC-HS remain incompletely understood. CHIP has been proposed as a potential underlying mechanism, as it may promote inflammation and perhaps exacerbate the development of hematologic imbalance ³⁰.

Previous studies have reported variable CHIP prevalences from 24% to 56.4% among patients undergoing CAR T-cell therapy ^{10-12, 14}. In our cohort, the observed prevalence of CHIP ranged from 13.8% in patients with LBCL to 30.8% in patients with MM. The higher prevalences reported in other studies could be explained by methodological differences, such as the use of lower VAF thresholds (e.g. VAF >1%)¹⁰. Consistent with previous studies, we identified *DNMT3A* as the most frequent CHIP-associated mutation in CAR T-cell patients ^{10, 12}. Whether CHIP confers a clinically meaningful risk for inferior OS or PFS remains unresolved. Consistent with several previous studies we did not detect a significant association between CHIP and OS or PFS in patients with LBCL and MM ¹⁰⁻¹³. However, the available evidence is inconclusive. For instance, Miller et al. reported higher CR rates in patients younger than 60 years with CHIP (88.9% vs. 46.7%)¹⁴. Likewise, Teipel et al. observed superior OS in CHIP-positive patients (median OS not reached CHIP vs. 265 days no CHIP, p=0.003) ⁹.

Given the proposed role of CHIP in promoting a pro-inflammatory state, we investigated its association with CRS, ICANS, and IEC-HS. Although, previous studies suggested an association between CHIP and the occurrence of CRS and ICANS, we did not observe such a relationship in our cohort ^{12, 13}. However, univariable analysis suggested a significant association between CHIP and IEC-HS in patients with MM, consistent with the higher ferritin levels observed at day +7. Notably, in a previously reported case of severe hyperinflammatory syndrome progressing to IEC-HS, we also observed pronounced anti-BCMA CAR T-cell expansion in the presence of a *DNMT3A* mutation ²⁵.

The CAR-HEMATOTOX score identifies low ANC as risk factors for hematotoxicity and subsequent adverse events, which are associated with inferior PFS and OS ^{6, 31-34}. In our cohort, pre-treatment neutropenia was not associated with inferior OS or PFS, the incidence

of CRS, ICANS, or with IEC-HS frequency, but was associated with ICAHT. Based on our findings, the combination of pre-treatment neutropenia and CHIP, analogous to the concept of clonal cytopenia of undetermined significance (CCUS) may represent a potential marker of increased vulnerability to inflammatory complications. This hypothesis requires further validation in prospective studies in larger cohorts and meta-analyses.

Beyond the risk for IEC-related adverse events, CHIP is also associated with an increased risk of therapy-related myeloid malignancies^{35, 36}. The preexisting inflammatory state in the bone marrow may be further amplified by CAR T-cell-mediated immune activation^{18, 30}. It is therefore plausible that preexisting CHIP mutations could be exacerbated by this proinflammatory environment. However, we did not observe a significant increase in either VAF or the absolute number of CHIP mutations over time, and all sequenced patients who subsequently developed secondary myeloid neoplasms were, surprisingly, CHIP-negative.

Our study has several limitations. First, the relatively small sample size, particularly the limited number of CHIP-positive patients, which restricts statistical power and precludes robust subgroup analyses. Second, the retrospective design using a heterogeneous patient cohort and incomplete longitudinal sampling, with follow-up specimens not available for all patients. In addition, sequencing was not performed at uniform time points. These observations should therefore be interpreted with caution and warrant validation in larger, prospective cohorts.

In summary, our data suggest that the impact of CHIP on outcomes after CAR T-cell therapy may not be uniform. The clinical relevance of CHIP is likely influenced by the specific gene mutation, as different CHIP-associated alterations may have distinct effects on inflammatory signaling, CAR T-cell expansion, and hematologic risk. However, the limited number of CHIP-positive patients precluded the analysis of mutation-specific contributions, potentially explaining the divergent findings reported in previous studies. Further understanding of mutation-specific and patient-specific effects may help identify patients that may benefit from closer monitoring and refined risk strategies.

References

1. Mullard A. FDA approves first CAR T therapy. *Nat Rev Drug Discov.* 2017;16(10):669.
2. Locke FL, Miklos DB, Jacobson CA, et al. axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. *N Engl J Med.* 2022;386(7):640-654.
3. Einsele H, San-Miguel J, Dhakal B, et al. Cilta-cel in lenalidomide-refractory multiple myeloma (CARTITUDE-4): an updated analysis including overall survival from an open-label, multicentre, randomised, phase 3 trial. *Lancet Oncol.* 2026;27(2):254-268.
4. Müller F, Hagen M, Wirsching A, et al. CD19 CAR-T cells for treatment-refractory autoimmune diseases: the phase 1/2 CASTLE basket trial. *Nat Med.* 2026;32(3):1142-1151.
5. Pecher A-C, Hensen L, Schairer R, et al. CD19-targeting CAR T cell therapy in five patients with systemic sclerosis unsuitable for autologous stem cell transplantation. *EULAR Rheumatol Open.* 2026;2(2):100008.
6. 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.
7. Renninger J, Kurz L, Stein H. Mitigation and management of common toxicities associated with the administration of CAR-T therapies in oncology patients. *Drug Saf.* 2025;48(7):719-737.
8. Shouval R, Strouse C, Kim S, et al. Cytokine release syndrome and neurotoxicity following CD19 CAR-T in B-cell lymphoma. *Transplant Cell Ther.* 2025;31(7):419-433.
9. Teipel R, Kroschinsky F, Kramer M, et al. Prevalence and variation of CHIP in patients with aggressive lymphomas undergoing CD19-directed CAR T-cell treatment. *Blood Adv.* 2022;6(6):1941-1946.
10. Panagiota V, Kerschbaum JF, Penack O, et al. Clinical implications and dynamics of clonal hematopoiesis in anti-CD19 CAR T-cell treated patients. *Hemasphere.* 2023;7(10):e957.
11. Gustine JN, Branagan AR, Cirstea DD, et al. Impact of clonal hematopoiesis on clinical outcomes to BCMA CAR-T in multiple myeloma. *Blood Adv.* 2025;9(12):3026-3030.
12. Goldsmith SR, Shouse G, Wong FL, et al. Clonal hematopoiesis is associated with severe cytokine release syndrome in patients treated with chimeric antigen receptor T-Cell (CART) Therapy. *Transplant Cell Ther.* 2024;30(9):927.e1-927.e9.
13. Saini NY, Swoboda DM, Greenbaum U, et al. Clonal hematopoiesis is associated with increased risk of severe neurotoxicity in axicabtagene ciloleucel therapy of large B-Cell lymphoma. *Blood Cancer Discov.* 2022;3(5):385-393.
14. Miller PG, Sperling AS, Brea EJ, et al. Clonal hematopoiesis in patients receiving chimeric antigen receptor T-cell therapy. *Blood Adv.* 2021;5(15):2982-2986.
15. Nathan DI, Dougherty M, Bhatta M, Mascarenhas J, Marcellino BK. Clonal hematopoiesis and inflammation: a review of mechanisms and clinical implications. *Crit Rev Oncol Hematol.* 2023;192:104187.
16. Miller PG, Sperling AS, Gibson CJ, et al. Contribution of clonal hematopoiesis to adult-onset hemophagocytic lymphohistiocytosis. *Blood.* 2020;136(26):3051-3055.
17. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med.* 2017;377(2):111-121.
18. Ben Khelil M, Arbab A, Srikanthan J, et al. CAR T cell-mediated bone marrow inflammation causes hematotoxicity and favors clonal hematopoiesis. *Sci Transl Med.* 2025;17(817):eadu9790.
19. Mouhieddine TH, Sperling AS, Redd R, et al. Clonal hematopoiesis is associated with adverse outcomes in multiple myeloma patients undergoing transplant. *Nat Commun.* 2020;11(1):2996.
20. Prinzing B, Zebley CC, Petersen CT, et al. Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. *Sci Transl Med.* 2021;13(620):eabh0272.
21. Fraietta JA, Nobles CL, Sammons MA, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature.* 2018;558(7709):307-312.

22. Lee DW, Santomaso 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.
23. Rejeski K, Subklewe M, Aljurf M, et al. Immune effector cell-associated hematotoxicity: EHA/EBMT consensus grading and best practice recommendations. *Blood*. 2023;142(10):865-877.
24. 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.
25. Riedel A, Stanger AMP, Kimmich LM, et al. Extensive non-clonal CAR T-cell expansion causing fatal hyperinflammatory syndrome with immune effector cell-associated hemophagocytic lymphohistiocytosis, cytokine release syndrome, and neurotoxicity syndrome. *Haematologica*. 2026;111(1):418-422.
26. Krishnamurthy K, Chai J, Liu X, Wang Y, Naeem R, Goldstein DY. Clinical validation of the Ion Torrent Oncomine Myeloid Assay GX v2 on the Genexus Integrated Sequencer as a stand-alone assay for single-nucleotide variants, insertions/deletions, and fusion genes: challenges, performance, and perspectives. *Am J Clin Pathol*. 2024;162(5):480-491.
27. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
28. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*. 2017;19(1):4-23.
29. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9-16.
30. Avigan ZM, Catlett J, Bodnar S, et al. Clonal hematopoiesis and inflammation predict hematologic toxicity and secondary myeloid malignancies after B-Cell maturation antigen-directed chimeric antigen receptor T-cell therapy. *Clin Cancer Res*. 2025;31(20):4333-4344.
31. Rejeski K, Hansen DK, Bansal R, et al. The CAR-HEMATOTOX score as a prognostic model of toxicity and response in patients receiving BCMA-directed CAR-T for relapsed/refractory multiple myeloma. *J Hematol Oncol*. 2023;16(1):88.
32. Rejeski K, Wang Y, Albanyan O, et al. The CAR-HEMATOTOX score identifies patients at high risk for hematological toxicity, infectious complications, and poor treatment outcomes following brexucabtagene autoleucel for relapsed or refractory MCL. *Am J Hematol*. 2023;98(11):1699-1710.
33. 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.
34. Riedel A, Phely L, Hug S, et al. Immunological consequences of CAR T-cell therapy: an analysis of infectious complications and immune reconstitution. *Blood Adv*. 2025;9(13):3149-3158.
35. Warren JT, Link DC. Clonal hematopoiesis and risk for hematologic malignancy. *Blood*. 2020;136(14):1599-1605.
36. Galli E, Rossi M, Pansini I, et al. Predicting therapy-related myeloid neoplasms after CAR-T with the Clonal Haematopoiesis Risk Score (CHRS). *Br J Haematol*. 2025;206(1):372-374.

Tables

Table 1: Patient Characteristics

	Large B-cell Lymphoma			Follicular and Mantle Cell Lymphoma			Multiple Myeloma		
	Absolute	Relative (%)	Range	Absolute	Relative (%)	Range	Absolute	Relative (%)	Range
N	64	56.6		10	8.8		39	34.5	
Median age (years)	63		19-79	68		53-76	67		46-82
Median prior therapy lines	2		1-5	2		2-4	3		1-9
Median Karnofsky	90		60-100	90		70-100	90		70-100
Male	43	67.2		7	70.0		21	53.8	
Female	21	32.8		3	30.0		18	46.2	
PD	39	61.9		6	60.0		21	53.8	
SD	5	7.9		0	0		5	12.8	
PR	15	23.8		4	40.0		12	30.8	
CR	4	6.3		0	0		1	2.6	
CHIP	4	13.8		2	28.6		8	30.8	
no CHIP	25	86.2		5	71.4		18	69.2	
DNMT3A	2	50.0		1	50.0		4	50.0	
TP53	1	25.0		0	0		2	25.0	
PPM1D	1	25.0		1	50.0		1	12.5	
ASXL1	0	0		0	0		1	12.5	
Ide-cel	0	0		0	0		9	23.1	
Cilta-cel	0	0		0	0		30	76,9	
Brexu-cel	0	0		5	50.0		0	0	
Tisa-cel	35	54.7		3	30.0		0	0	
Axi-cel	9	14.1		1	10.0		0	0	
Liso-cel	20	31.3		1	10.0		0	0	
PreCART Neutropenia (<1500/ μ l)	15	27.8		3	30.0		12	30.8	
PreCART Neutropenia (<500/ μ l)	5	7.8		1	10.0		3	7.7	
Median CAR-HEMATOTOX Score	1		0-7	0		0-7	1		0-5

Median normalized LDH	2,61		1.37-11.95	2.1		1.64-3.98	1.77		1.11-5.59
early infection (<d30) (patients)	20	31.3		4	40.0	4	15	38.5	
early bacterial infection	21	77.8		6	100	6	14	77.8	
early viral infection	3	11.1		0	0	0	3	16.7	
early fungal infection	3	11.1		0	0	0	1	5.6	
late infection (>d30) (patients)	11	18.0		0	0	0	10	28.6	
late bacterial infection	6	40.0		0	0	0	9	60.0	
late viral infection	9	60.0		0	0	0	5	33.3	
late fungal infection	0	0		0	0	0	1	6.7	
CRS	61	95.3		10	100		29	74.4	
CRS >2	7	10.9		3	30.0		4	10.3	
ICANS	30	46.9		4	40.0		5	12.8	
ICANS >2	13	20.3		2	20.0		2	5.1	
ICAHT early	58	90.6		9	90.0		36	92.3	
ICAHT early >2	18	28.1		4	40.0		7	17.9	
ICAHT late	46	75.4		5	50.0		25	71.4	
ICAHT late >2	18	29.5		1	10.0		11	31.4	
IEC-HS	1	1.6		0	0		3	7.7	
Median Hb (d180) g/dl	11.8		6.9-15.9	12.7		8.5-15.4	12		7.3-143
Median Platelets(d180) (G/l)	151		21-508	174.5		124-352	145.5		12-217
Median ANC (d180) 1/ μ l	1920		50-5540	2460		1640-4070	1780		30-9700
G-CSF Use	27	44.3		6	60.0		25	69.4	
Secondary Malignancy	6	9.4		0	0		4	10.3	
NRM	8	12.5		0	0		4	10.3	
CRS	1	12.5		0	0		0	0	
ICANS	1	12.5		0	0		0	0	
IEC-HS	1	12.5		0	0		2	50.0	
Infection	2	25.0		0	0		1	25.0	
Other	3	37.5		0	0		1	25.0	

Abbreviations: PD – progressive disease; SD – stable disease; PR – partial remission; CR – complete remission; Ide-cel – idecabtagene vicleucel; Cilta-cel – ciltacabtagene autoleucel; Axi-cel – axicabtagene ciloleucel; Tisa-cel – tisagenlecleucel; Liso-cel – lisocabtagene maraleucel; Brexu-cel – brexucabtagene autoleucel; CHIP – clonal hematopoiesis

of indeterminate potential; DNMT3A – DNA methyltransferase 3 alpha; TP53 – tumor protein p53; ASXL1 – additional sex combs-like 1; PPM1D – protein phosphatase, Mg²⁺/Mn²⁺ dependent 1D; SF3B1 – splicing factor 3b subunit 1; LDH – lactate dehydrogenase; CRS – cytokine release syndrome; ICANS – immune effector cell associated-neurotoxicity syndrome; IEC-HS – immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome; ICAHT – immune effector cell associated hematotoxicity; G-CSF – granulocyte colony stimulating factor; ANC – absolute neutrophil count; Hb – hemoglobin; NRM – Non-relapse mortality

Table 2: Univariable risk analysis for immune effector cell–associated adverse events (IEC-AEs) in the complete cohort, the CD19-targeted populations, and the BCMA-targeted population. Odds ratios (ORs) are shown with 95% confidence intervals in brackets.

All Subgroups OR (95-CI)	CRS (n=62-113)	CRS >2° (n=62-113)	ICANS (n=62-113)	ICANS>2° (n=62-113)	ICAHT (d<30) (n=62-113)	ICAHT>2° (d<30) (n=62-113)	ICAHT (d>30) (n=60-108)	ICAHT>2° (d>30) (n=60-108)	IEC-HS (n=62-113)	Early Infection (n=62-112)	Late Infection (n=57-103)
Karnofsky Score	0.98 0.92- 1.03	0.92 0.87-0.97	0.98 0.95-1.01	0.99 0.94-1.03	0.97 0.90-1.03	0.95 0.91-0.99	0.96 0.92-0.99	0.94 0.90-0.98	0.97 0.91-1.05	0.95 0.91-0.98	1.00 0.96-1.05
CHIP	2.22 0.35- 43.47	0.54 0.03- 3.57	2.00 0.59- 6.83	2.35 0.43-11.16	0.87 0.10-18.33	2.80 0.62-11.82	4.84 1.14-33.53	1.27 0.25- 5.23	6.27 0.94-52.23	0.30 0.04- 1.28	1.26 0.30 -4.69
Neutropenia	1.22 0.5-5.80	0.73 0.16- 2.54	0.93 0.37-2.22	1.18 0.35-3.55	NA NA	3.20 1.29-7.99	2.49 0.91- 8.05	2.96 1.19-7.41	0.68 0.03-4.84	0.74 0.29- 1.79	0.92 0.27- 2.71
CAR- HEMATOTOX Score	1.07 0.78- 1.48	1.11 0.73- 1.61	0.88 0.61-1.23	1.00 0.61- 1.48	1.99 1.02-5.71	1.43 0.98-2.11	1.50 1.00-2.50	1.93 1.32-3.03	1.49 0.90 -2.45	1.15 0.84-1.60	1.32 0.91 - 1.93
LDH normalized	1.17 0.85- 2.02	1.35 1.09- 1.70	1.15 0.96- 1.41	1.31 1.06- 1.64	1.24 0.85- 2.54	1.42 1.15- 1.84	1.20 0.95- 1.67	1.47 1.18-1.97	1.19 0.81- 1.57	1.17 0.96- 1.43	1.08 0.84-1.35
Progressive Disease	0.88 0.25- 2.84	1.30 0.42-4.48	2.20 0.97- 5.24	1.82 0.62-6.10	1.49 0.39- 5.66	1.79 0.74- 4.56	0.72 0.29- 1.68	2.42 0.99-6.39	0.45 0.06- 2.81	0.70 0.32-1.55	1.06 0.40- 2.97
CD19 Subgroup OR (95-CI)	CRS (n=36-74)	CRS >2° (n=36-74)	ICANS (n=36-74)	ICANS>2° (n=36-74)	ICAHT (d<30) (n=36-74)	ICAHT>2° (d<30) (n=36-74)	ICAHT (d>30) (n=34-71)	ICAHT>2° (d>30) (n=34-71)	IEC-HS (n=36-74)	Early Infection (n=33-74)	Late Infection (n=31-68)
Karnofsky Score	0.94 0.80-1.05	0.91 0.84-0.96	0.99 0.95-1.03	0.99 0.94-1.04	0.97 0.90-1.04	0.95 0.91- 0.99	0.97 0.93-1.02	0.93 0.88-0.97	NA NA	0.94 0.90-0.98	1.00 0.95-1.06
CHIP	NA NA	NA NA	1.75 0.29-14.03	2.50 0.29-17.15	0.36 0.03-8.52	0.8 0.04-6.35	4.67 0.65-95.33	0.96 0.05-7.96	NA NA	0.40 0.02-2.95	NA NA
Neutropenia	0.63 0.06-14.03	0.75 0.11-3.39	1.24 0.42-3.64	1.77 0.48-5.97	NA NA	9.20 2.90-32.46	2.14 0.60-10.18	2.45 0.75-7.85	NA NA	1.46 0.47-4.38	0.35 0.02-2.11
CAR- HEMATOTOX Score	0.92 0.45-2.95	1.51 1.01-2.47	1.10 0.76-1.67	0.96 0.58-1.44	0.92 0.60-1.56	3.54 1.73-10.52	1.48 0.92-3.00	2.11 1.28-4.28	NA NA	1.11 0.71-1.77	1.37 0.78-2.42
LDH normalized	0.99 0.68-2.15	1.40 1.11-1.82	1.06 0.86-1.31	1.25 1.00-1.57	3.00 1.07-16.78	1.36 1.09-1.80	1.25 0.95-1.91	1.64 1.24-2.42	6.63 1.26->100	1.23 0.99-1.56	1.19 0.91-1.52
Progressive Disease	0.80 0.04-8.70	1.54 0.39-7.64	2.41 0.92-6.69	1.94 0.58-7.67	0.62 0.08-3.09	2.79 0.94-9.55	0.32 0.08-1.04	2.12 0.70- 7.38	NA NA	0.81 0.30-2.24	1.58 0.39-7.91

BCMA Subgroup OR (95-CI)	CRS (n=26-39)	CRS >2° (n=26-39)	ICANS (n=26-39)	ICANS>2° (n=26-39)	ICAHT (d<30) (n=26-39)	ICAHT>2° (d<30) (n=26-39)	ICAHT (d>30) (n=25-37)	ICAHT>2° (d>30) (n=25-37)	IEC-HS (n=26-39)	Early Infection (n=26-38)	Late Infection (n=25-35)
Karnofsky Score	1.00 0.92-1.09	0.98 0.87-1.10	0.95 0.83-1.06	1.01 0.86-1.22	0.94 0.79-1.08	0.98 0.89-1.09	0.91 0.82-0.99	0.98 0.90-1.06	1.01 0.90-1.15	0.95 0.88-1.03	0.98 0.90-1.07
CHIP	2.00 0.24-43.12	2.43 0.09-67.50	NA NA	NA NA	NA NA	NA NA	4.80 0.63-100.6	1.40 0.16-9.88	10.20 1.05-233.9	0.22 0.01-1.68	4.67 0.75-33.95
Neutropenia	2.11 0.42-15.73	0.73 0.03-6.44	0.53 0.03-4.08	NA NA	NA NA	0.32 0.02-2.20	3.33 0.69-24.77	4.00 0.92-19.04	NA NA	0.20 0.03-0.95	1.42 0.29-6.49
CAR-HEMATOTOX Score	0.61 0.33-1.03	NA NA	0.58 0.10-1.40	NA NA	3.38 0.88-68.86	0.47 0.03-1.51	2.23 1.09-7.78	2.23 1.28-4.70	0.98 0.45-1.82	1.10 0.68-1.77	1.27 0.75-2.16
LDH normalized	0.84 0.40-1.91	0.95 0.18-2.43	1.00 0.25-2.37	1.17 0.14-3.42	0.49 0.19-1.30	1.72 0.79-4.02	0.95 0.46-2.16	1.22 0.56-2.58	0.76 0.10-2.17	1.08 0.51-2.22	1.12 0.25-4.53
Progressive Disease	0.71 0.15-3.04	0.84 0.09-7.67	1.33 0.20-11.11	0.85 0.03-22.58	NA NA	0.58 0.10-3.06	2.10 0.52-8.97	3.11 0.72-16.74	0.25 0.01-2.17	0.56 0.15-2.09	0.79 0.18-3.50

NA: Logistic regression was not performed because of perfect separation or linear dependence

Table 3: Exploratory multivariable risk analysis for immune effector cell–associated adverse events (IEC-AEs) in the complete cohort, the CD19-targeted population, and the BCMA-targeted population. Odds ratios (ORs) are shown with 95% confidence intervals in brackets.

All Subgroups OR (95-CI) (n)	CRS (n=62)	CRS >2° (n=62)	ICANS (n=62)	ICANS>2° (n=62)	ICAHT (d<30) (n=62)	ICAHT>2° (d<30) (n=62)	ICAHT (d>30) (n=60)	ICAHT>2° (d>30) (n=60)	IEC-HS (n=62)	Early Infection (n=62)	Late Infection (n=57)
CHIP (14)	0.66 0.18-2.41	1.16 0.34-3.97	0.66 0.18-2.56	1.86 0.53-7.15	0.42 0.10-1.52	0.71 0.19-2.48	0.99 0.28-3.50	1.35 0.37-4.79	0.78 0.20-2.80	1.84 0.50-6.96	0.35 0.07-1.32
Neutropenia (30)	0.30 0.08-1.04	1.43 0.45-4.64	3.08 0.79-16.61	3.51 1.03-14.30	0.64 0.16-2.63	3.73 1.13-14.13	0.69 0.20-2.25	1.41 0.42-4.66	3.14 0.94-11.27	5.81 1.67-24.49	0.88 0.24-3.03
LDH normalized	1.56 1.06-3.09	0.99 0.76-1.26	0.89 0.67-1.17	0.90 0.68-1.16	2.61 1.27-7.41	0.98 0.75-1.28	1.13 0.88-1.52	1.12 0.88-1.49	0.82 0.56-1.08	1.03 0.79-1.37	1.09 0.84-1.48
CD19 Subgroup OR (95-CI) (n)	CRS (n=36)	CRS >2° (n=36)	ICANS (n=36)	ICANS>2° (n=36)	ICAHT (d<30) (n=36)	ICAHT>2° (d<30) (n=36)	ICAHT (d>30) (n=35)	ICAHT>2° (d>30) (n=35)	IEC-HS (n=36)	Early Infection (n=36)	Late Infection (n=34)
CHIP (6)	NA	NA	1.98 0.31-16.81	5.15 0.48-59.70	NA	1.92 0.07-30.75	5.34 0.72-110.5	2.41 0.10-32.19	NA	0.55 0.03-4.52	NA
Neutropenia (18)	NA	NA	4.56 0.76-45.52	5.76 0.78-53.42	NA	11.04 1.46-114.1	1.12 0.18-7.40	2.15 0.14-21.26	NA	3.03 0.51-17.94	NA
LDH normalized	NA	NA	0.95 0.69-1.29	1.19 0.86-1.63	NA	1.30 0.94-1.88	1.12 0.85-1.58	1.48 1.08-2.30	NA	1.18 0.89-1.64	NA
BCMA Subgroup OR (95-CI) (n)	CRS (n=26)	CRS >2° (n=26)	ICANS (n=26)	ICANS>2° (n=26)	ICAHT (d<30) (n=26)	ICAHT>2° (d<30) (n=26)	ICAHT (d>30) (n=25)	ICAHT>2° (d>30) (n=25)	IEC-HS (n=26)	Early Infection (n=26)	Late Infection (n=25)
CHIP (8)	NA	NA	NA	NA	NA	NA	0.24 0.01-2.06	0.93 0.12-9.57	NA	NA	0.24 0.03-1.55
Neutropenia (12)	NA	NA	NA	NA	NA	NA	0.33 0.03-2.49	0.29 0.03-2.31	NA	NA	0.71 0.09-5.86
LDH normalized	NA	NA	NA	NA	NA	NA	0.21 0.01-1.44	0.55 0.08-3.58	NA	NA	0.61 0.10-3.45

NA: Logistic regression was not performed because of perfect separation or linear dependence

Figure Legends

Figure 1: Study overview and causes of death according to pretreatment neutropenia.

A) Schematic overview of the longitudinal study design **B)** Shown are the causes of death in patients treated with CD19-directed CAR T cells for aggressive lymphoma, stratified by the presence of pretreatment neutropenia $<1500/\mu\text{L}$ ($n = 9$) versus an absolute neutrophil count (ANC) $>1500/\mu\text{L}$ ($n = 24$). Notably, patients with pretreatment neutropenia exhibited a lower proportion of relapse-related deaths (55.56% vs. 79.17%), with a relative shift toward infection-related deaths (22.22% vs. 4.17%), while immune effector cell-mediated toxicities remained largely comparable between groups (11.11% vs. 8.34%). **C)** Causes of death in multiple myeloma (MM) patients stratified by ANC ($<1500/\mu\text{L}$: $n = 3$; $>1500/\mu\text{L}$: $n = 8$). A similar shift toward infections as the cause of death was observed in patients with pretreatment neutropenia (66.67% vs. 0%).

Figure 2: Analysis of overall survival, progression-free survival and incidence of non-relapse mortality according to pretreatment neutropenia status.

A) Top: Kaplan–Meier analysis of overall survival according to pretreatment neutrophil count in patients with large B-cell lymphoma. No significant difference between groups was observed in the log-rank test ($p = 0.91$). **Bottom:** Kaplan–Meier analysis of overall survival in MM patients stratified by ANC. No significant difference was observed using a cutoff of $1500/\mu\text{L}$ ($p = 0.56$). **B) Top:** Cumulative incidence of non-relapse mortality (NRM) according to pretreatment neutropenia in patients with aggressive lymphoma. No significant difference in NRM was observed between groups in the Fine–Gray competing risks model ($p = 0.30$). **Bottom:** Cumulative incidence of NRM according to pretreatment neutropenia in MM patients, showing no evidence of increased NRM over time in neutropenic patients (Fine–Gray model, $p = 0.67$). **C)** Event-free survival for lymphoma patients (**upper panel**) and multiple myeloma patients (**lower panel**). Using a neutrophil cutoff of $1500/\mu\text{L}$, no significant differences were observed ($p = 0.42$ and $p = 0.73$).

Figure 3: Dynamics of CHIP mutations and their association with CAR expansion and inflammation.

A) Top: Absolute number of CHIP mutations per patient. Three patients show new CHIP mutations after CAR T therapy. **Bottom:** Difference in variant allele frequency (VAF) in CHIP analyses performed before and after CAR administration. Newly detected CHIP mutations are shown as +VAF. **B) Top:** Relative CD19 CAR expansion as a proportion of the total lymphocyte count on day +7 according to CHIP status (CHIP+ $n = 3$, CHIP– $n = 25$). A Mann–Whitney U test showed no significant difference in CAR expansion between CHIP-positive and CHIP-negative patients (median: 43.10% vs. 24.70%; $p = 0.91$). **Bottom:** Corresponding analysis of relative BCMA CAR expansion on day +7 (CHIP+ $n = 8$, CHIP– $n = 14$). Likewise, no significant difference in CAR expansion was observed between the two groups (median: 37.17% vs. 43.50%; $p = 0.76$). **C) Top:** CD4/CD8 CAR ratios in anti-CD19 CAR-treated patients stratified by CHIP status (CHIP+ $n = 2$, CHIP– $n = 22$). **Bottom:** CD4/CD8 CAR ratios in anti-BCMA CAR-treated patients stratified by CHIP status (CHIP+ $n = 8$, CHIP– $n = 13$). No significant difference was detected (Mann–Whitney U test; median: 0.36 vs. 0.81; $p = 0.97$). **D) Top:** Ferritin levels in the anti-CD19 CAR cohort according to CHIP status (CHIP+ $n = 4$, CHIP– $n = 24$). No significant difference was observed (Mann–Whitney U test; median: 25.00 $\mu\text{g/L}$ vs. 69.50 $\mu\text{g/L}$; $p = 0.48$). **Bottom:** Ferritin levels in the anti-BCMA CAR cohort according to CHIP status (CHIP+ $n = 6$, CHIP– $n = 15$). Ferritin levels were significantly higher in CHIP-positive patients than in CHIP-negative patients (Mann–Whitney U test; median: 130.50 $\mu\text{g/L}$ vs. 34.00 $\mu\text{g/L}$; $p = 0.04$).

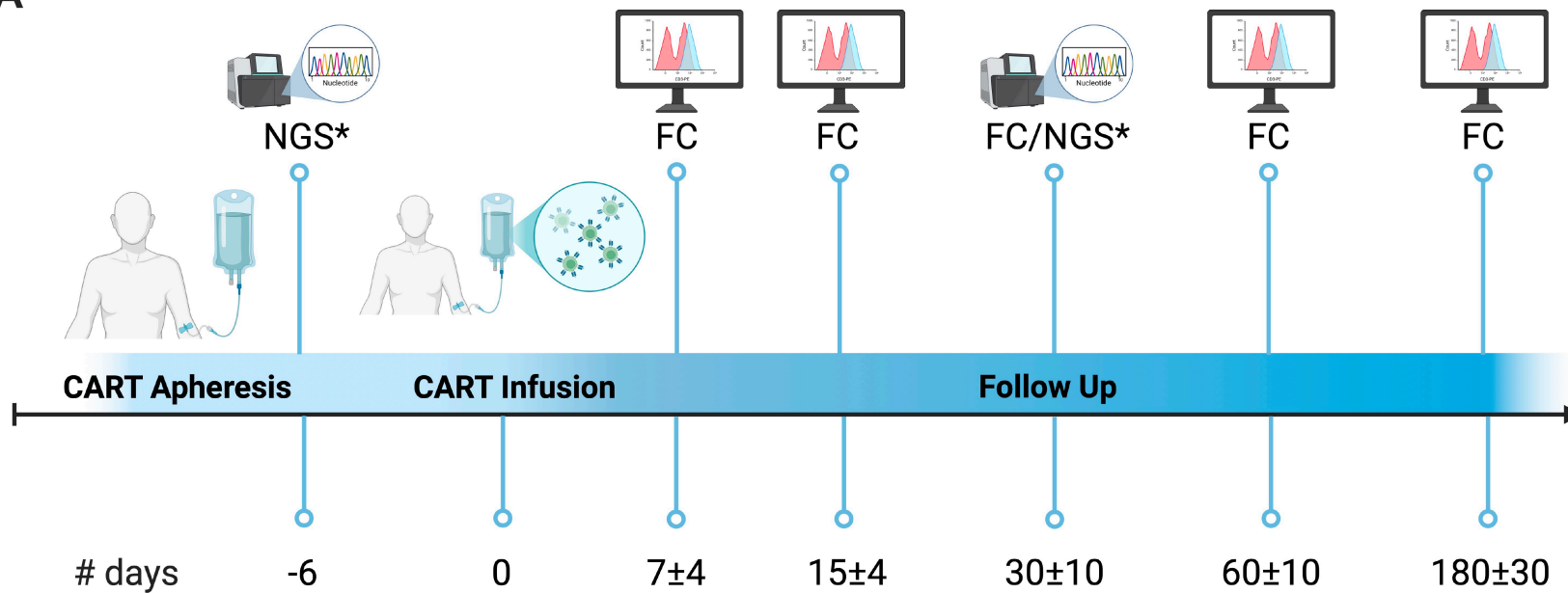
Figure 4: Analysis of overall survival and progression-free survival according to CHIP status

Kaplan–Meier curves for overall survival (OS) of patients with large B-cell lymphoma (**Top**) and multiple myeloma (**Bottom**) according to CHIP status. No significant differences were

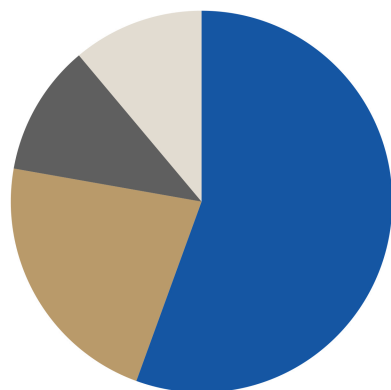
observed (log-rank: $p = 0.64$ and 0.10). **B)** Progression-free survival (PFS) in both cohorts (lymphoma **top**, myeloma **bottom**), do not reveal significant differences (log-rank: $p = 0.79$ and 0.11)

Figure 5: Explorative analysis of risk factors for immune effector cell-associated adverse events.

Log2 heat map of the univariable analysis of risk factors for immune effector cell-associated adverse events in CD19- and BCMA-directed CAR T-cell therapy. CHIP emerged as a potential risk factor for IEC-HS in BCMA-directed CAR T-cell therapy. Significance is indicated by asterisks. See also Table 2

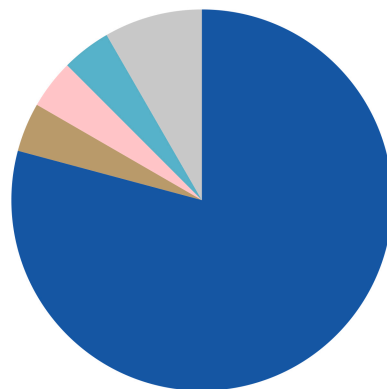
A**B**

Pretreatment ANC <1500/μl



55.56% Relapse
22.22% Infection
11.11% Other
11.11% IEC-HS

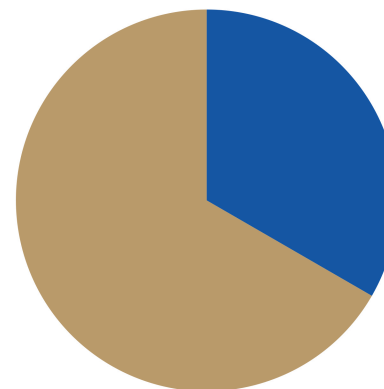
Pretreatment ANC >1500/μl



79.17% Relapse
04.17% Infection
04.17% CRS
04.17% ICANS
08.33% Other

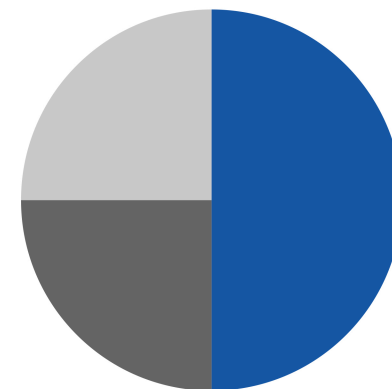
C

Pretreatment ANC <1500/μl

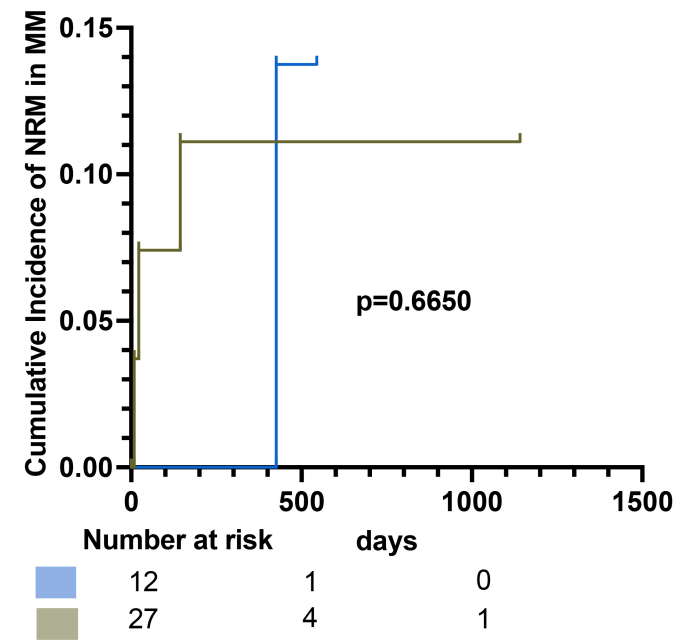
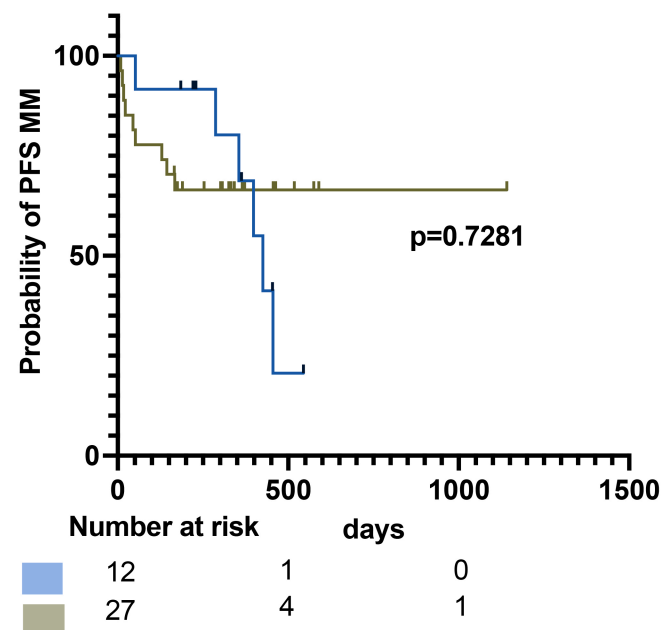
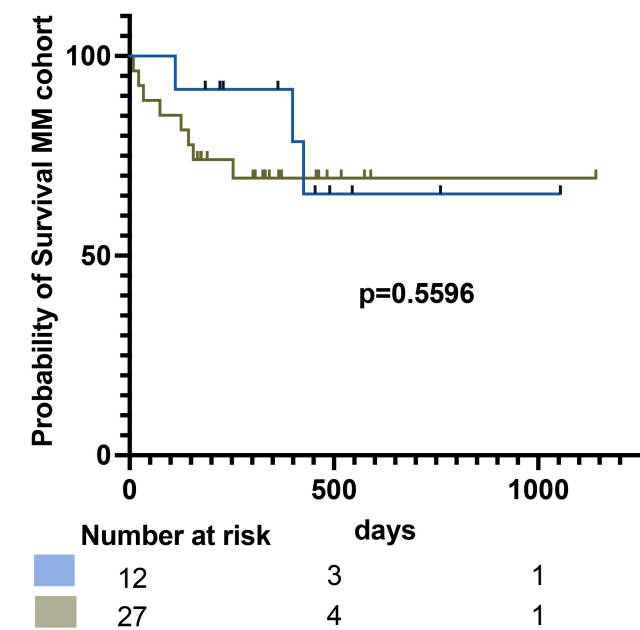
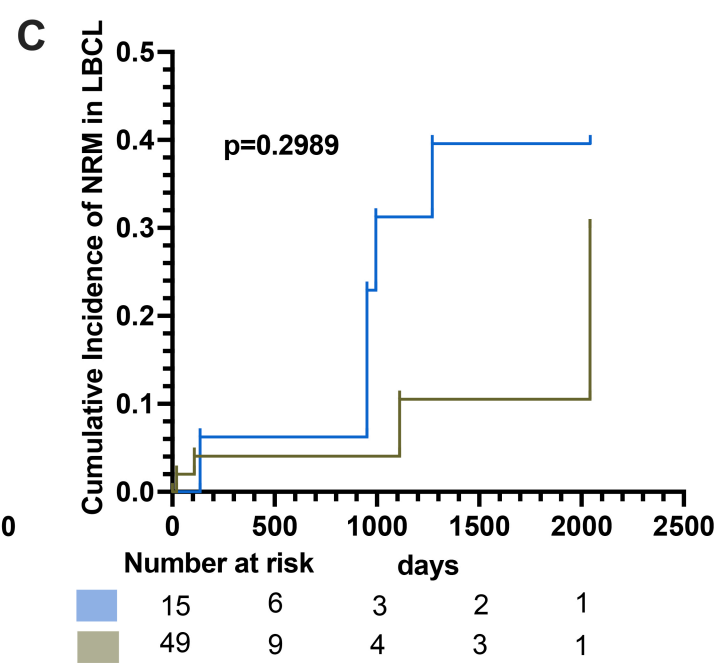
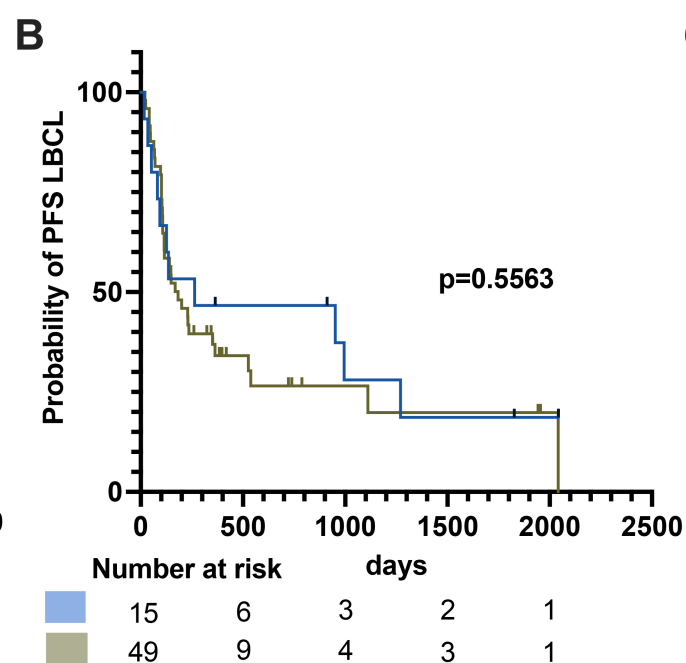
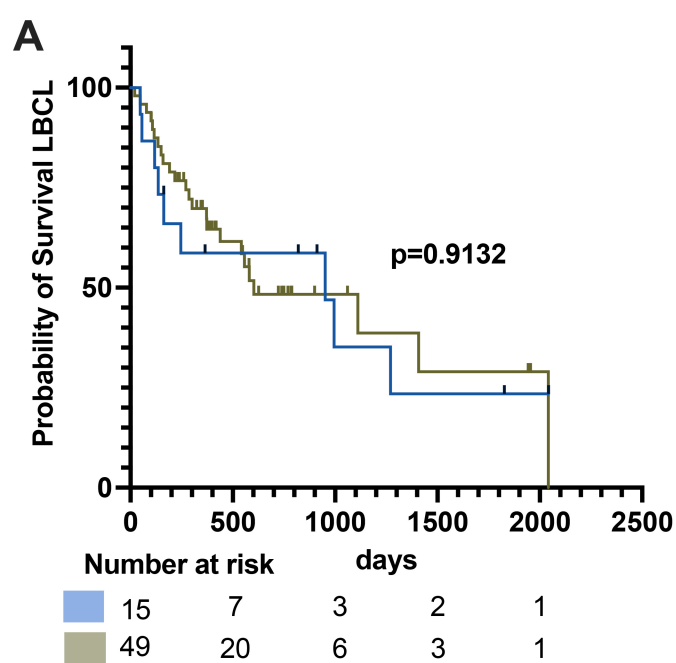


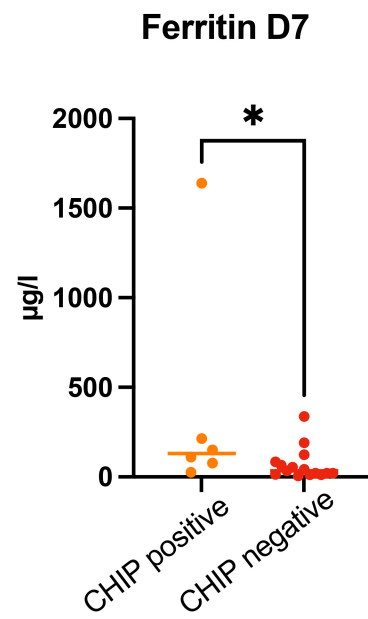
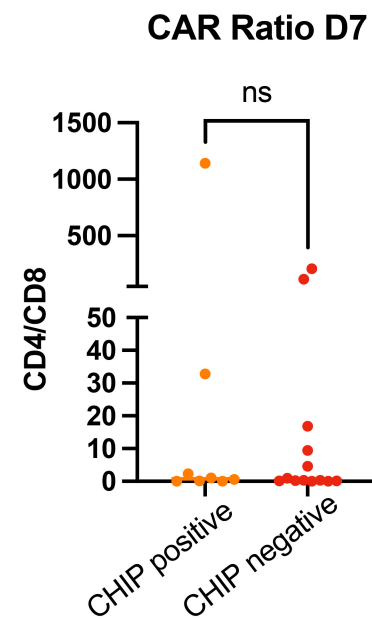
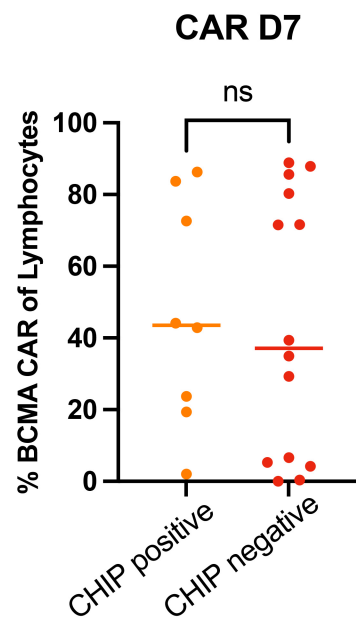
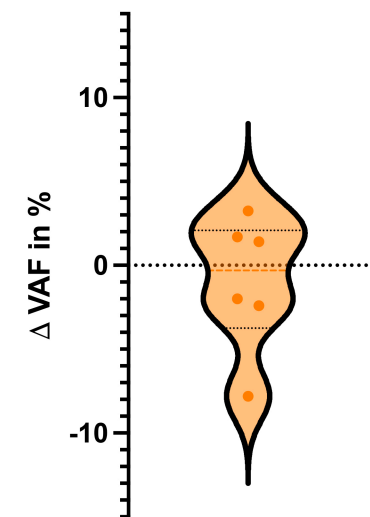
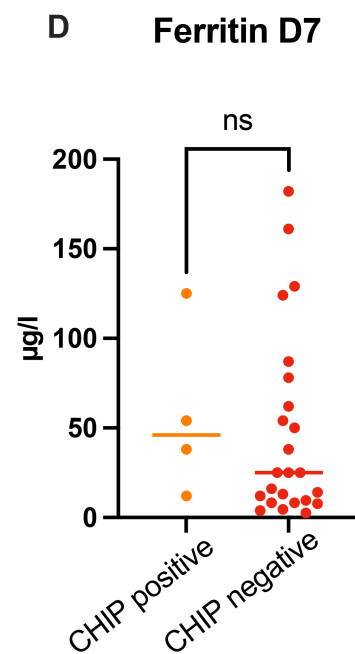
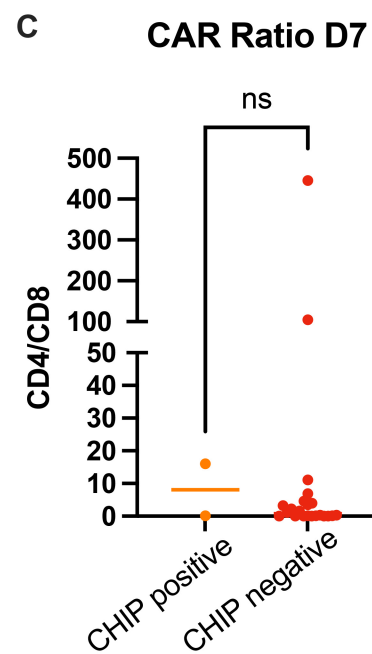
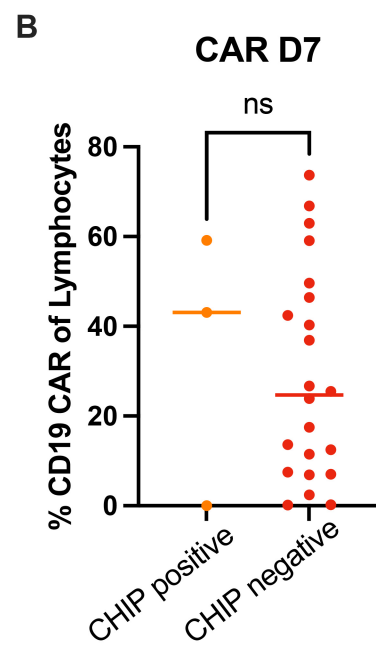
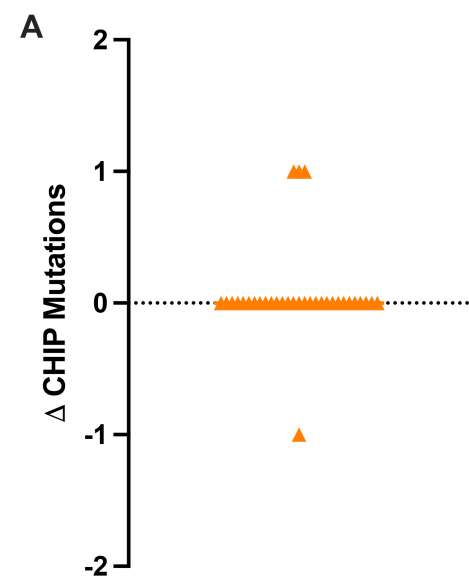
33.33% Relapse
66.67% Infection

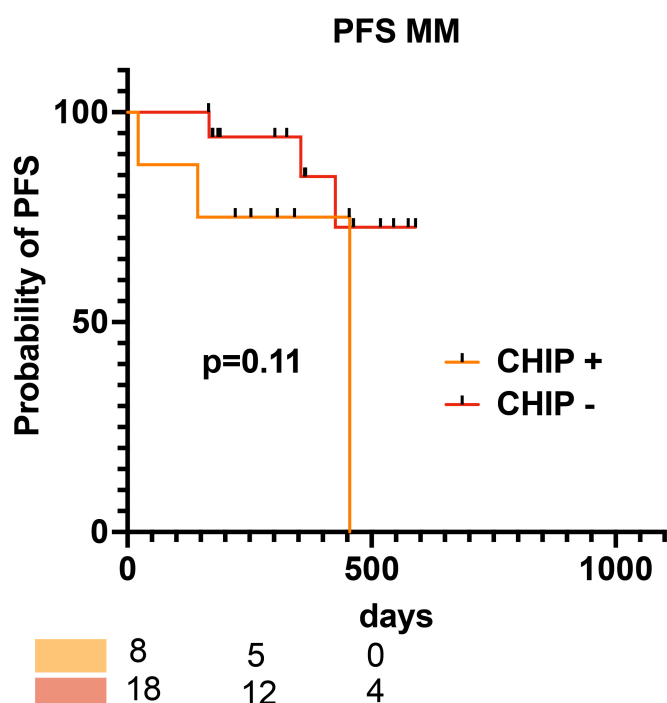
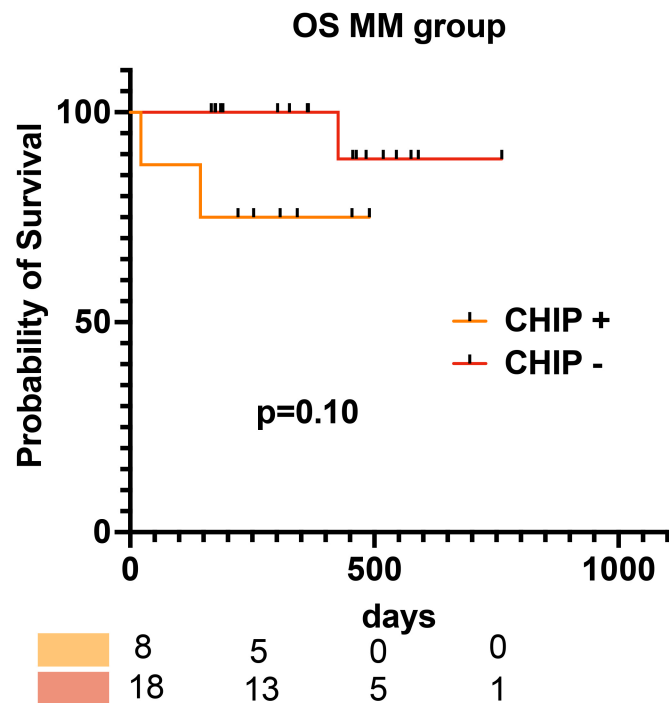
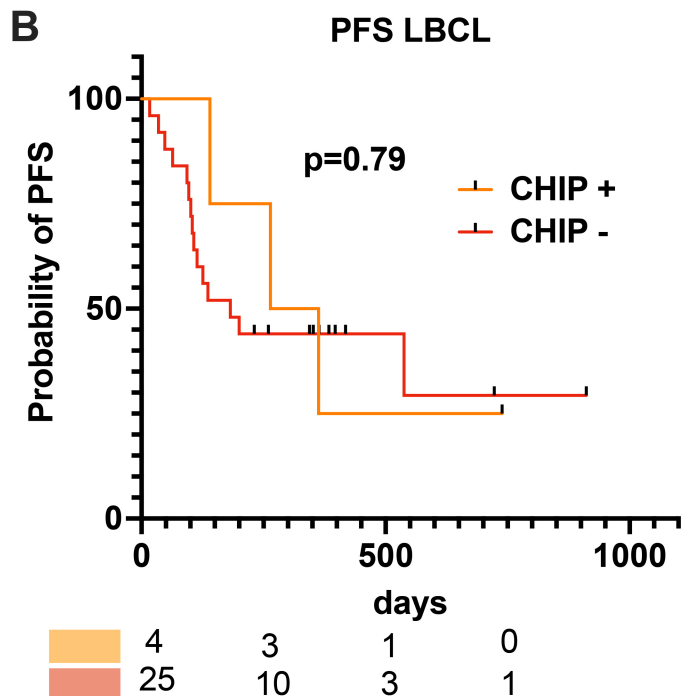
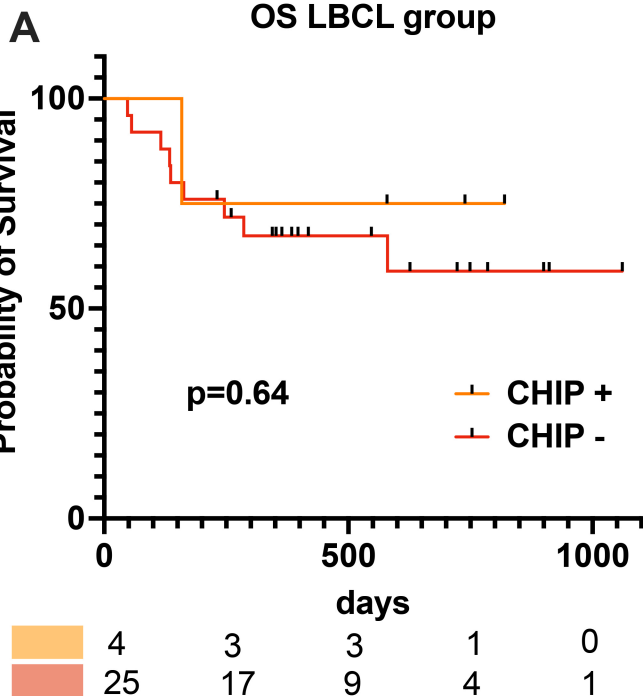
Pretreatment ANC >1500/μl



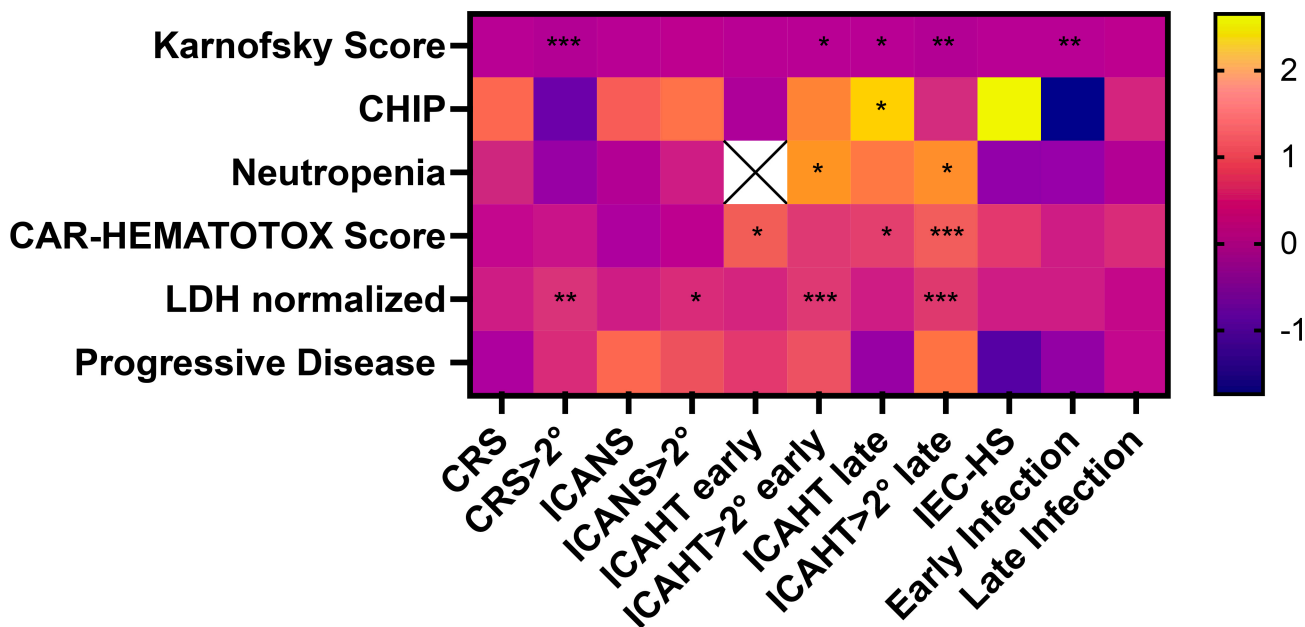
50.00% Relapse
25.00% IEC-HS
25.00% Other



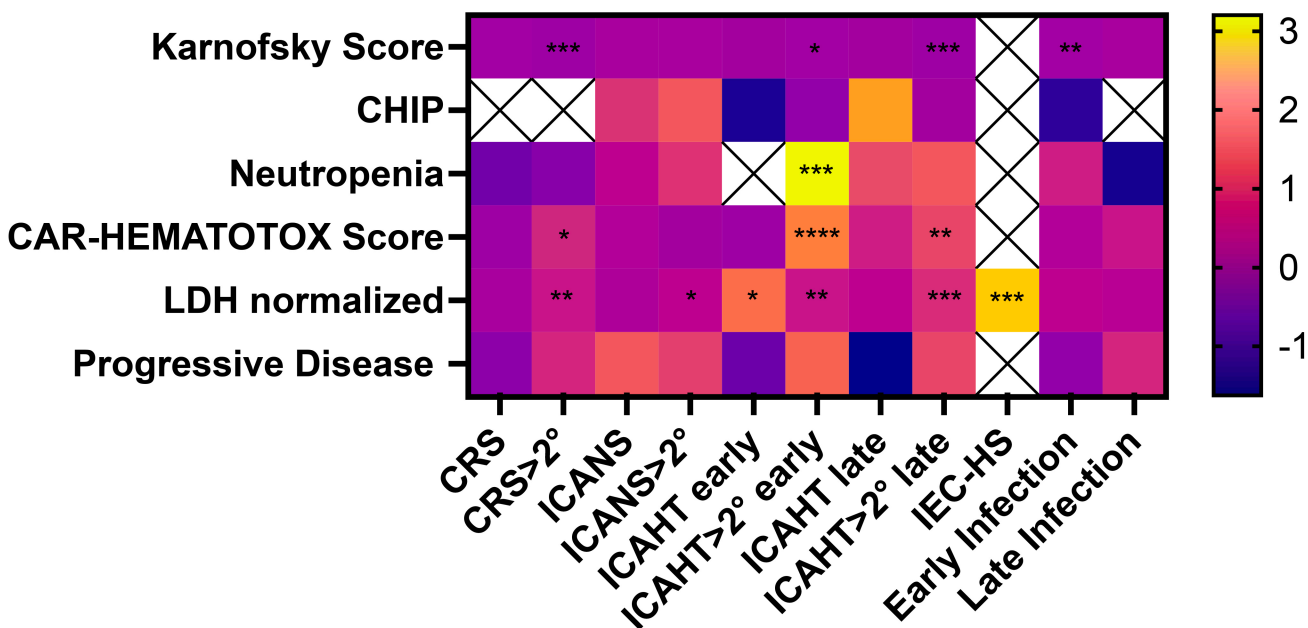




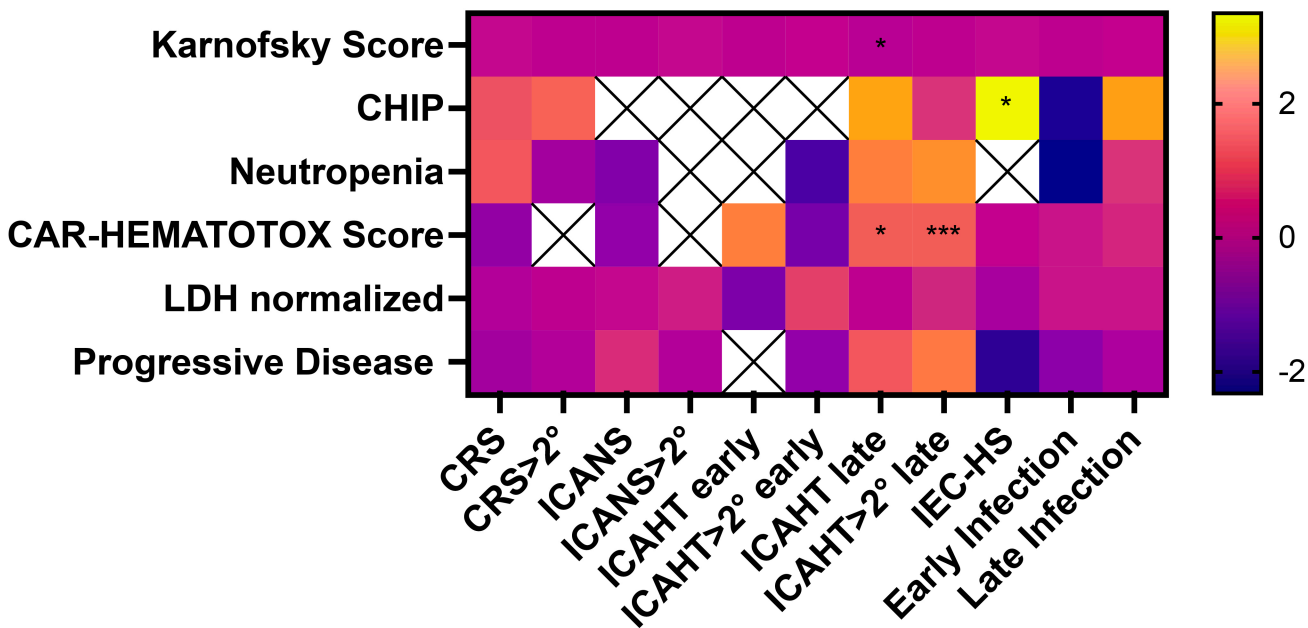
All Subgroups



CD19 Subgroup



BCMA Subgroup



The role of CHIP and pre-treatment neutropenia in patients treated with chimeric antigen receptor T cells for hematological malignancies

Running Title: CHIP and Outcomes after CART

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contributed equally to this study

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Supplementary Methods

Peripheral blood mononuclear cell isolation

Peripheral Blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Sepmate, StemCell Technologies according to manufacturer's protocol and Pancoll, Pan-Biotech). If necessary, PBMCs were frozen in 90% fetal bovine serum (FBS, Gibco) and 10% DMSO (AppliChem).

Next generation sequencing (NGS) of peripheral blood

Genomic DNA extracted from frozen PBMC pellets using commercially available kits was subjected to targeted NGS using the Ion Torrent™ Genexus™ Integrated Sequencer with the Oncomine™ Myeloid Assay GX v2. Samples were analyzed prior to CAR T-therapy, after therapy, or at both time points. The panel covers 45 genes and 30 fusion drivers²⁶. Sequence reads were aligned to the *Homo sapiens* reference genome (GRCh37/hg19).

Cell number was not normalized. DNA concentration was determined using a Qubit fluorometer (minimum used DNA quantity 1.9 ng), while DNA quality was assessed during sequencing data analysis by evaluating read length distributions and the read ratio of endogenous spike-ins, both serving as indicators of high-molecular-weight DNA. Standard sequencing quality control included assessment of raw read accuracy, mean read length, mean depth, and mapped reads. Variants were called for single nucleotide variants and insertion/deletions and classified according to the American College of Medical Genetics and Genomics (ACMG) criteria and only Tier 1 and 2 variants were included^{27, 28}.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 10 (San Diego, USA), and R (version 4.4.1). Variables for multivariate analysis were selected based on clinical plausibility,

significance in univariable analysis and prior evidence. Distribution between groups was calculated with Fisher's exact test. CAR T-expansion and hospitalization were tested for normality (Shapiro-Wilk) and subsequently analyzed with Mann-Whitney U tests. T-cell courses were compared using mixed-effects models. Survival was analyzed using log-rank tests and illustrated with Kaplan-Meier curves, non-relapse mortality (NRM) was assessed using Fine-Gray models. Predictive factors were evaluated by univariable and multivariate regression with ORs and 95%-confidence intervals (CI). LDH levels were scaled by a factor of 10, in order to facilitate interpretation of effect sizes and improve model stability.

Supplementary Tables

Supplementary Table 1: Antibodies used for Flow cytometry of peripheral blood

	Conjugate	Clone	Manufacturer
CD45	FITC	HI30	BD Biosciences
CD3	APC	UCHT1	BD Biosciences
CD4	BV605	RPA-T4	BD Biosciences
CD8	BV786	RPA-T8	BD Biosciences
CD19 CAR Anti Biotin	PE		Miltenyi
BCMA CAR Anti Biotin	PE		Miltenyi

Supplementary Table 2: Comparison of sequenced and non-sequenced patients

	LBCL (NGS)			LBCL (no NGS)				MM (NGS)			MM (no NGS)			
	Absolute	Relative (%)	Range	Absolute	Relative (%)	Range	P	Absolute	Relative (%)	Range	Absolute	Relative (%)	Range	P
N	29			35			0.44	26			13			
Median age (years)	62		35-77	64		19-79		65		46-74	70		59-82	0.02
Median prior therapy lines	1		1-5	2		1-5	0.01	2.5		1-8	3		2-9	0.11
Median Karnofsky	90		60-100	80		60-100	0.48	90		70-100	80		70-100	0.04
Male	20	68.97		23	65.71		>0.99	13	50.00		8	61.54		0.73
Female	9	31.03		12	34.29			13	50.00		5	38.46		
PD	19	65.52		20	57.14		0.44	13	50.00		8	61.54		0.73
SD	1	3.45		4	11.43			4	15.38		1	7.69		
PR	7	24.14		8	22.86			8	30.77		4	30.77		
CR	1	3.45		3	8.57			1	3.85		0	0		
PreCART Neutropenia (<1500/ μ l)	8	27.59		7		20.00	0.56	8	44.44		4	30.76		>0.99

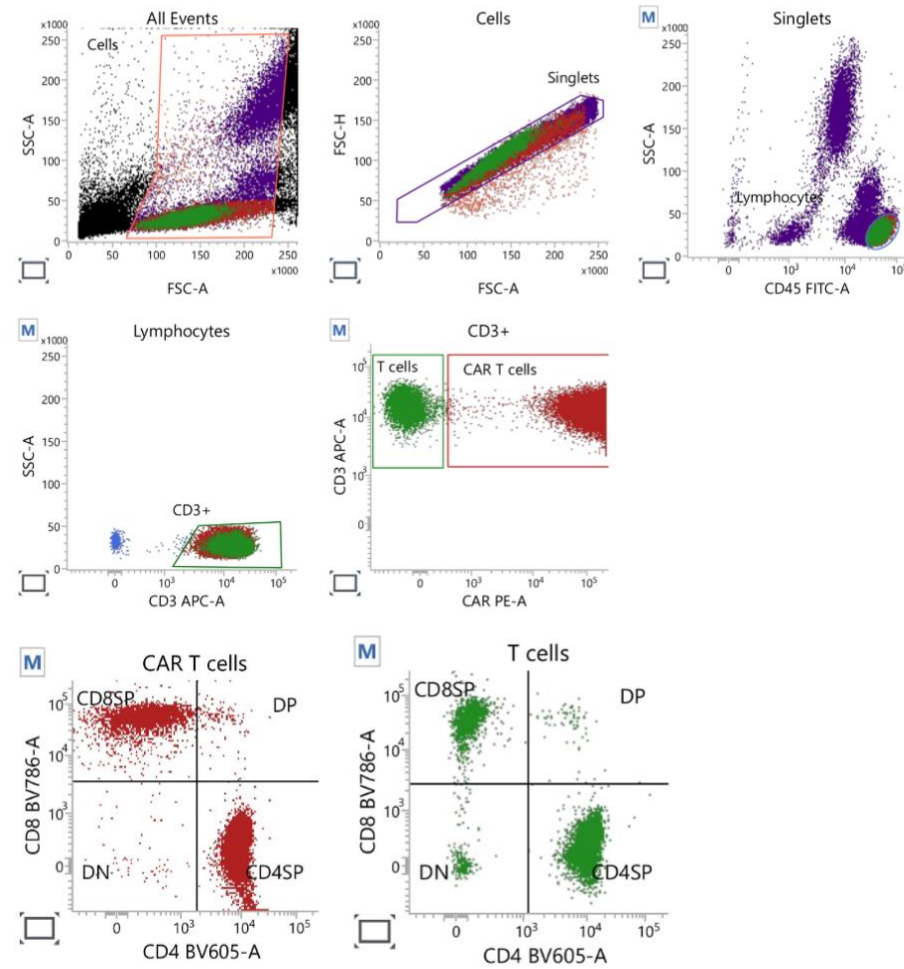
Median HEMATOTOX Score	1		0-7	1		0-4	0.69	1		0-5	2.5		0-5	0.21
Median normalized LDH	2.01		1.37- 11.95	2.73		1.49- 10.35	0.10	1.80		1.11- 3.16	1.77		1.15- 5.59	0.87

Abbreviations: PD – progressive disease; SD – stable disease; PR – partial remission; CR – complete remission; LDH – lactate dehydrogenase; LBCL – Large B-cell lymphoma; MM – Multiple Myeloma; NGS – Next Generation Sequencing

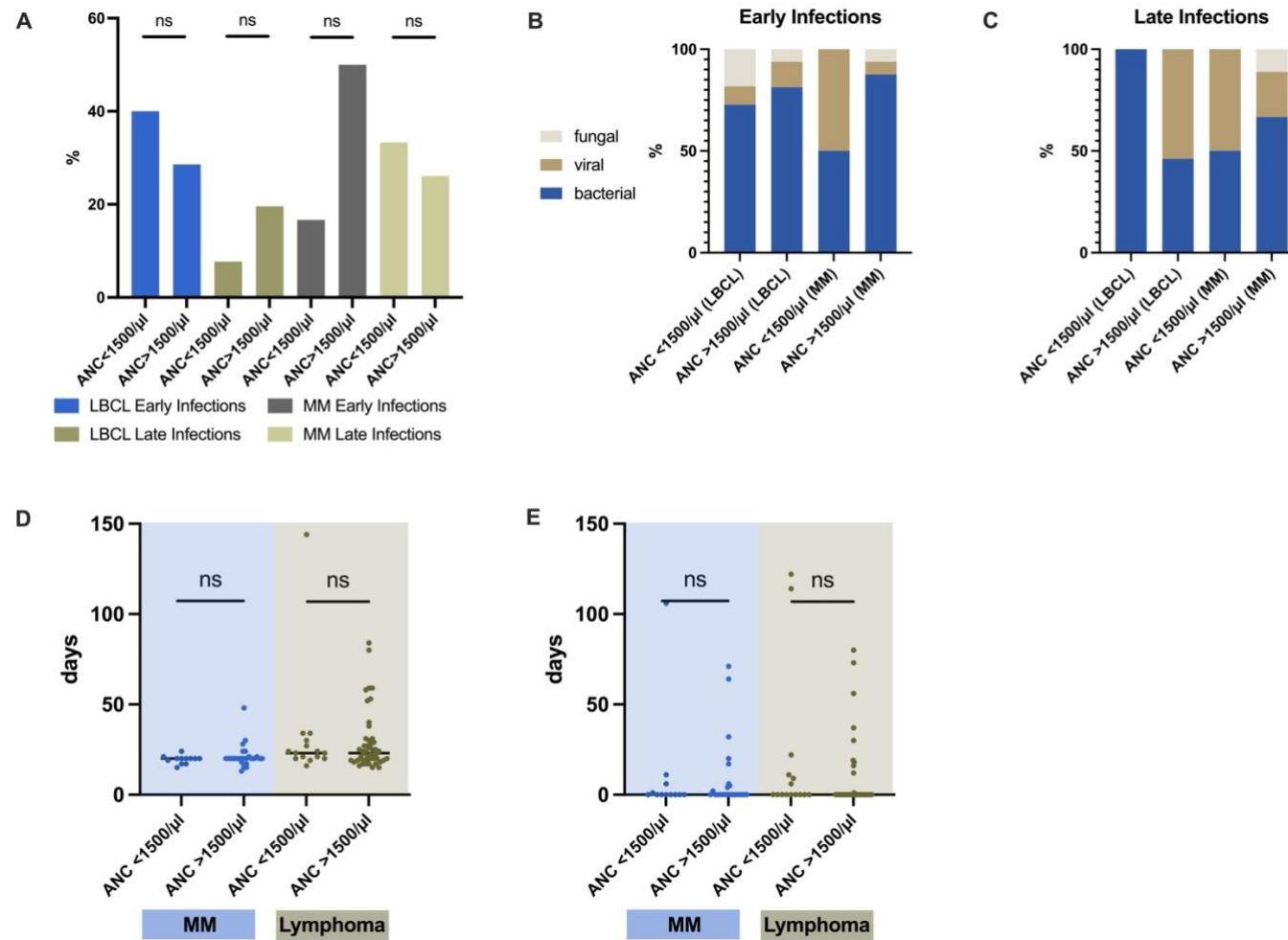
Supplementary Table 3: Patient characteristics according to CHIP status in the aggressive B-cell lymphoma group and the MM group.

	Large B-cell Lymphoma			Multiple Myeloma		
	CHIP Positive (n=4)	CHIP Negative (n=25)	P	CHIP Positive (n=8)	CHIP Negative (n=18)	P
Median age years	68.5 (4)	57 (25)	0.09	72 (8)	62.5 (18)	0.02
Median prior therapy lines	1.5 (4)	1 (25)	0.99	3.5 (8)	2.5 (18)	0.50
Median Karnofsky Score %	100 (4)	90 (25)	0.07	80 (8)	95 (18)	0.05
Male %	75.0 (3)	68.0 (17)	0.99	37.5 (3)	76.9 (10)	0.67
PD %	75.0 (3)	66.7 (16)	0.99	50.0 (4)	50.0 (9)	0.99
Median LDH normalized baseline U/L	1.9 (4)	2.0 (25)	0.27	1.7 (8)	1.8 (18)	0.93
CRP baseline mg/dl	0.3 (4)	0.6 (25)	0.97	0.1 (8)	0.2 (18)	0.84
Ferritin baseline	24.0 (3)	7.1 (14)	0.68	31 (7)	11.6 (10)	0.14
HAEMATOTOX Score	0 (2)	1 (16)	0.16	1 (7)	1 (13)	0.67

Supplementary Figures



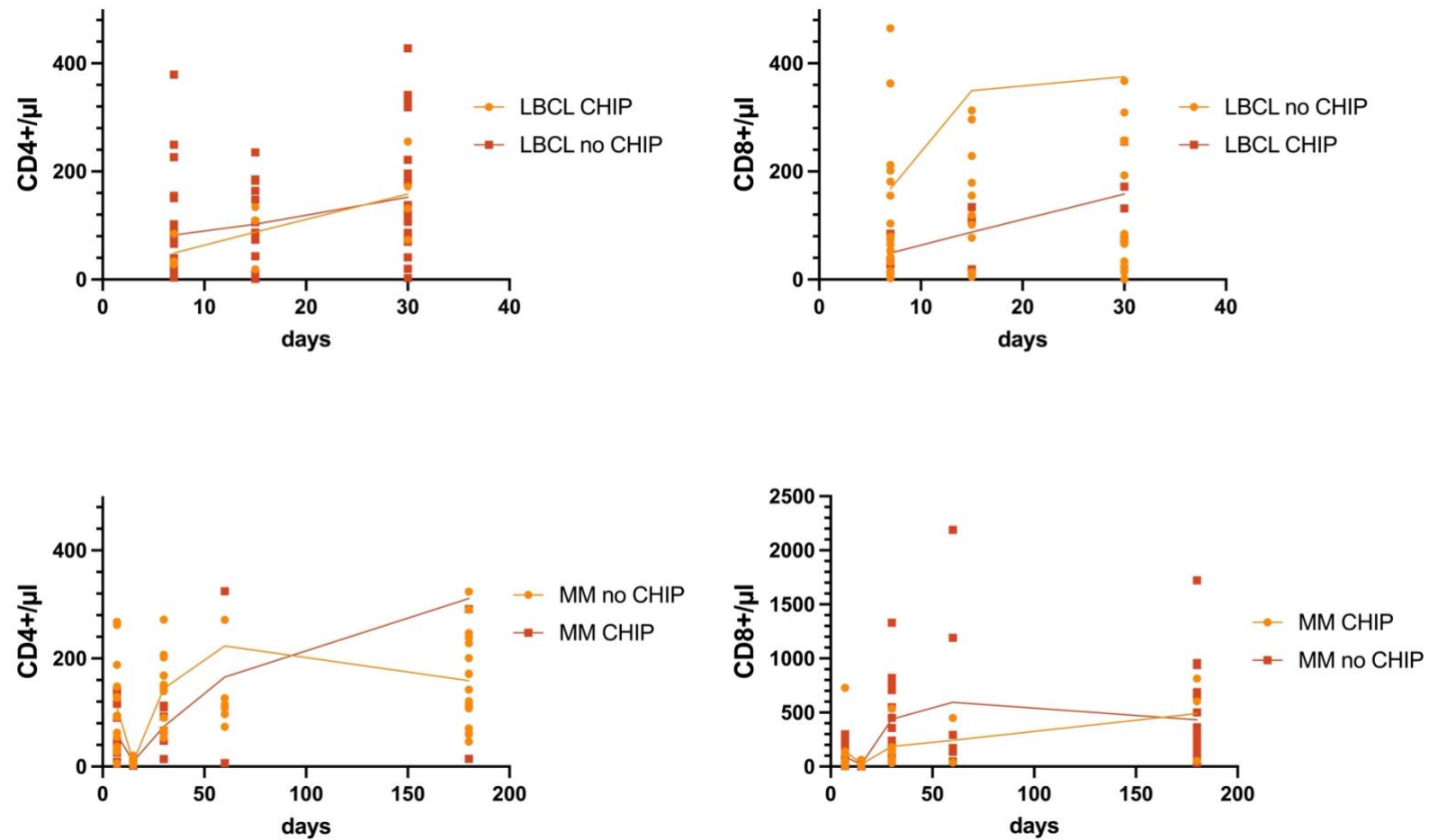
Supplementary Figure 1: Representative flow cytometry gating strategy



Supplementary Figure 2: Infectious complications and hospitalization according to pretreatment neutropenia.

A) Analysis of early (≤ 30 days post-infusion) and late (> 30 days post-infusion) infection rates showed no significant differences between patients with and without pre-treatment neutropenia in the LBCL cohort (Fisher's exact test: early $n = 6$ vs. 14 , $p = 0.53$; late $n = 1$ vs. 9 , $p = 0.43$) or the MM cohort (Fisher's

exact test: early n=2 vs. 13, p=0.08; late n= 4 vs. 6, p=0.71). B) Early onset (<30d post-infusion) infections in LBCL with neutropenia and MM patients. D) Late onset (<30d post-infusion) infections in LBCL with neutropenia and MM patients. **D)** Early hospitalization days during CAR T-cell therapy. **E)** Hospitalization days after discharge until progression or death. No differences were observed according to pretreatment neutrophil count.



Supplementary Figure 3: Dynamics of T-cell subsets according to CHIP status.

CD4⁺ and CD8⁺ non-CAR T-cell counts in CHIP-positive and CHIP-negative MM and LBCL patients. No differences in T-cell reconstitution were observed between groups, as assessed using a mixed-effects model (CD4⁺ LBCL: $p = 0.71$; CD4⁺ MM: $p = 0.90$; CD8⁺ LBCL: $p = 0.55$; CD8⁺ MM: $p = 0.23$).