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A novel dose-dense strategy for CD19-directed CAR T-cell therapy is associated with durable responses without increased toxicity in patients with B-cell non-Hodgkin lymphoma

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De-identified participant data and the data dictionary can be made available at the request of qualified investigators, who are required to obtain approval from the Institutional Review Committee of the Fred Hutchinson Cancer Center to use the data. Requests can be made to the corresponding author.

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This study is the first to report a dose-dense CD19-directed CAR T-cell redosing strategy in R/R B-NHL. Using concurrent trial cohorts treated at the same dose level, we show that administering an early second infusion at day 14 may increase CAR T-cell exposure and elicit long-term response without added toxicity, providing clinical proof-of-concept to guide future dosing approaches.

Author contributions:

C.J.T. and D.G.M. conceptualized and designed the study; V.O.M., C.J.T. and J.G. collected and assembled the data; V.O.M. and J.G. analyzed and interpreted the data; all authors were responsible for writing the manuscript and approval of the final version; and all authors are accountable for all aspects of the work.

Conflict of interest disclosures:

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Bristol Myers Squibb company); has stock options in A2 Biotherapeutics, and Navan Technologies; reports memberships with compensation in A2 Biotherapeutics; is a member of the scientific advisory board of Navan Technologies, Chimeric Therapeutics, and Genentech; and is a member and chair of the Bristol Myers Squibb Lymphoma Steering Committee; a member of the JCAR017 EAP-001 safety review committee; a member of the Bristol Myers Squibb CLL strategic council; a member of the ImmPACT Bio clinical advisory board for the CD19/CD20 bispecific CAR T-Cell Therapy Program; a member of the Gilead Sciences scientific review committee for the Research Scholars Program in Hematologic Malignancies; and a clinical advisory board member for Interius Biotherapeutics, Inc; and reports memberships without compensation withthe Bristol Myers Squibb JCAR017-BCM-0 scientific steering committee.

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Abstract

CD19-directed chimeric antigen receptor (CAR) T-cell therapy induces durable remissions in only 30-40% of patients with relapsed or refractory (R/R) B-cell non-Hodgkin lymphoma (B-NHL), highlighting a major need to improve outcomes. In dose-escalation studies, higher CAR T-cell doses improved tumor control but caused prohibitive toxicities. Having established the maximum tolerated JCAR014 dose in patients with B-NHL at 2 x 10^6 CAR+ cells/kg, we hypothesized that a second infusion at day 14 ("dose-dense") at the same dose and without repeat lymphodepletion, would be safe and enhance antitumor efficacy. We report outcomes from the pilot dose-dense cohort of a phase 1/2 trial (ClinicalTrials.gov identifier: NCT01865617) with 8-year follow-up.

Two CAR T-cell products, each containing 2 x 10^6 CAR+ cells/kg, were manufactured for all 20 treated patients; 17 received both infusions. Any-grade cytokine release syndrome (CRS) and neurotoxicity (NT) occurred in 10 (59%) and 3 (18%) of dose-dense patients, respectively, and—except for one grade 2 CRS—events followed the first infusion only. Despite no additional lymphodepletion, CAR T-cell re-expansion after the second infusion occurred in 16 (94%) patients. By Lugano criteria, overall and complete response rates were 47% (8/17) and 41% (7/17), respectively. Among responders, the 8-year duration-of-response rate was 63% (95% CI: 37-100), comparing favorably with the 26% (95% CI: 14-48) observed in patients treated with a single infusion.

In conclusion, early redosing on day 14 was feasible, safe and led to durable responses in patients with R/R B-NHL.

Introduction

CD19-directed chimeric antigen receptor (CAR) T-cell therapy has transformed the treatment of relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL) with curative potential. Three products—tisagenlecleucel, axicabtagene ciloleucel, and lisocabtagene maraleucel—are currently FDA-approved in the second-line setting or beyond.[1-3] However, these CD19-directed CAR T-cell therapies achieve durable responses in only 30-40% of patients with R/R B-NHL,[4-6] reflecting a vital need to understand mechanisms of resistance and design strategies to improve outcomes. The administration of higher doses of CAR T-cells is associated with superior antitumor efficacy, but also with a greater risk of severe cytokine release syndrome (CRS) and neurotoxicity (NT).[7-11] We hypothesized that the administration of a higher cumulative CAR T-cell dose might decouple the superior antitumor effects of higher CAR T-cell dose from toxicity. To test this hypothesis, we conducted a pilot study in patients with R/R B-NHL to evaluate the feasibility, safety and efficacy of lymphodepletion chemotherapy followed by two consecutive CAR T-cell infusions on days 0 and 14 (2 x 10^6 CAR T-cells/kg each; total infused dose 4 x 10^6 CAR T-cells/kg; dose-dense [DD] cohort). In addition, we performed exploratory safety, efficacy and cellular kinetic comparisons to a similar patient population concurrently enrolled to receive a single infusion.

Methods

Study design and participants

This open-label, phase 1/2 trial (NCT01865617) assessed the feasibility, safety, and efficacy of lymphodepletion chemotherapy followed by JCAR014, an autologous, second-generation CD19-directed CAR T-cell product incorporating

a 4-1BB costimulatory domain and fixed 1:1 CD4+:CD8+ T-cell ratio, in adults with R/R B-cell malignancies.[7,12-15] Eligible patients had indolent or aggressive non-Hodgkin lymphomas (NHL) refractory to first-line chemoimmunotherapy, had relapsed after autologous or allogeneic hematopoietic stem-cell transplantation (HCT), or were ineligible for autologous HCT. The trial included dose-escalation single-infusion cohorts at three dose levels: 0.2 x 10^6 (DL1), 2 x 10^6 (DL2), or 20 x 10^6 (DL3) CAR T-cells/kg, as previously reported,[12] and a concurrent exploratory "dose-dense" cohort treated at DL2.

Between March and July 2016, patients in the dose-dense cohort received a single lymphodepletion course followed by two JCAR014 infusions of 2 x 10^6 CAR T-cells/kg each on days 0 and 14 (cumulative dose of 4 x 10^6 CAR T-cells/kg). The timing of the second infusion was specified in the protocol amendment introducing the dose-dense strategy for B-NHL. The protocol allowed administration between days 10 and 21 following the first infusion, if patients had no dose-limiting toxicity, had recovered to ≤ grade 2 CRS and ≤ grade 1 neurotoxicity, and were afebrile for at least 24 hours. Day 14 was selected as a standardized midpoint within this window to ensure consistency across patients while allowing adequate monitoring and resolution of early toxicities. No prophylactic steroids were administered. The protocol was approved by the Fred Hutchinson Cancer Center (FHCC) Institutional Review Board and conducted according to the Declaration of Helsinki. All patients provided informed consent.

CAR T-cell manufacturing and cellular kinetics

Autologous CAR T-cells were manufactured from leukapheresis material as previously described.[7,12] The CAR construct co-expressed a truncated epidermal growth factor receptor (EGFRt) to facilitate quantification.[16] CD4+ and

CD8+ CAR T-cells were identified by EGFRt-based flow cytometry, and absolute counts were derived from the percentage of CAR T-cells and same-day lymphocyte counts. Expansion and persistence were determined by flow cytometry and quantitative PCR (qPCR) for integrated transgene sequences (lower limit of detection 10 copies/μg DNA).[7,12] Loss of persistence was defined as the first qPCR value below threshold without subsequent re-emergence. B-cell aplasia (BCA) was defined as absence of any measured B cells by flow cytometry and B-cell recovery (BCR) as their reappearance.

Bridging and lymphodepletion

Bridging therapy after leukapheresis was allowed for rapidly progressive disease and was investigator-selected, with systemic corticosteroids minimized when feasible. Lymphodepleting chemotherapy was administered intravenously before the first infusion only. Regimens consisted of cyclophosphamide, fludarabine, etoposide, and/or bendamustine, alone or in combination. Lymphodepletion regimen selection was protocol-permitted and investigator-selected to accommodate underlying disease and clinical factors, reflecting the early-phase nature of the trial. For the dose-dense cohort, fludarabine/cyclophosphamide was specified as the preferred regimen unless clinically inappropriate.

CAR T-cell infusion

Patients received their first CAR T-cell infusion two to three days after completing lymphodepletion (day 0). Patients were eligible for a second infusion between days 10 and 21 if they had no dose-limiting toxicity, were afebrile for at least 24 hours, and had resolution or improvement of CRS to ≤ grade 2 and NT to ≤ grade 1.

Outcomes and statistical analysis

Adverse events, including CRS and NT, were graded and managed by CTCAE v4.0 and Lee et al., 2014, as previously described.[14,17] Response was assessed by PET-CT according to Lugano criteria at prespecified time points including months 1, 6, and 12 after the last CAR T-cell infusion (i.e., after the second infusion in the dose-dense cohort). Additional assessments were conducted at the discretion of the treating clinician.

Efficacy and survival outcomes were analyzed using standard descriptive and time-to-event methods; full statistical details are provided in the Supplementary Methods. BCA and BCR were analyzed using cumulative incidence estimates with death treated as a competing event and compared between cohorts using Gray's test.[18] Post-hoc analyses included exploratory comparisons of key safety, efficacy and cellular kinetics between the dose-dense and the single-infusion cohorts. For longitudinal analyses of CAR T-cell expansion, linear mixed-effects models (LMM) were used to account for repeated measurements within patients. Models included fixed effects for time (continuous), group (e.g., ICAHT status or infusion cohort), and their interaction, with a random intercept for each patient. Differences in temporal trajectories between groups were assessed using likelihood ratio tests (LRT) comparing models with and without the interaction term.

Results

Patient characteristics

Of 24 screened patients, 21 underwent leukapheresis, and 20 received lymphodepletion chemotherapy followed by CAR T-cell infusion, of whom 17

proceeded to receive the second infusion on day 14 (dose-dense cohort; Figure 1, Table 1). In the dose-dense cohort, the median age was 59 years (range, 43-70). Lymphoma subtypes were as follows: de novo or transformed diffuse large B-cell lymphoma in 8 (47%), high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements in 4 (24%), follicular lymphoma in 3 (18%), and mantle cell lymphoma in 2 (12%) patients. Patients had received a median of four prior systemic regimens (range, 1-9), including autologous and allogeneic HCT in 7 (41%) and 3 (18%) of cases, respectively.

Prior to lymphodepletion, 6 (35%) had an ECOG performance status ≥ 1, 9 (53%) had abnormally elevated lactate dehydrogenase (LDH), 15 (88%) had extranodal involvement, and 9 (53%) carried an intermediate- or high-risk International Prognostic Index (IPI) score.

Procedures

Two CAR T-cell products were generated successfully for all patients intended for dose-dense treatment and who received lymphodepletion chemotherapy (n=20). Three patients did not receive the second dose due to: rapid disease progression (n=2), grade 4 CRS with grade 3 NT (n=1). Six of the 17 patients treated with both infusions (35%) received investigator-selected bridging therapy after leukapheresis and prior lymphodepletion, which consisted of chemotherapy in three (18%), ibrutinib in two (12%), and systemic corticosteroids in one (6%) patient (Suppl. Table 1). Lymphodepletion was predominantly fludarabine/cyclophosphamide-based (16/17, 94%), with only one patient receiving bendamustine alone (6%) (Suppl. Table 2). The median time from apheresis to the first infusion was 19 days (IQR, 17-21), and the median time from first to second infusion was 14 days (IQR, 14-14).

Safety of early redosing

Any-grade CRS and NT occurred in 10 (59%) and 3 (18%) dose-dense patients, respectively; all CRS and NT events resolved with supportive care alone, without tocilizumab or corticosteroids, and no grade ≥3 CRS or NT was observed. After the second infusion, only one patient experienced CRS (grade 2), which started one day later and resolved within 48 hours with supportive care alone, and no NT events occurred after the second infusion.

Efficacy after early redosing

Among the 17 dose-dense patients, the best overall response rate (ORR) was 47%, and the complete response (CR) rate was 41%. After a median follow-up of 8 years (95% CI: 7.93-NA), the median duration of response (DOR) for patients with CR or partial response (PR) was not reached (95% CI: 2.7-NA), with an estimated 8-year DOR of 63% (95% CI: 37-100). The median progression-free survival (PFS) was 3 months (95% CI: 1.4-NA), with an 8-year PFS probability of 29% (95% CI: 14-61). The median overall survival (OS) was 19 months (95% CI: 7.2-NA), with an 8-year OS probability of 29% (95% CI: 14-61; Figure 2A-D). Survival was markedly superior in responders: median OS was not reached (95% CI: 73-NA) vs. 3.8 months (95% CI: 3.1-NA) for non-responders ($p < 0.0006$), with an 8-year OS rate of 63% compared with 0% for non-responders ($p < 0.001$).

CAR T-cell expansion and persistence

Because only one patient developed CRS after the second CAR T-cell infusion, we hypothesized that redosing without repeat lymphodepletion might not result in additional in vivo CAR T-cell re-expansion. To investigate this, we comprehensively analyzed the longitudinal cellular kinetics of CD8+ and CD4+

CAR T-cells in the peripheral blood using multiparameter flow cytometry. After the first infusion, the median time to CAR T-cell count peak (Cmax) was 14 days for CD8+ (IQR, 12-22) and 12 days for CD4+ CAR T-cells (IQR, 10-14.5). Median Cmax values were 15 CD8+ (IQR, 4-66) and 12 CD4+ (IQR, 5-41) CAR T-cells/μL. Inspection of individual kinetic curves (Figure 3) showed a biphasic shape in 16 of 17 patients (94%), suggesting re-expansion of CAR T-cells in the blood following the second infusion. Overall, CAR T-cell Cmax occurred after the first infusion in 10 patients (59%) and 13 patients (76%) for CD8+ and CD4+ CAR T-cells, respectively. In the remaining patients, Cmax occurred after the second infusion (7 patients [41%] for CD8+ and 4 [24%] for CD4+ CAR T cells). These findings suggest that despite the absence of repeat lymphodepletion and low rates of CRS, early CAR T-cell redosing can lead to CAR T-cell re-expansion in the blood.

Next, we assessed the long-term CAR T-cell persistence. The median time to loss of measurable CAR transgene by qPCR in the blood was 12.9 months (95% CI: 3.2-32.2), while the CD8+ and CD4+ CAR T-cell persistence measured by flow cytometry was detected for a median of 7.2 months (95% CI: 3.2-31.1) and 12.9 months (95% CI: 4.2-32.2), respectively. Long-term responders exhibited the most durable CAR transgene persistence, with a median time to loss of persistence of 32.1 months (95% CI: 31-NA).

Long-term persistence of CAR T-cell function was reflected by sustained BCA in a subset of patients. The probability of ongoing BCA at 3, 6, and 12 months was 82% (95% CI: 55-100), 59% (95% CI: 20.4-93.1), and 25% (95% CI: 0.0-74.4), respectively.

Dose-dense versus single-infusion

To compare safety and efficacy between the dose-dense and single-infusion cohorts, we performed post-hoc exploratory comparisons between the 17 dose-dense patients and patients with R/R B-NHL concurrently enrolled and treated with a single infusion (n = 52). Three patients initially assigned to the dose-dense cohort, but who received only the first infusion were analyzed in the single-infusion cohort. Patient selection for these analyses is detailed in Suppl. Figure 1.

Baseline demographics and disease features between the dose-dense and single-infusion cohorts were generally balanced, except for a lower proportion of bulky disease (≥5 cm) in dose-dense patients (Table 1). Bridging therapy administration, lymphodepletion regimen type and intensity, and median vein-to-vein time did not significantly differ between groups (Suppl. Tables 1 and 2).

No grade ≥3 CRS or NT events occurred in dose-dense patients, compared with 3 (6%) and 4 (8%) cases, respectively, observed in patients treated with a single-infusion (Table 2). Immune effector cell-associated hematotoxicity (ICAHT) within 30 days post CAR T-cell infusion was comparable between cohorts. The incidence of any-grade ICAHT was 81% in the single-infusion cohort and 71% in the dose-dense cohort, while severe ICAHT occurred in 19% and 6% of patients, respectively, with no significant differences in grade distribution (Suppl. Figure 2; p = 0.59). When CAR T-cell expansion kinetics were examined according to ICAHT development, expansion curves were broadly overlapping between patients with and without ICAHT in both cohorts, without a consistent visual separation during early or peak expansion phases (Suppl. Figure 3 and 4). BCR

did not differ significantly between cohorts (p = 0.3). However, numerically lower cumulative incidence of BCR was consistently observed in the dose-dense cohort across all evaluated time points (3, 6, 9, and 12 months; Suppl. Table 3).

Complete response rates were similar in dose-dense (41%) and single-infusion (42%) patients. No patient with baseline bulky disease (≥5 cm) achieved complete response. Bulky disease was present in 11 (21%) patients in the single-infusion cohort and in none of the dose-dense patients (p = 0.054), suggesting a potential imbalance that may have influenced comparisons of early response outcomes. To explore early response kinetics, we examined the depth of response at the first disease assessment (Suppl. Table 4). Because dose-dense patients received a planned second infusion on day 14, first restaging occurred 14 days later when referenced to infusion #1. Depth of tumor reduction at first assessment was comparable and numerically deeper in dose-dense patients (median −70% vs −53% tumor cross-sectional area reduction; p = 0.10). However, responses were more durable in dose-dense patients: median DOR was not reached in dose-dense patients (8-year DOR, 63%) compared to a median of 8.5 months (8-year DOR, 26%) in patients treated with single-infusion (p = 0.16; Figure 4A). Progression-free survival and overall survival were also longer in dose-dense patients: the 8-year PFS was 29% (95% CI: 14-61) in dose-dense patients while it was 16% (95% CI: 8.2-30) in single-infusion patients (p = 0.54; Figure 4B). Among responders, the median OS was not reached in dose-dense patients (8-year OS, 63%) while it was 24.1 months (8-year OS, 35%) in single-infusion patients (p = 0.19; Figure 4D).

Despite similar complete response rates between the dose-dense and single-infusion cohorts, patterns of CD19 expression at relapse differed. Among patients with evaluable samples, CD19-negative relapses were more frequent in the dose-dense group (4 of 9 [44%] vs 2 of 18 [11%] after single infusion), whereas CD19-positive relapses were less common in the dose-dense group (5 of 9 [56%] vs 16 of 18 [89%] after single infusion), although this did not reach statistical significance ($p = 0.14$). Given the higher frequency of CD19-negative relapses observed in the dose-dense cohort, we performed an exploratory analysis among dose-dense patients with relapse/progression. CAR T-cell expansion by qPCR and early cellular exposure (AUC day 0–30) differed according to CD19 status at relapse/progression, with higher AUC day 0–30 in patients with CD19-negative than CD19-positive relapse (median 675,949 vs 78,715; $p = 0.011$; Suppl. Figure 5). Post-relapse therapies were heterogeneous and are summarized in Suppl. Table 5. Overall survival after documented relapse/refractoriness was dismal (median 5.2 months, 95% CI 3.9–13.4) and was shortest in patients with CD19-negative relapse/refractoriness (median 2.0 months, 95% CI 1.7–not estimable; Suppl. Figure 6).

The improved durability of responses in dose-dense patients suggested more robust CAR T-cell in vivo expansion and persistence compared to single-infusion patients. To test this hypothesis, we compared the in vivo CAR T-cell kinetics between the two groups. We measured comparable peak CAR T-cell expansion (Cmax) in the dose-dense and single-infusion cohorts (CD8+ CAR T-cells/μL, median, 15 vs 19; CD4+ CAR T-cells/μL, median, 12 vs 10; Figure 5; Suppl. Table 6). However, compared with the single-infusion cohort, dose-dense patients had higher exposure—day 0–30 area under the curve (median CD8+ CAR T-

cells·day/μL, 101 vs 66 [p = 0.46]; CD4+ CAR T-cells·day/μL, 78 vs 34 [p = 0.13]) and day 14–30 AUC (median CD8+ CAR T-cells·day/μL, 26 vs 15 [p = 0.10]; CD4+ CAR T-cells·day/μL, 15 vs 5 [p = 0.038])—and longer CAR transgene persistence (median 12.9 vs 5.6 months, respectively; p = 0.58; Figure 6), although this only reached statistical significance for the day 14–30 AUC CD4+ CAR T cells.

We next hypothesized that the more prolonged in vivo persistence in dose-dense patients could reflect the presence of CAR T-cells harboring less differentiated effector states. To explore this, we compared the CAR T-cell naïve and memory subsets in the peripheral blood using flow cytometry. At day +28 after CAR T-cell infusion (after the first infusion for dose-dense patients), we measured significantly lower frequencies of CD8+ EMRA CAR T-cells in the dose-dense cohort compared to the single-infusion cohort (median, 6% vs 17%, p = 0.04) and higher frequencies of CD8+ central memory CAR T-cells (median, 21% versus 11%, p=0.10). In contrast, no other statistically significant or consistent differences were observed across CD8+ or CD4+ CAR T-cell subsets (Suppl. Table 7).

Because differences in differentiation states in end-manufacturing CAR T cells could impact in vivo CAR T-cell persistence, we next compared the percentages of naïve and memory CAR T-cell subsets between the single-infusion and dose-dense cohort. We found that the frequency of naïve, central memory, effector memory, and effector subsets within both CD4+ and CD8+ CAR T-cell compartments were comparable between the single-infusion and dose-dense cohorts, with no statistically significant differences across comparisons (Suppl. Table 8).

Discussion

CD19-directed CAR T-cell therapy yields durable remissions in only 30-40% of patients with R/R B-NHL, highlighting a key unmet need. The administration of higher CAR T-cell doses generally enhances antitumor efficacy, but at the cost of higher rates of CRS and neurotoxicity. Innovative dosing strategies that raise cellular exposure without increasing toxicity are therefore needed.

Having previously determined the single-infusion maximum tolerated JCAR014 dose of 2 x 10^6 CAR T-cells/kg, we sought to study the outcomes of a novel dose-dense approach in patients with R/R B-NHL receiving JCAR014. First, we showed the feasibility of manufacturing two infusion products, which was achieved successfully in all patients. Dose-dense CAR T-cell therapy did not increase the incidence or severity of CRS and NT compared to patients who received a single infusion. Despite the absence of additional lymphodepletion between the two CAR T-cell infusions and the low rate of CRS, we measured CAR T-cell re-expansion in 94% of patients.

Our exploratory analyses suggest early CAR T-cell redosing may translate into more durable responses. Although we observed comparable overall and complete response rates, the 8-year DOR was 63% in the dose-dense cohort compared to 26% in the single-infusion cohort. These data are consistent with the hypothesis that higher day 0-30 and 14-30 AUC and prolonged CAR T-cell persistence observed in the dose-dense cohort may contribute to improved antitumor activity without increasing toxicity. In this context, the numerically lower cumulative incidence of BCR observed in the dose-dense cohort, although not statistically significant, may reflect more sustained CAR T-cell functional persistence and ongoing B-cell suppression, and may represent a

pharmacodynamic marker consistent with the improved durability of response observed with this strategy. Furthermore, comparative analyses of CAR T-cell differentiation phenotypes in both the end-manufacturing product and post-infusion peripheral blood at day 28 showed largely comparable distribution between cohorts. While this observation suggests a quantitative rather than qualitative mechanism for the enhanced persistence observed with early redosing, distinct CAR T-cell functional states may not be captured by phenotypic changes. Future studies incorporating single-cell transcriptomic and epigenomic methods will help characterize CAR T-cell functional states.[19] In addition, serial ctDNA monitoring may help capture MRD dynamics and better quantify whether early redosing deepens molecular remission beyond imaging-based response assessments. Notably, depth of tumor reduction by imaging at the first disease assessment was numerically deeper in dose-dense patients, consistent with augmented early cellular exposure. We also observed a higher proportion of CD19-negative relapses among dose-dense patients, despite similar complete response rates, raising the possibility that intensified early CAR T-cell pressure may shift relapse patterns toward antigen-negative escape in a subset of tumors. This interpretation is supported by exploratory analyses within the dose-dense cohort showing higher CAR T-cell exposure in patients with CD19-negative than CD19-positive relapses.

The timing of redosing in our study was biologically and clinically informed. Within the same clinical trial platform, prior experience suggested that retreatment at approximately day 28 could be administered with minimal toxicity with evidence of antitumor activity. Based on these observations, the dose-dense strategy hypothesized that administering the second infusion earlier—during the transition

from peak expansion to early contraction—might increase cumulative CAR T-cell exposure and persistence while maintaining safety. Our dose-dense approach should be distinguished from other CAR T-cell formulations using “split dosing”, “fractionated dosing”, and “hyper fractionation”, typically referring to the administration of 2–3 CAR T-cell doses over a short time period (e.g., 3–10 days), with incrementally higher doses of CAR T-cells.[9,20-29] CAR T-cell fractionation has been mostly applied to patients with acute lymphoblastic leukemia or chronic lymphocytic leukemia, in whom higher rates of severe toxicities have been reported. In contrast, the dose-dense approach was investigated in B-NHL patients who received the same CAR T-cell dose twice, 14 days apart, following a single course of lymphodepletion chemotherapy. A similar concept was explored in a small cohort within the TRANSCEND-NHL-001 study,[3,5] where six patients received two equal infusions of lisocabtagene maraleucel 14 days apart after one lymphodepleting regimen at a substantially lower fixed dose (50×10^6 CAR T-cells per infusion). That experience did not provide detailed data on the incidence of CRS or NT after the second infusion, on the impact of the second infusion on CAR T-cell expansion and persistence, or on comparisons with a single-infusion cohort at the same dose level; therefore, the effect of dose-dense redosing on safety and cellular kinetics remained largely undefined. Our data complement and extend this lower-dose experience. In addition, other sequential CAR T-cell strategies targeting different antigens (e.g., CD19/CD20 in lymphoma or CD19/CD22 in B-ALL) have been explored to address relapse through antigen diversification, representing a conceptually related but biologically distinct approach.[30,31] Our study has several limitations. This was an exploratory cohort with a small sample size, and our observation of improved outcomes in the dose-

dense cohort could have been confounded by other factors impacting clinical outcomes, such as tumor burden or lymphoma subtypes. Specifically, bulky disease was present only in the single-infusion cohort and may have adversely affected comparisons of early response outcomes between cohorts. Bridging therapy was used in a minority of patients, which likely reflects the short vein-to-vein time and the requirement for a fresh product infusion in this cohort; bridging practices may vary across institutions. Lymphodepletion is also a known determinant of CAR T-cell expansion; although regimen heterogeneity was protocol-permitted, the distribution of lymphodepletion strategies did not meaningfully differ between cohorts, and the small number of non–fludarabine/cyclophosphamide cases precludes regimen-specific conclusions. In addition, multiple mechanisms of CAR T-cell failure described in other series—including antigen-low or antigen-negative escape and a profoundly immunosuppressive tumor microenvironment—are unlikely to be fully overcome by simply increasing cumulative CAR T-cell exposure or improving product fitness. Thus, our dose-dense strategy is likely to address only a subset of resistance mechanisms, primarily those related to early functional exhaustion and loss of persistence. Nevertheless, the observations of CAR T-cell re-expansion and more prolonged in vivo CAR T-cell persistence in patients treated on the dose-dense cohort suggest CAR T-cells from the second infusion may have contributed to enhanced antitumor activity. Lastly, our clinical trial investigated the use of the investigational product JCAR014, which is distinct from lisocabtagene maraleucel and is not approved by the FDA. However, two single-arm clinical trials investigating the role of a similar dose-dense approach are currently underway using commercially available CAR T-cell products: the first is

evaluating redosing with tisagenlecleucel 30-60 days after the initial dose in children and young adults with R/R B-ALL (NCT05460533, REFUEL trial); the second is investigating the early retreatment with axicabtagene ciloleucel 7-14 days after the initial dose in adults with high-risk, second-line R/R B-NHL (NCT05794958, Axi-Cel-2). We hope our findings can be replicated in these studies.

In conclusion, we demonstrated the feasibility, safety, and long-term efficacy of a novel dose-dense CAR T-cell redosing strategy in patients with R/R B-NHL. These findings support this approach to improve the therapeutic index of CAR T-cell therapies, with ongoing clinical trials applying this redosing strategy to commercially available CAR T-cell therapies.

References:

1. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N Engl J Med. 2017;377(26):2531-2544.
2. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. N Engl J Med. 2019;380(1):45-56.
3. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet. 2020;396(10254):839-852.
4. Jain MD, Spiegel JY, Nastoupil LJ, et al. Five-Year Follow-Up of Standard-of-Care Axicabtagene Ciloleucel for Large B-Cell Lymphoma: Results From the US Lymphoma CAR T Consortium. J Clin Oncol. 2024;42(30):3581-3592.
5. Abramson JS, Palomba ML, Gordon LI, et al. Two-year follow-up of lisocabtagene maraleucel in relapsed or refractory large B-cell lymphoma in TRANSCEND NHL 001. Blood. 2024;143(5):404-416.
6. Landsburg DJ, Frigault MJ, Heim M, et al. Real-world outcomes with tisagenlecleucel in aggressive B-cell lymphoma: subgroup analyses from the CIBMTR registry. J Immunother Cancer. 2025;13(2):e009890.
7. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. J Clin Invest. 2016;126(6):2123-2138.

8. Gardner RA, Finney O, Annesley C, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood. 2017;129(25):3322-3331.
9. Frey NV, Shaw PA, Hexner EO, et al. Optimizing Chimeric Antigen Receptor T-Cell Therapy for Adults With Acute Lymphoblastic Leukemia. J Clin Oncol. 2020;38(5):415-422.
10. Lei W, Xie M, Jiang Q, et al. Treatment-Related Adverse Events of Chimeric Antigen Receptor T-Cell (CAR T) in Clinical Trials: A Systematic Review and Meta-Analysis. Cancers (Basel). 2021;13(15):3912.
11. Rotte A, Frigault MJ, Ansari A, Gliner B, Heery C, Shah B. Dose-response correlation for CAR-T cells: a systematic review of clinical studies. J Immunother Cancer. 2022;10(12):e005678.
12. Turtle CJ, Hanafi LA, Berger C, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. Sci Transl Med. 2016;8(355):355ra116.
13. Turtle CJ, Hay KA, Hanafi LA, et al. Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor-Modified T Cells After Failure of Ibrutinib. J Clin Oncol. 2017;35(26):3010-3020.
14. Hirayama AV, Gauthier J, Hay KA, et al. The response to lymphodepletion impacts PFS in patients with aggressive non-Hodgkin lymphoma treated with CD19 CAR T cells. Blood. 2019;133(17):1876-1887.
15. Hirayama AV, Gauthier J, Hay KA, et al. High rate of durable complete remission in follicular lymphoma after CD19 CAR-T cell immunotherapy. Blood. 2019;134(7):636-640.

16. Wang X, Chang WC, Wong CW, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood. 2011;118(5):1255-1263.
17. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188-195.
18. Vaquera-Alfaro HA, Jeon Y, Wu QV, et al. A Practical Guide to Competing Risk Analysis for Transplant and Cell Therapy Research. Transplant Cell Ther. 2026;32(4):489-500.
19. Fiorenza S, Zheng Y, Purushe J, et al. Histone marks identify novel transcription factors that parse CAR-T subset-of-origin, clinical potential and expansion. Nat Commun. 2024;15(1):8309.
20. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725-733.
21. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368(16):1509-1518.
22. Hu Y, Wu Z, Luo Y, et al. Potent Anti-leukemia Activities of Chimeric Antigen Receptor-Modified T Cells against CD19 in Chinese Patients with Relapsed/Refractory Acute Lymphocytic Leukemia. Clin Cancer Res. 2017;23(13):3297-3306.
23. Geyer MB, Rivière I, Sénéchal B, et al. Safety and tolerability of conditioning chemotherapy followed by CD19-targeted CAR T cells for relapsed/refractory CLL. JCI Insight. 2019;5(9):e122627.
24. Ying Z, Huang XF, Xiang X, et al. A safe and potent anti-CD19 CAR T cell therapy. Nat Med. 2019;25(6):947-953.

25. Ortíz-Maldonado V, Rives S, Castellà M, et al. CART19-BE-01: A Multicenter Trial of ARI-0001 Cell Therapy in Patients with CD19+ Relapsed/Refractory Malignancies. Mol Ther. 2021;29(2):636-644.
26. Roddie C, Dias J, O'Reilly MA, et al. Durable Responses and Low Toxicity After Fast Off-Rate CD19 Chimeric Antigen Receptor-T Therapy in Adults With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia. J Clin Oncol. 2021;39(30):3352-3363.
27. Oliver-Caldés A, González-Calle V, Cabañas V, et al. Fractionated initial infusion and booster dose of ARI0002h, a humanised, BCMA-directed CAR T-cell therapy, for patients with relapsed or refractory multiple myeloma (CARTBCMA-HCB-01): a single-arm, multicentre, academic pilot study. Lancet Oncol. 2023;24(8):913-924.
28. Martínez-Cibrián N, Ortiz-Maldonado V, Español-Rego M, et al. The academic point-of-care anti-CD19 chimeric antigen receptor T-cell product varnimcabtagene autoleucel (ARI-0001 cells) shows efficacy and safety in the treatment of relapsed/refractory B-cell non-Hodgkin lymphoma. Br J Haematol. 2024;204(2):525-533.
29. Roddie C, Sandhu KS, Tholouli E, et al. Obecabtagene Autoleucel in Adults with B-Cell Acute Lymphoblastic Leukemia. N Engl J Med. 2024;391(23):2219-2230.
30. Xue F, Liu R, Fu Z, et al. Sequential CD19-20 CAR T-cell therapy for refractory/relapsed diffuse large B-cell lymphoma. Cytotherapy. 2025;27(8):910-916.

31. Schultz L, Aftandilian C, Baggott C, et al. Sequential CD19 and CD22 CART for relapsed and refractory B cell ALL: Phase I results. Blood. 2025;146(Suppl 1):2374.

Tables and Figures:

Table 1. Baseline patient characteristics

Characteristic	Dose-Dense cohort ($2 + 2 \times 10^6$ CAR T-cells/kg) N = 17	Single-Infusion cohort (2×10^6 CAR T-cells/kg) N = 52	p-value[1]
Age (years), median (range)	59 (43 to 70)	59 (27 to 71)	>0.9
Male sex, n (%)	12 (71)	39 (75)	0.8
Ethnicity, n (%)			**0.046**
White	12 (71)	47 (92)	
Asian	3 (18)	1 (2)	
Black or African American	1 (6)	2 (4)	
American Indian or Alaska Native	1 (6)	1 (2)	
Altered ECOG PS (≥1), n (%)	6 (35)	26 (50)	0.3
Disease subtype, n (%)			0.12
DLBCL	8 (47)	33 (67)	
HGBCL	4 (24)	2 (4)	
FL	3 (18)	8 (16)	
MCL	2 (12)	6 (12)	
Aggressive lymphoma, n (%)	14 (82)	44 (85)	>0.9
Prior therapy lines, median (range)	4 (1 to 9)	4 (1 to 8)	>0.9
Prior autologous HCT, n (%)	7 (41)	21 (40)	>0.9
Prior allogeneic HCT, n (%)	3 (18)	7 (13)	0.7
Best response to first line, n (%)			0.4
Complete response	6 (35)	19 (37)	
Partial response	9 (53)	19 (37)	
No response	2 (12)	14 (27)	
Refractory to first line, n (%)	2 (12)	14 (27)	0.3
No-CR to first line, n (%)	8 (47)	19 (37)	0.4
Early relapse (<12 months) after first line, n (%)	2 (12)	7 (13)	>0.9
Bulky (≥5 cm), n (%)	0 (0)	11 (21)	0.054
Tumor cross-sectional area (mm^2), median (IQR)	2,593 (1,969–4,829)	3,700 (2,027–7,354)	0.2
Extranodal disease, n (%)	15 (88)	44 (85)	>0.9
LDH (U/L), median (IQR)[2]	232 (164–366)	259 (166–362)	0.9
High LDH, n (%)[2]	9 (53)	31 (60)	0.6
IPI, n (%)			0.3
0	0 (0)	3 (6)	
1	4 (24)	5 (10)	
2	4 (24)	22 (42)	
3	8 (47)	16 (31)	
4	1 (6)	6 (12)	
IPI 3 or 4, n (%)	9 (53)	22 (42)	0.4
Platelets ($x10^3/\mu L$), median (IQR)[2]	165 (99–195)	139 (81–214)	0.7

Hemoglobin (g/dL), median (IQR)[2]	11.0 (9.8–12.2)	11.05 (9.75–12.80)	0.5
ANC (x10^3 cells/µL), median (IQR)[2]	3.58 (2.33–4.82)	3.25 (2.17–5.35)	0.8
ALC (x10^3 cells/µL), median (IQR)[2]	0.95 (0.58–1.08)	0.71 (0.44–1.24)	0.4
CRP (mg/L), median (IQR)[2]	8 (3–46)	21 (4–38)	0.7
Ferritin (ng/mL), median (IQR)[2]	119 (87–819)	341 (149–746)	0.4
CAR-HEMATOTOX risk, n (%)[2]			0.8
– **High**	7 (41%)	24 (46%)	
– **Low**	10 (59%)	27 (52%)	
– **Unknown**	0 (0%)	1 (1.9%)	
Vein-to-vein time (days), median (IQR)	19 (17–21)	19 (17–27)	>0.9
Time from 1st to 2nd infusion (days), median (IQR)	14 (14–14)	NA	
Patient weight (kg), median (IQR)	82 (61–88)	82 (71–94)	0.5
Absolute first CAR T-cell dose (x10^6 CAR+ cells), median (IQR)	164 (121–176)	165 (143–188)	0.5
Absolute 1st + 2nd CAR T-cell dose (x10^6 CAR+ cells), median (IQR)	328 (243–369)	165 (143–188)	**<0.001**

Abbreviations: DLBCL, diffuse large B-cell lymphoma; HGBCL, high-grade B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; HCT, hematopoietic stem-cell transplantation; CR, complete response; IPI, International Prognostic Index; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; CRP, C-reactive protein. [1] Wilcoxon rank sum test; Fisher's exact test; Pearson's Chi-squared test. [2]Values correspond to the baseline pre-lymphodepletion assessment.

Table 2. Feasibility and safety of early CAR T-cell redosing

Characteristic	Dose-Dense cohort ($2 + 2 \times 10^6$ CAR T-cells/kg) N = 17	Single-Infusion cohort (2×10^6 CAR T-cells/kg) N = 52
CRS (any grade), n (%)	10 (59)	26 (50)
Severe CRS (grade ≥3), n (%)	0 (0)	3 (6)
Neurotoxicity (any grade), n (%)	3 (18)	18 (35)
Severe neurotoxicity (grade ≥3), n (%)	0 (0)	4 (8)
Day 0-30 ICAHT, n (%)		
– **Any grade**	12 (71)	42 (81)
– **Grade ≥3**	1 (6)	10 (19)
ORR (CR or PR), n (%)	8 (47)	31 (60)
CRR, n (%)	7 (41)	22 (42)
Best response, n (%)		
Complete response	7 (41)	22 (42)
Partial response	1 (6)	9 (17)
Stable disease	1 (6)	7 (13)
Progressive disease	8 (47)	14 (27)

Abbreviations: CRS, cytokine release syndrome; **ICAHT**, Immune effector cell-associated hematotoxicity; ORR, overall response rate; CR, complete response; PR, partial response; CRR, complete response rate.

Figure 1. Dose-dense CONSORT Diagram

Abbreviations: CRS, cytokine release syndrome; NT, neurotoxicity; CR, complete response; PR, partial response.

Figure 2. DOR, PFS, and OS in dose-dense patients with early CAR T-cell redosing. (A) DOR in patients achieving CR or PR, (B) PFS, (C) OS, and (D) OS in patients according to response (CR/PR vs PD/SD).

Abbreviations: DOR, duration of response; PFS, progression-free survival; OS, overall survival; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease.

Figure 3. Early CAR T-cell count kinetics in dose-dense patients. (A) CD8+ CAR T-cell expansion. (B) CD4+ CAR T-cell expansion. (C) CD8+ and CD4+ CAR T-cell expansion peaks in individual patients.

Footnote: (A and B) Dashed black lines indicate the timepoint of the first and the second CAR T-cell infusions. The red line depicts the average expansion pattern across dose-dense patients. (C) Red and green arrows point to CD8+ and CD4+ CAR T-cell expansion peaks following the second CAR T-cell infusion in individual patients. The dashed blue line indicates the time point of the second infusion.

Figure 4. DOR, PFS, and OS in dose-dense (DD) vs single-infusion (SI) patients. (A) DOR in patients achieving CR or PR, (B) PFS, (C) OS, and (D) OS in patients achieving CR or PR.

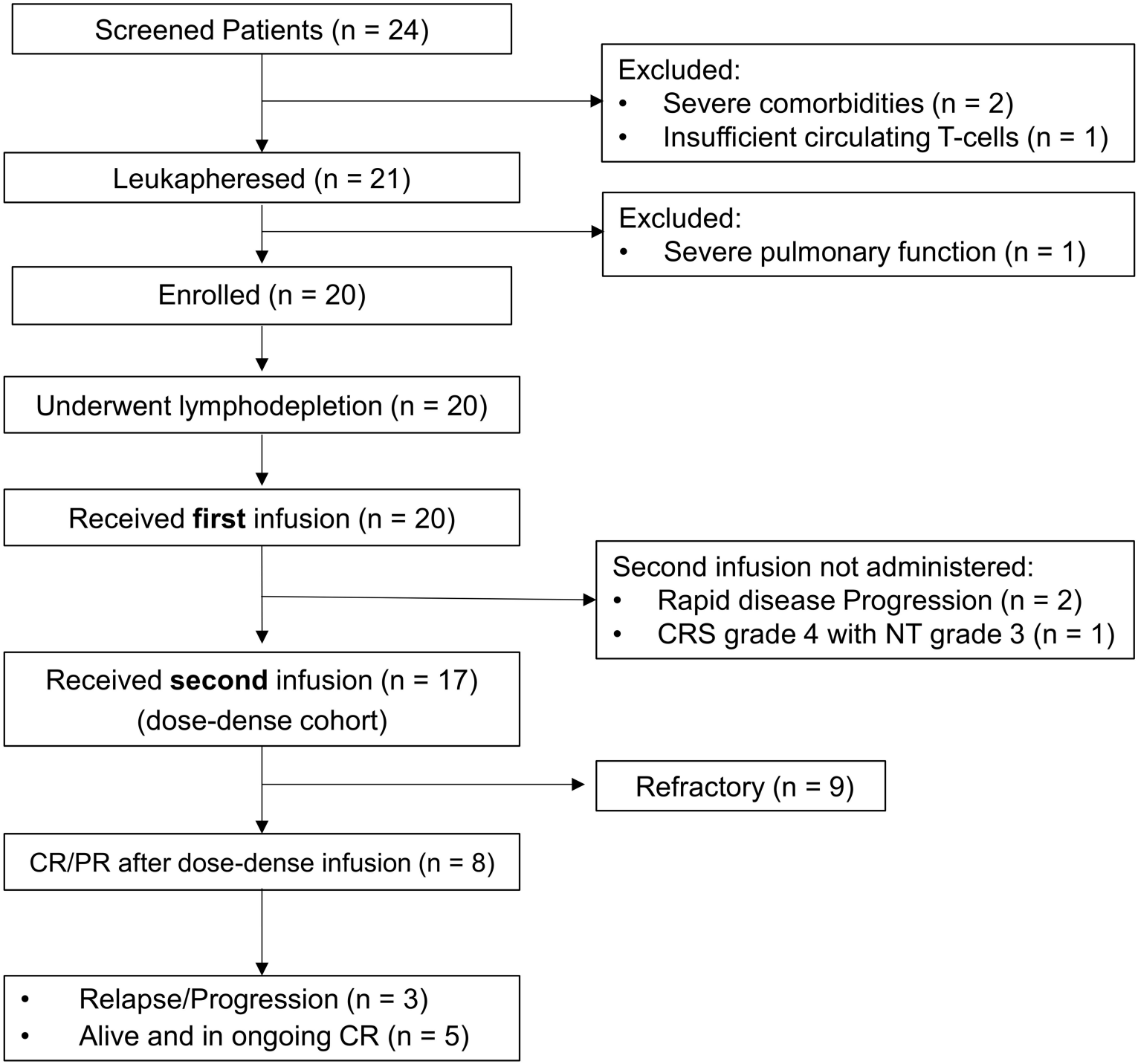
Abbreviations: DOR, duration of response; PFS, progression-free survival; OS, overall survival; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease; DD, dose-dense; SI, single-infusion.

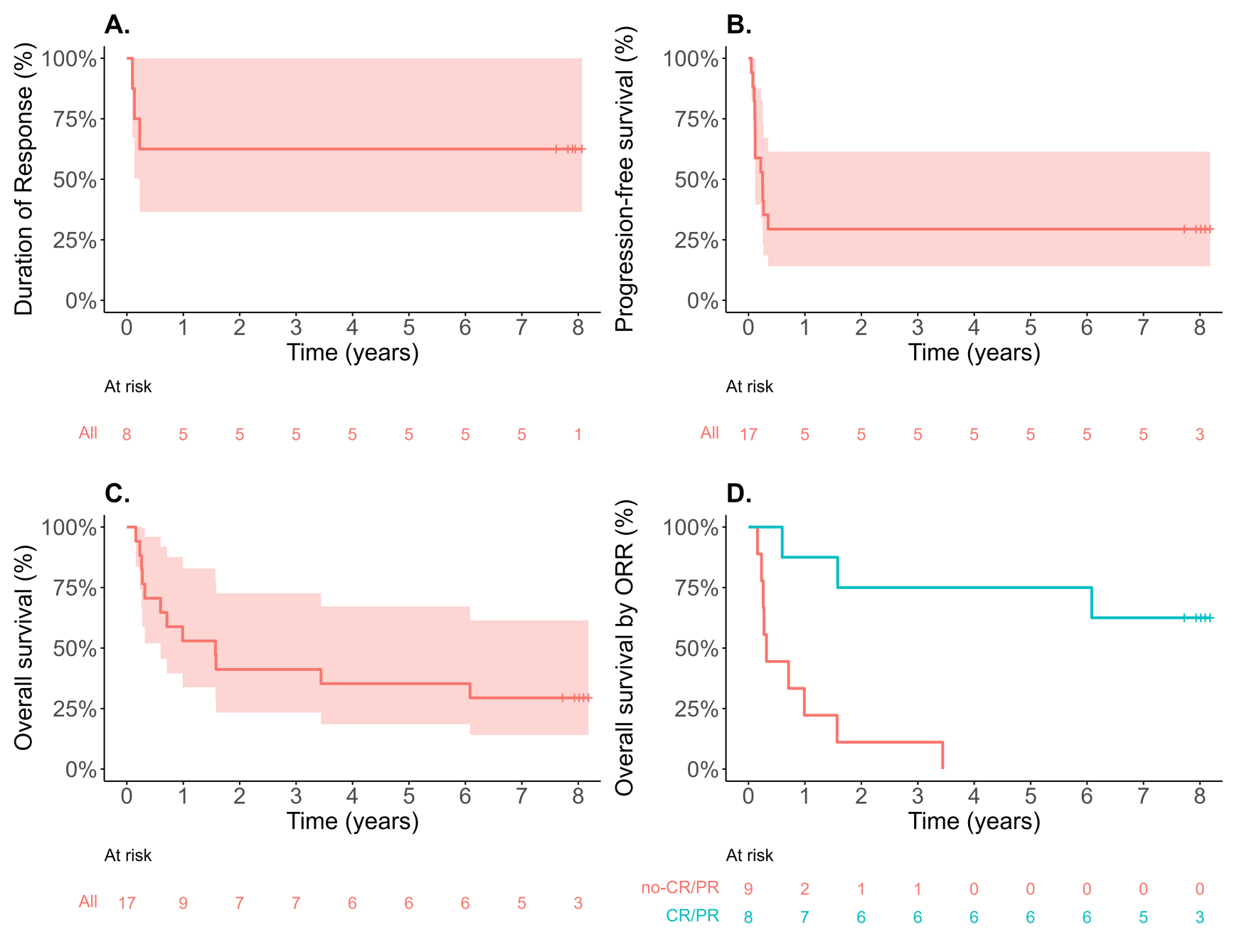
Figure 5. Early CAR T-cell expansion, AUC (days 14–30), Cmax, and long-term CAR T-cell persistence in dose-dense vs single-infusion patients. (A) CD8+ CAR T-cell early expansion curves, (B) CD4+ CAR T-cell early expansion curves, (C) CD8+ CAR T-cell AUC (days 14–30), (D) CD4+ CAR T-cell AUC (days 14–30), (E) CD8+ CAR T-cell Cmax, (F) CD4+ CAR T-cell Cmax, (G) CAR transgene Cmax, and (H) CAR T-cell persistence by qPCR.

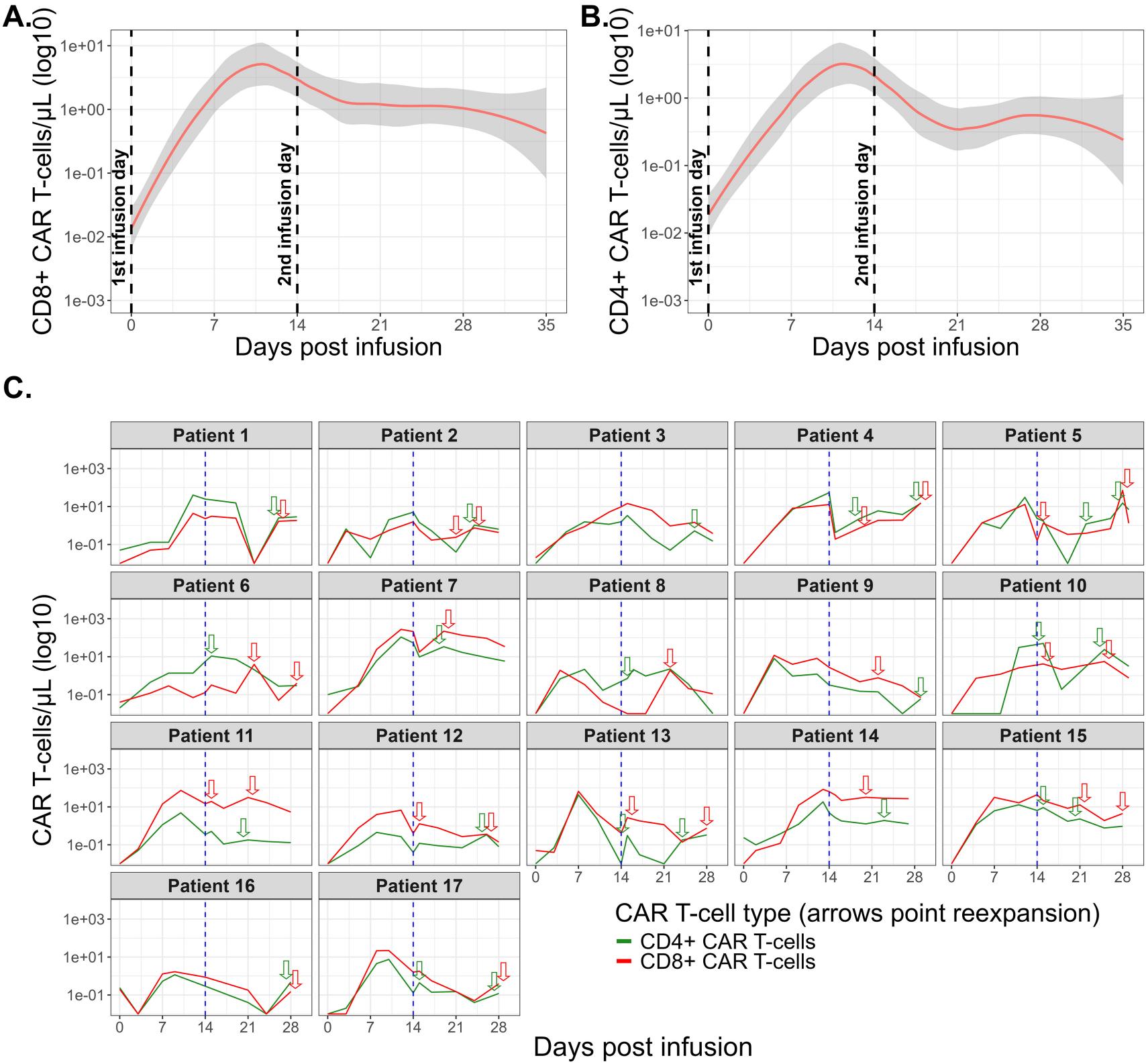
Abbreviations: DD, dose-dense; SI, single-infusion; Cmax, peak CAR T-cell count in blood; AUC, area under the curve.

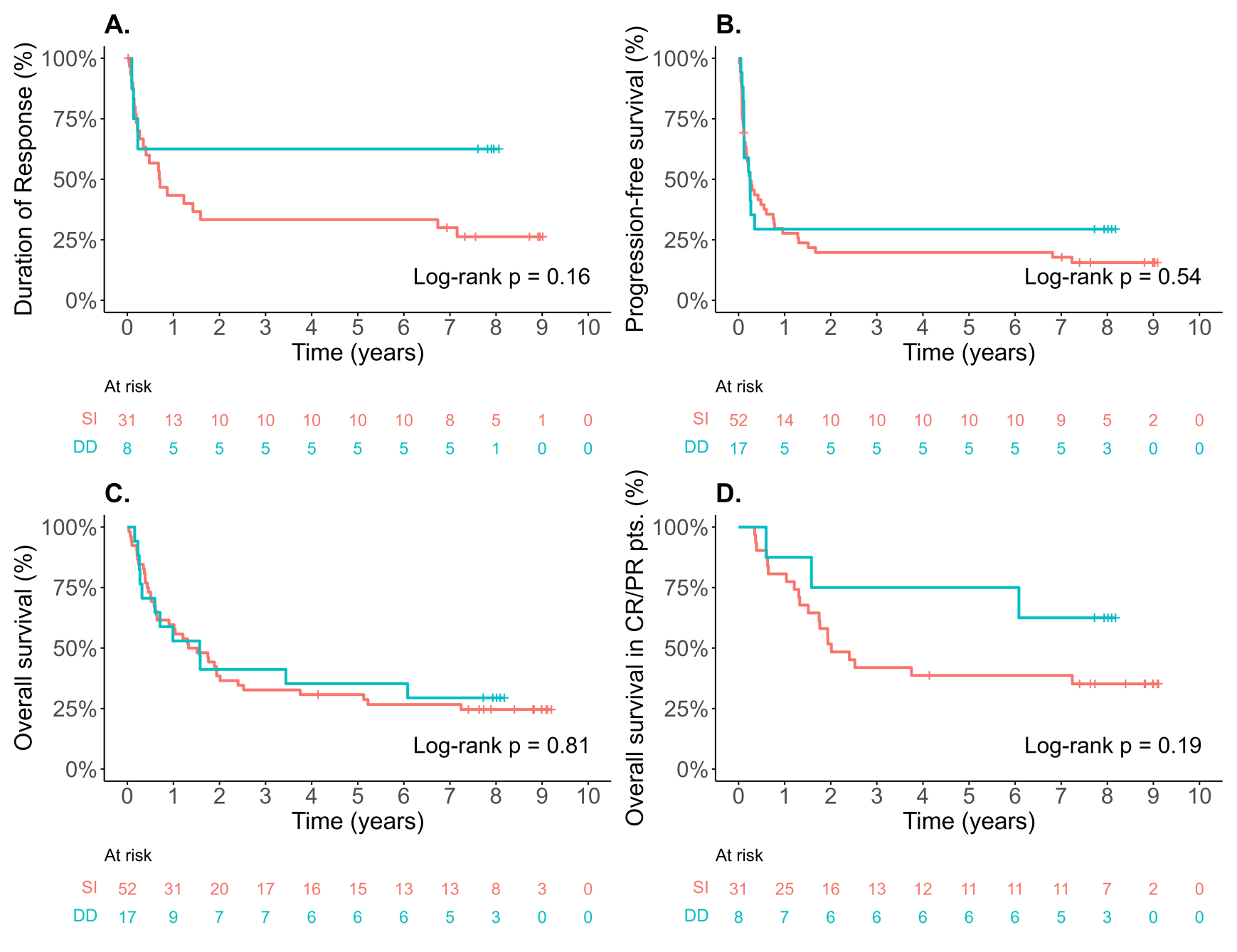
Figure 6. Long-term CAR T-cell persistence in dose-dense vs single-infusion patients. CAR T-cell persistence by qPCR.

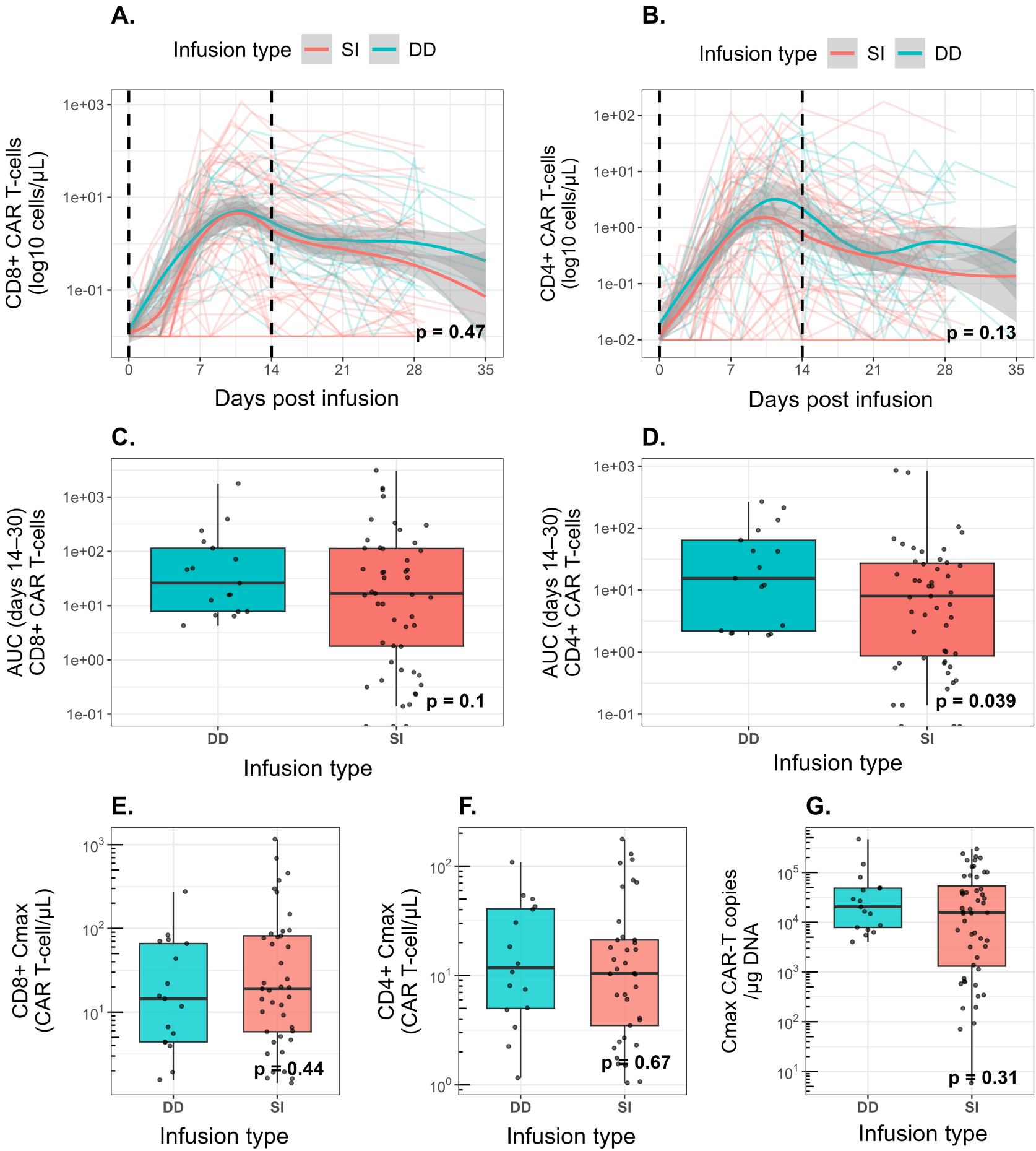
Abbreviations: DD, dose-dense; SI, single-infusion.

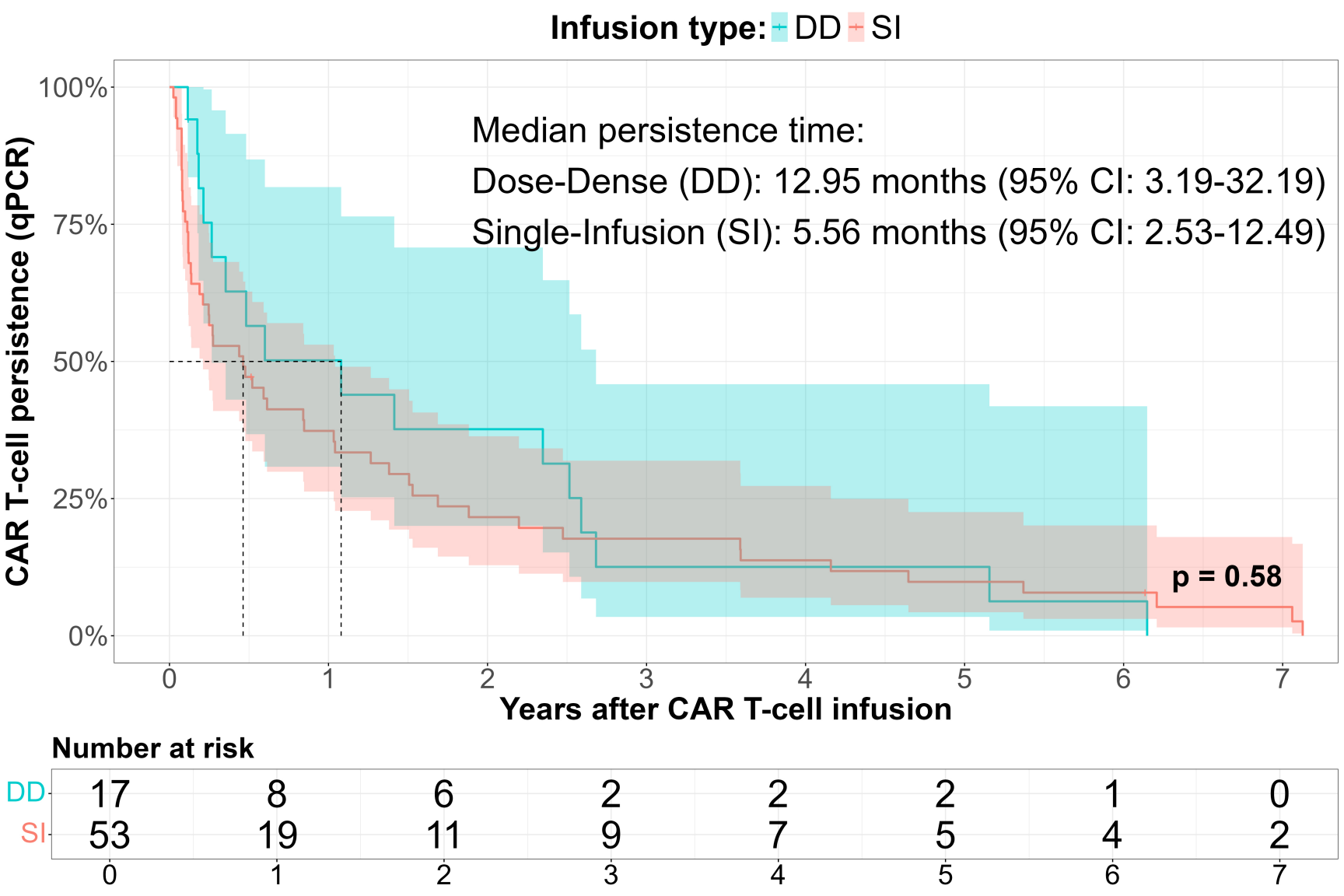

Screened Patients (n = 24)
Excluded:
• Severe comorbidities (n = 2)
• Insufficient circulating T-cells (n = 1)
Leukapheresed (n = 21)
Excluded:
• Severe pulmonary function (n = 1)
Enrolled (n = 20)
Underwent lymphodepletion (n = 20)
Received **first** infusion (n = 20)
Second infusion not administered:
• Rapid disease Progression (n = 2)
• CRS grade 4 with NT grade 3 (n = 1)
Received **second** infusion (n = 17)
(dose-dense cohort)
Refractory (n = 9)
CR/PR after dose-dense infusion (n = 8)
• Relapse/Progression (n = 3)
• Alive and in ongoing CR (n = 5)


A.
Duration of Response (%)
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8
Time (years)
At risk
All 8 5 5 5 5 5 5 5 1
B.
Progression-free survival (%)
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8
Time (years)
At risk
All 17 5 5 5 5 5 5 5 3
C.
Overall survival (%)
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8
Time (years)
At risk
All 17 9 7 7 6 6 6 5 3
D.
Overall survival by ORR (%)
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8
Time (years)
At risk
no-CR/PR 9 2 1 1 0 0 0 0 0
CR/PR 8 7 6 6 6 6 6 5 3


A.
CD8+ CAR T-cells/µL (log10)
1e+01
1e+00
1e-01
1e-02
1e-03
1st infusion day
2nd infusion day
0
7
14
21
28
35
Days post infusion
B.
CD4+ CAR T-cells/µL (log10)
1e+01
1e+00
1e-01
1e-02
1e-03
1st infusion day
2nd infusion day
0
7
14
21
28
35
Days post infusion
C.
CAR T-cells/µL (log10)
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Patient 15
Patient 16
Patient 17
1e+03
1e+01
1e-01
0
7
14
21
28
CAR T-cell type (arrows point reexpansion)
CD4+ CAR T-cells
CD8+ CAR T-cells
Days post infusion


A.
Duration of Response (%)
100%
75%
50%
25%
0%
Log-rank p = 0.16
0 1 2 3 4 5 6 7 8 9 10
Time (years)
At risk
SI 31 13 10 10 10 10 10 8 5 1 0
DD 8 5 5 5 5 5 5 5 1 0 0
B.
Progression-free survival (%)
100%
75%
50%
25%
0%
Log-rank p = 0.54
0 1 2 3 4 5 6 7 8 9 10
Time (years)
At risk
SI 52 14 10 10 10 10 10 9 5 2 0
DD 17 5 5 5 5 5 5 5 3 0 0
C.
Overall survival (%)
100%
75%
50%
25%
0%
Log-rank p = 0.81
0 1 2 3 4 5 6 7 8 9 10
Time (years)
At risk
SI 52 31 20 17 16 15 13 13 8 3 0
DD 17 9 7 7 6 6 6 5 3 0 0
D.
Overall survival in CR/PR pts. (%)
100%
75%
50%
25%
0%
Log-rank p = 0.19
0 1 2 3 4 5 6 7 8 9 10
Time (years)
At risk
SI 31 25 16 13 12 11 11 11 7 2 0
DD 8 7 6 6 6 6 6 5 3 0 0


A.
Infusion type
SI
DD
CD8+ CAR T-cells
(log10 cells/µL)
1e+03
1e+01
1e-01
p = 0.47
0
7
14
21
28
35
Days post infusion
B.
Infusion type
SI
DD
CD4+ CAR T-cells
(log10 cells/µL)
1e+02
1e+01
1e+00
1e-01
1e-02
p = 0.13
0
7
14
21
28
35
Days post infusion
C.
AUC (days 14–30)
CD8+ CAR T-cells
1e+03
1e+02
1e+01
1e+00
1e-01
p = 0.1
DD
SI
Infusion type
D.
AUC (days 14–30)
CD4+ CAR T-cells
1e+03
1e+02
1e+01
1e+00
1e-01
p = 0.039
DD
SI
Infusion type
E.
CD8+ Cmax
(CAR T-cell/µL)
10^3
10^2
10^1
p = 0.44
DD
SI
Infusion type
F.
CD4+ Cmax
(CAR T-cell/µL)
10^2
10^1
10^0
p = 0.67
DD
SI
Infusion type
G.
Cmax CAR-T copies
/µg DNA
10^5
10^4
10^3
10^2
10^1
p = 0.31
DD
SI
Infusion type


Infusion type: DD SI
Median persistence time:
Dose-Dense (DD): 12.95 months (95% CI: 3.19-32.19)
Single-Infusion (SI): 5.56 months (95% CI: 2.53-12.49)
p = 0.58
CAR T-cell persistence (qPCR)
100%
75%
50%
25%
0%
0
1
2
3
4
5
6
7
Years after CAR T-cell infusion
Number at risk
DD 17 8 6 2 2 2 1 0
SI 53 19 11 9 7 5 4 2
0
1
2
3
4
5
6
7

Supplementary Material for manuscript "A novel dose-dense strategy for CD19-directed CAR T-cell therapy is associated with durable responses without increased toxicity in patients with B-cell non-Hodgkin lymphoma" by Ortiz-Maldonado et al.

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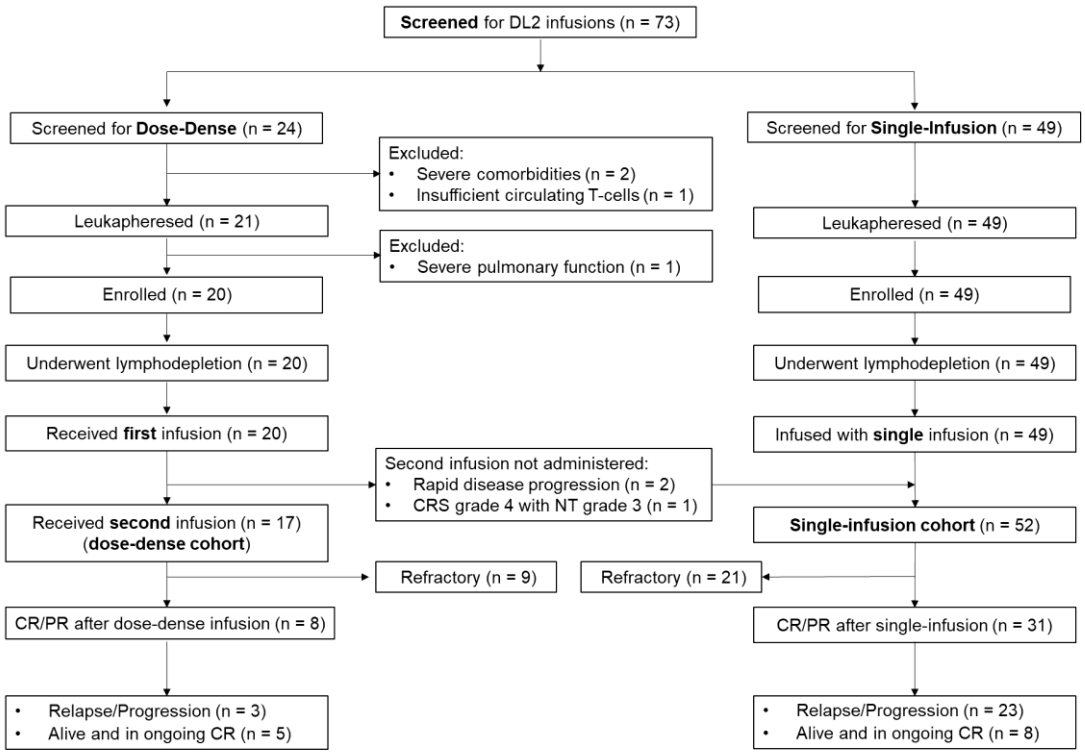
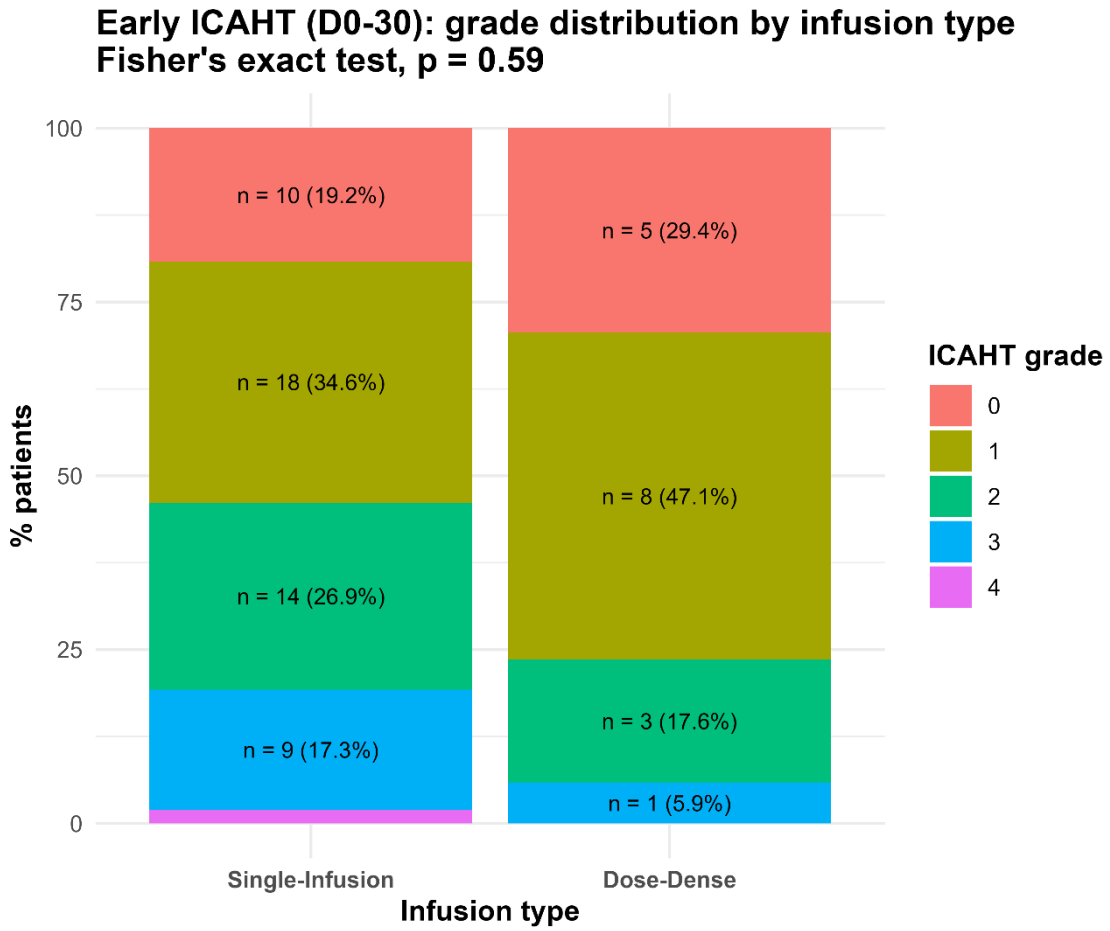
Supplementary Figures

Supplementary Figure 1: Dose-dense and single-infusion CONSORT Diagram

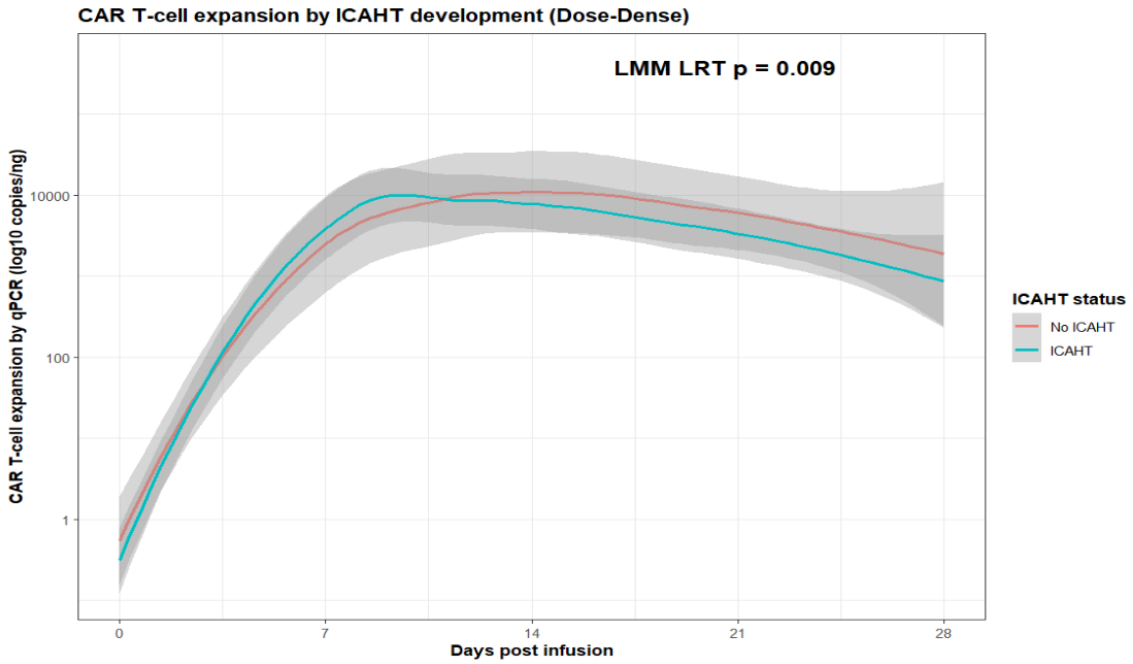


Abbreviations: DL2, dose level 2; CR, complete response; PR, partial response; CRS, cytokine release syndrome; NT, neurotoxicity.

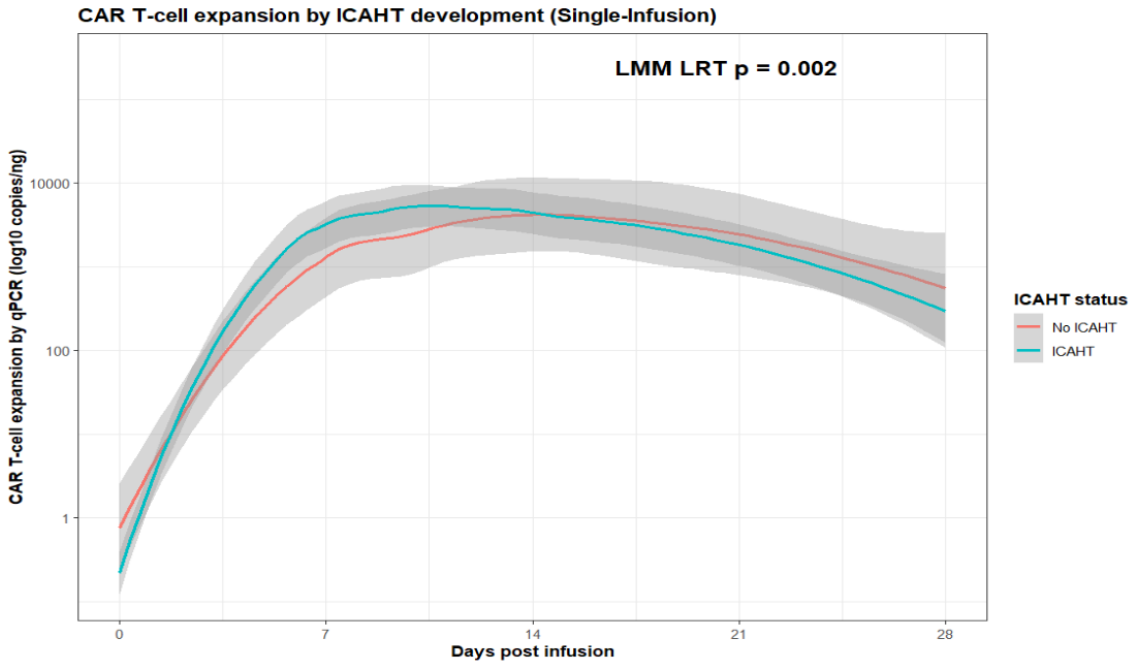
Supplementary Figure 2: Overall distribution of day 0-30 ICAHT in the dose-dense and single-infusion cohorts.



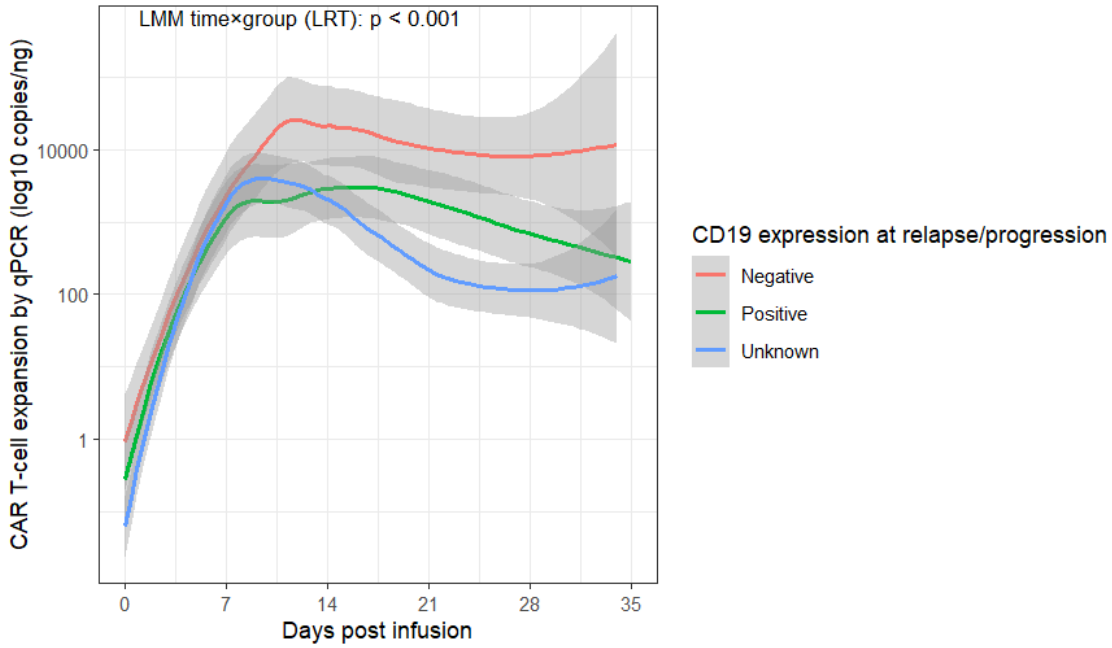
Supplementary Figure 3: CAR T-cell expansion by ICAHT development in the dose-dense cohort



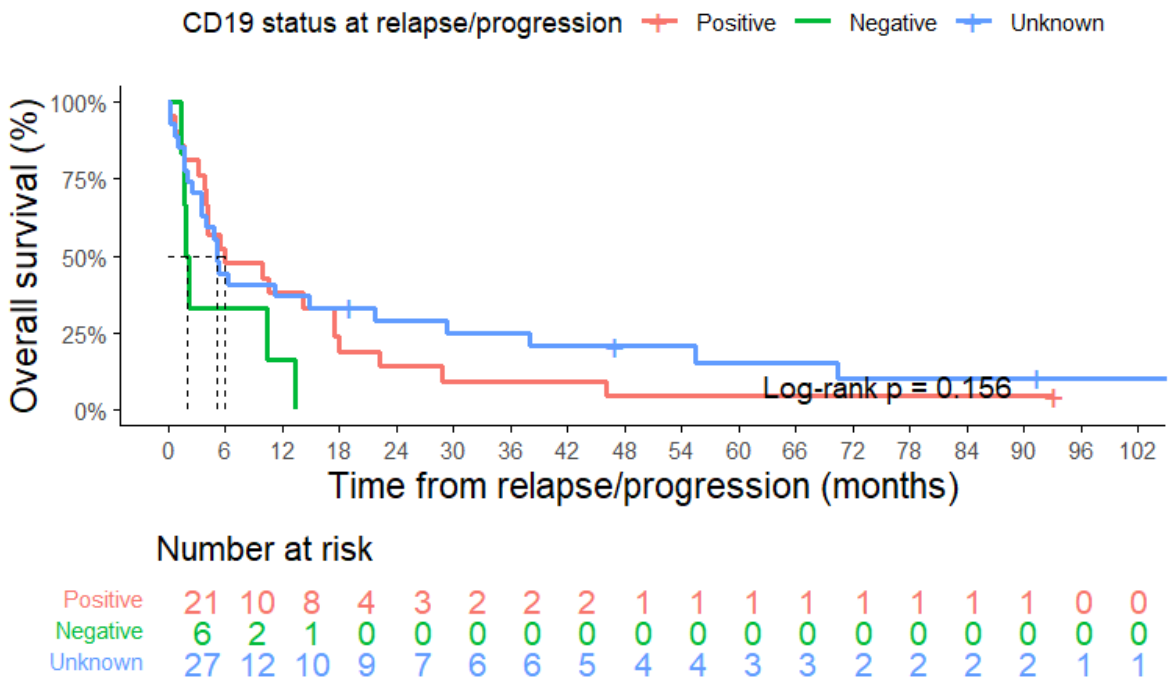
Supplementary Figure 4: CAR T-cell expansion by ICAHT development in the single-infusion cohort



Supplementary Figure 5: CAR T-cell expansion in the dose-dense cohort by CD19 expression at relapse or progression.



Supplementary Figure 6: Overall survival in the dose-dense plus single-infusion population after relapse or refractoriness by CD19-expression status



Supplementary Tables

Supplementary Table 1: Bridging therapy in dose-dense and single-infusion cohorts.

Characteristic	Dose-Dense cohort ($2 + 2 \times 10^6$ CAR T-cells/kg) N = 17	Single-Infusion cohort (2×10^6 CAR T-cells/kg) N = 52
Bridging therapy, n (%)	6 (35)	21 (40)
Bridging therapy type, n (%)		
Bendamustine	1 (17)	0 (0)
Dexamethasone	1 (17)	3 (14)
GemOx	0 (0)	2 (9.5)
Ibrutinib	2 (33)	2 (9.5)
Ibrutinib + radiotherapy	0 (0)	1 (5)
Ibrutinib + vincristine	0 (0)	1 (5)
Ibrutinib, Bendamustine + rituximab	0 (0)	1 (5)
Intrathecal methotrexate	0 (0)	1 (5)
Lenalidomide	0 (0)	1 (5)
Lenalidomide + methylprednisolone	0 (0)	1 (5)
Methylprednisolone	0 (0)	2 (9.5)
Ofatumumab	0 (0)	1 (5)
Ofatumumab, Lenalidomide	0 (0)	1 (5)
R-EPOCH	1 (17)	0 (0)
R-GCD	0 (0)	1 (5)
R-GDP	0 (0)	1 (5)
Rituximab	0 (0)	2 (9.5)
Vincristine + dexamethasone, Radiotherapy + dexamethasone	1 (17)	0 (0)

Abbreviations: GemOx, gemcitabine and oxaliplatin; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin; R-GCD, rituximab, gemcitabine, cisplatin, dexamethasone; R-GDP, rituximab, gemcitabine, dexamethasone and cisplatin.

Supplementary Table 2: Lymphodepletion regimens in dose-dense and single-infusion cohorts.

Lymphodepletion Characteristic	Dose-Dense cohort (2 + 2 x10^6 CAR T-cells/kg) N = 17	Single-Infusion cohort (2 x10^6 CAR T-cells/kg) N = 52
Lymphodepletion base, n (%)		
- Flu/Cy-based	16 (94)	45 (87)
- Non-Flu/Cy	1 (6)	7 (13)
High cyclophosphamide dose (>30 mg/kg), n (%)	10 (59)	33 (63)
Lymphodepletion type, n (%)		
- Cy. (60 mg/kg/day) x1 day followed by Flu. (25 mg/m^2/day) x3 days	10 (59)	25 (48)
- Cy. (300 mg/m^2/day) and Flu. (30 mg/m^2/day) concurrently x3 days	2 (12)	16 (31)
- Cy. (30 mg/kg/day) x1 day followed by Flu. (25 mg/m^2/day) x3 days	4 (24)	3 (6)
- Cy. (60 mg/kg/day) x1 day followed by Flu. (25 mg/m^2/day) x5 days	0 (0)	1 (2)
- Cy. (4 g/m^2/day) x1 day followed by Etop. (200 mg/m^2/day) x3 days	0 (0)	4 (8)
- Cy. (2 g/m^2/day) x1 day	0 (0)	3 (6)
- Bendamustine (90 mg/m^2/day) x2 days	1 (6)	0 (0)

Abbreviations: Flu, fludarabine; Cy, cyclophosphamide; Etop, etoposide.

Supplementary Table 3: Cumulative incidence of B-cell recovery in dose-dense and single-infusion cohorts.

	3-month B-cell recovery CI	6-month B-cell recovery CI	9-month B-cell recovery CI	12-month B-cell recovery CI	p-value[1]
Cohort					0.3
Single-Infusion cohort (2×10^6 CAR T-cells/kg)	15% (6.5−27)	24% (13−37)	30% (18−44)	30% (18−44)	
Dose-Dense cohort ($2 + 2 \times 10^6$ CAR T-cells/kg)	5.9% (0.35−24)	12% (1.8−32)	20% (4.1−45)	20% (4.1−45)	

Abbreviations: CI, cumulative incidence; [1] Gray's Test

Supplementary Table 4: Response kinetics in dose-dense and single-infusion cohorts.

	Dose-Dense cohort (2 + 2 x10^6 CAR T-cells/kg) N = 17	**Single-Infusion cohort (2 x10^6 CAR T-cells/kg)** N = 52	**p-value[1]**
- Days to first assessment, median (IQR)	42 (41 to 43)	28 (27 to 29)	<0.001
- Tumor cross-sectional area reduction (%) at first assessment, median (IQR)	-70 (-86 to -35)	-53 (-72 to -9)	0.1
- Days to CR/PR, median (IQR)	43 (42 to 48)	28 (28 to 29)	<0.001
- Days to CR, median (IQR)	42 (42 to 52)	28 (28 to 29)	0.002

Abbreviations: CR, complete response; PR, partial response. [1]Wilcoxon rank sum test

Supplementary Table 5: Post-relapse regimens in dose-dense and single-infusion cohorts.

	Dose-Dense cohort (2 + 2 x10^6 CAR T-cells/kg) N = 12	Single-Infusion cohort (2 x10^6 CAR T-cells/kg) N = 43
Second JCAR014, n (%)	3 (25%)	12 (27.9%)
Allogeneic HCT, n (%)	1 (8.3%)	2 (4.7%)
Anti-4-1BB + Rituximab	1 (8.3%)	0 (0%)
Autologous HCT	0 (0%)	1 (2.3%)
CD19-directed antibody-drug conjugate	0 (0%)	1 (2.3%)
Copanlisib	0 (0%)	1 (2.3%)
Hydroxyurea	0 (0%)	1 (2.3%)
Ibrutinib	1 (8.3%)	1 (2.3%)
Intrathecal Methotrexate	0 (0%)	1 (2.3%)
Methotrexate + Ara-C	0 (0%)	1 (2.3%)
Mitomycin + Second JCAR014 + allogeneic HCT	0 (0%)	1 (2.3%)
Nivolumab	0 (0%)	2 (4.7%)
PI3K-delta inhibitor	0 (0%)	1 (2.3%)
R-Bendamustine	0 (0%)	1 (2.3%)
R-EPOCH	0 (0%)	2 (4.7%)
R-EPOCH + Fludarabine + allogeneic HCT	1 (8.3%)	0 (0%)
R-GEMOX	0 (0%)	1 (2.3%)
R-ICE + Second JCAR014	0 (0%)	1 (2.3%)
R-Lenalidomide	0 (0%)	1 (2.3%)
Radiotherapy	1 (8.3%)	1 (2.3%)
Radiotherapy + Intrathecal Methotrexate + Second JCAR014	0 (0%)	1 (2.3%)
Radiotherapy + Revlimid	0 (0%)	1 (2.3%)
Revlimid	0 (0%)	1 (2.3%)
Revlimid + Rituximab	1 (8.3%)	0 (0%)
Revlimid + Rituximab + Second JCAR014	1 (8.3%)	0 (0%)
Rituximab	0 (0%)	1 (2.3%)
Second JCAR014	0 (0%)	1 (2.3%)
Second JCAR014 + allogeneic HCT	0 (0%)	2 (4.7%)
Second JCAR014 + anti-4-1BB + Rituximab	1 (8.3%)	0 (0%)
Second JCAR014 + Idelalisib	0 (0%)	1 (2.3%)
Second JCAR014 + LAG-3 Therapy	0 (0%)	1 (2.3%)
Second JCAR014 + Nivolumab	0 (0%)	1 (2.3%)
Second JCAR014 + Nivolumab + PD1	0 (0%)	1 (2.3%)
Second JCAR014 + Radiotherapy	0 (0%)	3 (7.0%)
Second JCAR014 + Radiotherapy + R-EPOCH	1 (8.3%)	0 (0%)
Second JCAR014 + Revlimid + Rituximab	0 (0%)	1 (2.3%)

Abbreviations: HCT, hematopoietic stem-cell transplantation

Supplementary Table 6: Cmax and AUC in dose-dense and single-infusion cohorts.

Characteristic	Dose-Dense cohort ($2 + 2 \times 10^6$ CAR T-cells/kg) N = 17	Single-Infusion cohort (2×10^6 CAR T-cells/kg) N = 52
CAR T-cell peak (Cmax), median (IQR)		
Transgene (qPCR, copies/μg DNA)	20,479 (7,837–48,289)	15,722 (1,253–63,723)
CD8+ (cells/μL)	15 (4–66)	19 (6–82)
CD4+ (cells/μL)	12 (5–41)	10 (3–21)
$AUC_{day0-30}$ CAR T-cell, median (IQR)		
Transgene (qPCR)	149,077 (61,151–434,989)	131,468 (8,520–550,193)
CD8+	101 (41.8–262.9)	66 (8.4–464.8)
CD4+	78 (27.8–160.2)	34 (5.4–129.6)
$AUC_{day14-30}$, median (IQR)		
Transgene (qPCR)	70,657 (40,100–211,178)	26,086 (2,562–156,642)
CD8+	26 (7.8–114)	15 (0.58–105.4)
CD4+	15 (2.2–63.9)	5 (0.64–25.1)

Abbreviations: Cmax, maximum concentration; AUC, area under the curve.

Supplementary Table 7: Day 28 CAR T-cell subsets in the peripheral blood in the dose-dense and single-infusion cohorts.

CAR T-cell subtypes	Dose-Dense cohort (2 + 2 x10^6 CAR T-cells/kg) N = 17	Single-Infusion cohort (2 x10^6 CAR T-cells/kg) N = 52	p-value[1]
Day 28 post infusion			
- CD8+ CAR T-cells			
- Naïve (%), median (IQR)	10 (0−25)	13 (3−32)	0.3
- Central memory (%), median (IQR)	21 (7−34)	11 (3−17)	0.10
- Effector memory (%), median (IQR)	44 (26−67)	44 (25−63)	0.7
- Effector RA+ (%), median (IQR)	6 (1−12)	17 (4−33)	**0.040**
- CD4+ CAR T-cells			
- Naïve (%), median (IQR)	6 (0−27)	22 (6−44)	0.10
- Central memory (%), median (IQR)	19 (8−40)	25 (11−36)	0.8
- Effector memory (%), median (IQR)	45 (8−71)	27 (12−67)	>0.9
- Effector RA+ (%), median (IQR)	2 (0−15)	4 (0−13)	>0.9

1 Wilcoxon rank sum test; naïve, central memory, and effector memory phenotypes were defined as CD45RA+CD62L+, CD45RA-CD62L+ cells, CD45RA-CD62L- cells, respectively, in a CD3+/CD4+/CD4+ and EGFRt+ or CD8+/CD8+ and EGFRt+ gate using multiparameter flow cytometry.

Supplementary Table 8: CAR T-cell subsets in dose-dense and single-infusion cohorts administered in the end-manufacturing product.

CAR T-cell subtypes	Dose-Dense cohort (2 + 2 x10^6 CAR T-cells/kg) N = 17	Single-Infusion cohort (2 x10^6 CAR T-cells/kg) N = 52	p-value[1]
CD8+ CAR T-cells			
- Naïve (%), median (IQR)	0.3 (0.1−5.3)	0.9 (0.1−5.5)	0.4
- Central memory (%), median (IQR)	52 (47−59)	53 (39−60)	>0.9
- Effector memory (%), median (IQR)	48 (34−50)	40 (34−50)	0.4
- Effector RA+ (%), median (IQR)	0.58 (0.05−2.36)	0.45 (0.14−2.22)	0.5
CD4+ CAR T-cells			
- Naïve (%), median (IQR)	2.2 (0.4−6.7)	0.9 (0.1−5.2)	0.3
- Central memory (%), median (IQR)	37 (24−51)	37 (27−48)	>0.9
- Effector memory (%), median (IQR)	49 (41−65)	59 (42−67)	0.5
- Effector RA+ (%), median (IQR)	2.10 (0.32−5.25)	0.90 (0.14−4.83)	0.3

1 Wilcoxon rank sum test; naïve, central memory, and effector memory phenotypes were defined as CD45RA+CD62L+, CD45RA-CD62L+ cells, CD45RA-CD62L- cells, respectively, in a CD3+/CD4+/CD4+ and EGFRt+ or CD8+/CD8+ and EGFRt+ gate using multiparameter flow cytometry.

Supplementary statistical plan

Safety, efficacy and statistical assessments:

General toxicities, toxicities of special interest (CRS and NT), and efficacy assessment were performed as previously described (Hirayama et al, Blood 2019). Response rates were reported with 95% confidence intervals (CI) calculated using the Clopper-Pearson method. Progression-free survival (PFS) was defined as the time from CAR T-cell infusion to disease progression, need for new anti-tumor therapy, or death, whichever occurred first. Duration of response (DOR) was defined as the time from response assessment after CAR T-cell infusion to relapse, disease progression, need for new anti-tumor therapy, or death, whichever occurred first. PFS, DOR and overall survival (OS) were censored at the last available follow-up. Kaplan-Meier analyses were used to estimate PFS and OS (median, point estimates, and 95% CIs). Differences in PFS, DOR, and OS between groups were assessed using the log-rank test. Follow-up time was estimated using the reverse Kaplan-Meier method (median and range), using the ´survfit´ function from the ´survival´ and ´survminer´ packages in R. Kaplan-Meier survival plots for PFS, DOR and OS were generated using the ´ggsurvplot´ function from the ´survminer´ package in R. CAR T-cell expansion curves were plotted using the LOESS method from the ´ggplot2´ package in R. Univariate logistic regression was performed to evaluate the association between independent variables with binary dependent variables, such as complete response (CR). Data were analyzed using RStudio (R Foundation, Vienna, Austria), version 2024.12.1. Additional statistical methods are detailed in the figure legends.