CD22-directed CAR T-cell therapy for large B-cell lymphomas progressing after CD19-directed CAR T-cell therapy: a dose-finding phase 1 study

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Summary

Background Outcomes are poor for patients with large B-cell lymphoma who relapse after CD19-directed chimeric antigen receptor (CAR) T-cell therapy (CAR19). CD22 is a nearly universally expressed B-cell surface antigen and the efficacy of a CD22-directed CAR T-cell therapy (CAR22) in large B-cell lymphoma is unknown, which was what we aimed to examine in this study.

Methods In this single centre, open-label, dose-escalation phase 1 trial, we intravenously administered CAR22 at two dose levels (1 million and 3 million CAR22-positive T cells per kg of bodyweight) to adult patients (aged ≥18 years) who relapsed after CAR19 or had CD19-negative large B-cell lymphoma. The primary endpoints were manufacturing feasibility, safety measured by the incidence and severity of adverse events and dose-limiting toxicities, and identification of the maximum tolerated dose (ie, the recommended phase 2 dose). This study is registered with ClinicalTrials.gov (NCT04088890) and is active, but closed for enrolment.

Findings From Oct 17, 2019, to Oct 19, 2022, a total of 41 patients were assessed for eligibility; however, one patient withdrew. 40 patients underwent leukapheresis and 38 (95%) had CAR T-cell products manufactured successfully. The median age was 65 years (range 25–84), 17 (45%) were women, 32 (84%) had elevated pretreatment lactate dehydrogenase, 11 (29%) had refractory disease to all previous therapies, and patients had received a median of four lines of previous therapy (range 3–8). Of the 38 patients treated, 37 (97%) had relapsed after previous CAR19. The identified maximum tolerated dose was 1 million CAR T cells per kg. Of 29 patients who received the maximum tolerated dose, no patients developed a dose-limiting toxicity or grade 3 or higher cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, or immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome.

Interpretation This trial identifies CD22 as an immunotherapeutic target in large B-cell lymphoma and demonstrates the durable clinical activity of CAR22 in patients with disease progression after CAR19 therapy. Although these findings are promising, it is essential to recognise that this is a phase 1 dose-finding study. Further investigations are warranted to establish the long-term efficacy and to delineate the patient subgroups that will derive the most benefit from this therapeutic approach.

Funding National Cancer Institute, National Institutes of Health, Stanford Cancer Institute, Leukemia & Lymphoma Society, Parker Institute for Cancer Immunotherapy, Lymph & Co, and the European Hematology Association.

Lancet 2024; 404: 353–63

Published Online July 9, 2024 https://doi.org/10.1016/S0140-6736(24)00746-3

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Introduction

Chimeric antigen receptor (CAR) T-cell therapies targeting CD19 (CAR19) have improved outcomes for relapsed or refractory large B-cell lymphoma. The long-term follow-up of patients given commercially available CAR19 therapies, including axicabtagene ciloleucel, lisocabtagene maraleucel, and tisagenlecleucel, has shown durable responses in 30–50% of patients with relapsed or refractory large B-cell lymphoma.[1–3] However, the outcomes of patients who relapse after CAR19 are poor, with a contemporaneous median overall survival of approximately 6 months at the outset of our trial.[4–8] Moreover, CD19 downregulation or loss has emerged as a mechanism of resistance against CAR19.[4,9,10] CD22 is a sialic acid binding adhesion molecule restricted to the B-cell lineage and expressed in nearly all B-cell malignancies.[11,12] Paediatric patients with B-cell acute lymphoblastic leukaemia (B-ALL) given CAR T cells targeting CD22, most of whom progressed after CAR19, had a 70% complete response rate.[13] Although CD22 has proven to be an effective therapeutic target for B-ALL, no CD22-directed therapy is approved for use in large B-cell lymphoma, and non-CAR CD22-directed therapies have only shown modest efficacy.[14] We designed a phase 1

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Research in context

Evidence before this study

We searched PubMed from database inception to Oct 1, 2023, with the terms "CD22" AND "chimeric antigen receptor" (OR "CAR") AND "lymphoma", without language or study type restrictions. Our search identified no published clinical data on autologous, CD22-directed chimeric antigen receptor (CAR) T-cell therapies (CAR22) for the treatment of large B-cell lymphoma, with the exception of our group's published report on the initial three patients treated in this study. We found multiple studies evaluating the overall survival of patients who progressed after CD19-directed CAR T-cell therapy (CAR19). Multiple publications have shown that the median overall survival for patients who progress after CAR19 is approximately 6 months with available standard-of-care therapies, which largely did not include CD20 × CD3 bispecific antibodies. More recent publications evaluating such bispecific antibodies for the treatment of patients with large B-cell lymphoma, which included 30–40% of patients who progressed after CAR19, showed complete response rates of 35–40%. Median overall survival for those who received bispecific antibodies after CAR19 has not been specifically reported, but both for patients who are CAR T naive and exposed, the combined median overall survival is approximately 9 months. Another study evaluated the reinfusion of CAR19 after initial CAR19 progression in patients with a mix of non-Hodgkin lymphoma subtypes, and showed a complete response rate of 19% and median overall survival of 9 months.

Added value of this study

This study by the CARdinal-22 investigators is the first clinical trial of a CD22-directed CAR T-cell therapy for patients with relapsed or refractory large B-cell lymphoma who have predominantly progressed after CAR19. CAR22 was successfully manufactured via an automated, closed system approach for nearly all patients. This study shows that patients who progressed after previous CAR T-cell therapies can respond durably to subsequent CAR T-cell therapy. Additionally, this study demonstrates that CD22 is an effective therapeutic target for large B-cell lymphoma. This study shows that heavily treated patients with large B-cell lymphoma who progress after CAR19 are able to reach durable remission after a single infusion of CAR22, which has a manageable safety profile.

Implications of all the available evidence

This study shows the promise of using a subsequent autologous CAR22 product, which showed durable efficacy after a single infusion in a heavily pretreated population of patients with large B-cell lymphoma. These results provide compelling initial evidence to indicate CAR22 might become a new standard of care for patients who relapse after CAR19 therapy.

See Online for appendix

dose-escalation study using a second-generation CAR T-cell therapy containing a fully humanised CD22 (m971) single chain variable fragment, a 4-1BB costimulatory domain, and CD3ζ activation domain (CAR22), for the treatment of adults with relapsed or refractory large B-cell lymphoma who progressed after CAR19 therapy or with CD19-negative disease.[15]

Methods

Study design and participants

This single centre, dose-finding, open-label study was conducted at Stanford University (Stanford, CA, USA). The protocol was approved by the institutional review board and registered with ClinicalTrials.gov (NCT04088890). Patients were recruited through our referral networks as well as through institutional recruitment. All patients provided written informed consent in accordance with the Declaration of Helsinki. Eligible adults (aged ≥18 years) were those who had an Eastern Cooperative Oncology Group performance status of 0–2 with adequate organ function and histologically confirmed large B-cell lymphoma (including diffuse large B-cell lymphoma, primary mediastinal large B-cell lymphoma, transformed indolent lymphomas, or grade 3B follicular lymphoma) with measurable disease after two or more previous lines of therapy, which must have included anthracycline-containing chemotherapy and an anti-CD20 monoclonal antibody. For patients who received previous CAR T-cell therapy the percentage of peripheral blood CAR+ T cells was required to be less than 5% (appendix p 3). Patients were excluded if they had an active infection, previous malignancies (unless disease-free for 3 years or more, or in remission for 1–2 years, at the principal investigator's discretion), neurological conditions that impair the ability to evaluate for neurotoxicity, a history of myocardial infarction, cardiac angioplasty or stenting, unstable angina within 12 months of enrolment, a history of hypersensitivity to agents used within this trial, primary immunodeficiency, or autoimmune disease requiring systemic treatment within the past 2 years. The protocol permitted an evaluation of CAR22 in a cohort of adult patients with B-cell acute lymphoblastic leukaemia, which was reported separately.[16]

Procedures

CAR T-cell products were manufactured in an automated, closed system Miltenyi CliniMACs Prodigy device (Miltenyi Biotec, San Jose, CA, USA). Leukapheresis material was enriched for CD4+ and CD8+ T cells, followed by activation, transduction with a lentiviral vector containing a single-cistron-encoded CD22.BB.z-CAR, and expansion for a total of 7–12 days. Bridging therapy after leukapheresis was permitted; if radiation was used, at least one measurable site was left untreated. Lymphodepletion conditioning consisted of

fludarabine, 30 mg/m² of body surface area per day, and cyclophosphamide, 500 mg/m² per day on days –5, –4, and –3, followed by an intravenous infusion of CAR22 at a target dose of 1×10^6 CAR+ cells per kg (dose level 1) or 3×10^6 CAR+ cells per kg (dose level 2) on day 0. A detailed schema is presented in the appendix (p 24).

Outcomes

The primary endpoints were manufacturing feasibility, safety measured by the incidence and severity of adverse events and dose-limiting toxicities, and identification of the maximum tolerated dose (ie, the recommended phase 2 dose; appendix p 4). Cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) were graded according to American Society for Transplantation and Cellular Therapy consensus criteria.[17] All other toxicities were graded according to the Common Terminology Criteria for Adverse Events (version 5.0).

Secondary endpoints included the investigator-reported overall response rate (complete or partial response) according to the Lugano classification,[18] duration of response, progression free survival (defined as the time from infusion to disease progression or death), overall survival, and the number of CAR22+ cells in blood. Exploratory post-hoc analyses included tumour surface CD22 expression by immunohistochemistry and flow cytometry, serum cytokine concentrations, and phenotypic characterisation of CAR22 products (appendix pp 6–10).

Statistical analysis

The trial protocol used a 3+3 dose-escalation design with an expansion cohort with a maximum acceptable dose-limiting toxicity rate of 30%; the definitions of dose-limiting toxicities are described in the appendix (pp 4–5). However, the investigators deviated from the 3+3 design as described (appendix p 11). An efficacy assessment was done that used a Minimax Simon two-stage design, which had 80% power at a one-sided 0·05 α level to distinguish between an active therapy with a 45% overall response rate and a therapy with a 25% or less overall response rate at 3 months after infusion. The therapy was deemed worthy of further investigation if a minimum of 14 of the maximum of 36 evaluable patients had an overall response rate at 3 months at the maximum tolerated dose. Time-to-event analyses were done using the Kaplan–Meier method; categorical groups were compared via a log-rank test. Clinical outcomes and biomarkers were evaluated via Wilcoxon rank-sum and Kruskal–Wallis tests; p values and 95% CIs were descriptive and were not adjusted for multiple testing. Statistical analyses were conducted using R version 4.2.1 and version 4.1.1, and Graphpad Prism version 10.2.0.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From Oct 17, 2019, to Oct 19, 2022, a total of 41 patients were enrolled and assessed for eligibility. One patient withdrew and pursued alternative therapy, and 40 patients underwent leukapheresis (figure 1). CAR22 was successfully manufactured and administered in 38 (95%) patients (appendix p 25). Two patients had insufficient T cells to start CAR22 manufacturing; one had previously received an anti-CD52 monoclonal antibody as part of a lymphodepletion conditioning regimen for another CAR therapy. 29 (76%) patients were treated at the dose 1 level and nine (24%) patients were treated at the dose 2 level. The median time from leukapheresis to CAR22 infusion was 18 days (range 15–476). The median follow-up time from date of CAR22 infusion to the data cutoff date of May 22, 2023, was 23·3 months (range 6·4–43·8).

The demographics and disease subtypes for all treated patients are described in table 1. The median age was 65 years (range 25–84), 17 (45%) were women, 32 (84%) had elevated pretreatment lactate dehydrogenase, 11 (29%) had refractory disease to all previous therapies, and patients had received a median of four lines of previous therapy (range 3–8). One patient had a history of previous CNS involvement that had since resolved, and at the time of study enrolment, no patients had any active CNS involvement. 37 patients (97%) had relapsed after CAR19, and one had CD19-negative disease. The median time from CAR19 infusion to CAR22 infusion was 212 days (range 50–1218 days,

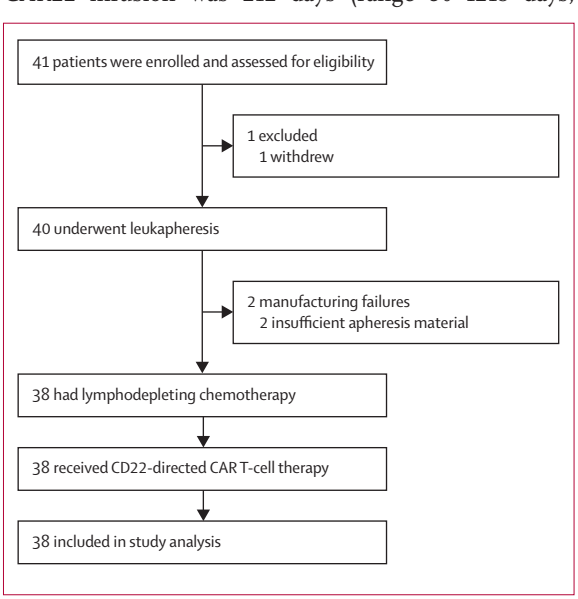


Figure 1: **Trial profile**
CAR=chimeric antigen receptor.

	All patients (N=38)	Dose level 1 (n=29)	Dose level 2 (n=9)
Age			
Median age, years	65 (25–84)	64 (25–84)	68 (36–76)
≥65 years	19 (50%)	14 (48%)	5 (56%)
Sex			
Female	17 (45%)	14 (48%)	3 (33%)
Male	21 (55%)	15 (52%)	6 (67%)
ECOG performance status score of 0–1*	38 (100%)	29 (100%)	9 (100%)
Race or ethnic group†			
Asian	3 (8%)	1 (3%)	2 (22%)
Black	2 (5%)	2 (7%)	0
White	27 (71%)	21 (72%)	6 (67%)
Hispanic or Latino	6 (16%)	5 (17%)	1 (11%)
Disease stage			
I or II	12 (32%)	8 (28%)	4 (44%)
III or IV	26 (68%)	21 (72%)	5 (56%)
Disease classification			
Diffuse large B-cell lymphoma, not otherwise specified	23 (60%)	16 (55%)	7 (78%)
Germinal centre B-cell like	11/23 (48%)	8/16 (50%)	3/7 (43%)
Activated B-cell like	12/23 (52%)	8/16 (50%)	4/7 (57%)
High grade B-cell lymphoma with MYC and BCL2 rearrangements	6 (16%)	6 (21%)	0
Large-cell transformation from indolent lymphoma‡	7 (18%)	5 (17%)	2 (22%)
Follicular lymphoma, grade 3B	1 (3%)	1 (3%)	0
Primary mediastinal large B-cell lymphoma	1 (3%)	1 (3%)	0
Previous therapies			
Median number of lines	4 (3–8)	4 (3–8)	4 (4–7)
History of refractory disease to all previous therapies	11 (29%)	8 (28%)	3 (33%)
(Continued from previous column)			
Relapse after autologous stem-cell transplantation	7 (18%)	3 (10%)	4 (44%)
Relapse after CAR T-cell therapy§	37 (97%)	28 (97%)	9 (100%)
Median duration of response after previous CAR T-cell therapy, months	3 (1–36)	3 (1–36)	3 (1–6)
Received bridging therapy¶	14 (37%)	11 (38%)	3 (33%)
Disease status at infusion			
Median tumour burden,‖ mm²	1705 (252–11165)	1617 (254–8539)	1994 (252–11165)
Median CD19 expression, H-score**	180 (0–300)	210 (0–300)	150 (90–300)
Elevated lactate dehydrogenase††	32 (84%)	23 (79%)	9 (100%)
Elevated ferritin concentrations††	16 (42%)	10 (34%)	6 (67%)
Elevated C-reactive protein††	27 (71%)	20 (69%)	7 (78%)
Vein-to-vein time	18 (15–476)	18 (15–476)	17 (16–30)

(Table 1 continues in next column)

Data are n (%) or median (range). CAR=chimeric antigen receptor. ECOG=Eastern Cooperative Oncology Group. *ECOG performance status scores are assessed on a five-point scale, with a score of 0 indicating no symptoms and increasing scores indicating greater disability. A score of 1 indicates that the patient is ambulatory but restricted from strenuous activity. †Race and ethnic group were self-reported by the patient. No patients identified as American Indian, Alaska Native, Native Hawaiian, or other Pacific Islander. ‡Transformed from follicular lymphoma (n=7) or marginal zone lymphoma (n=1). §In the trial cohort, patients received previous autologous CD19-28.z (n=28), CD19-41BB.z (n=6), CD20-19.41BB.z (n=2), CD19xxx.z (n=1), or CD22-19.41BB.z (n=1). One patient received no previous CAR T-cell therapy, and was enrolled after relapsing with CD19-negative disease after autologous stem-cell transplantation. One patient received two different autologous CAR T products before enrolment. ¶Bridging therapy was with corticosteroids (n=9), rituximab chemotherapy (n=2), corticosteroids plus radiation (n=2), or targeted therapy (n=1). ‖Tumor burden was determined using the sum of the product diameters of index lesions, according to Lugano criteria,[17] defined using the maximal diameters identified on cross-sectional computed tomography imaging. **Immunohistochemistry assessment of CD19 expression was available for 29 patients before infusion, encompassing 20 of 29 patients at dose level 1 and all nine patients at dose level 2. ††An elevated concentration was defined as a value above the upper limit of the normal range according to the local laboratory.

Table 1: **Baseline demographic and clinical characteristics of all treated patients**

appendix pp 12–18). 14 (37%) received bridging therapy (table 1; appendix p 25). The median vein-to-vein time was 18 days (range 15–476 days). The extended vein-to-vein time of 476 days was a result of one patient having a complete response after bridging therapy. CAR22 was infused after relapsing from bridging therapy, at the time of measurable disease.

The initial treatment of three patients at dose level 1 showed no dose-limiting toxicities. Subsequently, nine patients received treatment at dose level 2 and two had dose-limiting toxicities: one due to reversible grade 3 left ventricular systolic dysfunction with concurrent grade 3 pulmonary oedema, and one due to persistent grade 3 alanine aminotransferase and aspartate aminotransferase elevations associated with grade 4 IEC-HS. This latter patient, after treatment for IEC-HS, died of multi-organ failure due to *Klebsiella pneumoniae* septic shock. After de-escalation to dose level 1, an 26 additional patients (29 total) were treated with no dose-limiting toxicities observed.

Adverse events that were possibly related to CAR22 are reported in table 2. The most common grade 3 or higher adverse events were haematological events, including neutropenia (38/38 [100%]), anaemia (23/38 [61%]), and thrombocytopenia (24/38 [63%]). Recovery to grade 2 or lower cytopenias occurred within the first 60 days after infusion in most patients (appendix p 26). After CAR22 infusion, infections occurred in 16 (42%) patients, of which only two were grade 3 or higher (appendix p 19).

36 patients (95%) developed CRS; only one patient had grade 3 CRS (at dose level 2). 24 patients (63%) developed a single event of CRS (monophasic CRS) with a median time of onset of 1 day (range 0–11) after infusion, lasting for a median of 2 days (1–14). 12 patients (32%) developed a second onset of CRS (biphasic CRS) after the initial resolution. In these patients, the median time after CAR22 infusion to the initial onset of CRS was 1 day (range 0–4), lasting for a median of 2 days (1–9). The second onset of CRS occurred at a median of 12 days (10–21) after CAR22 infusion, lasting for a median of 3 days (1–7). Both patients who did not have CRS had progressive disease as a best response. Four patients (11%; three at dose level 1 and one at dose level 2) developed ICANS (two with grade 1 and two with grade 2 ICANS) after CAR22 infusion. No grade 3 or higher ICANS occurred. The median time to the initial onset of ICANS was 7 days (1–18). All ICANS events resolved in one day (in three patients) or two days (in one patient). For the management of CRS and ICANS, 82% (31/38) of patients received tocilizumab and 74% (28/38) received glucocorticoids (appendix p 20). Five patients (13%) required treatment for IEC-HS (two at dose level 1 and three at dose level 2). Four patients had grade 2 and one had grade 4 (at dose level 2). These patients developed hyperferritinaemia, transfusion-dependent hypofibrinogenaemia, grade 2 or higher transaminitis, and grade 3–4 cytopenias (table 2). The median time to onset of IEC-HS was 16 days (range 8–22). These abnormalities resolved to baseline after a median duration of 12 days (3–20). Patients were managed with high-dose corticosteroids (5/5 [100%]), anakinra (4/5 [80%]), and transfusion support including clotting factor replacement (5/5 [100%]).

Five patients (13%) died for non-relapse related reasons during the study. One patient had grade 5 septicaemia, one patient had unrelated heart failure (at dose level 1), and two patients (at dose level 2) who had each undergone four previous lines of therapy developed treatment-related myeloid neoplasms at a minimum of 22 months after CAR22 infusion. One patient (at dose level 1) died from unknown causes after being lost to follow-up at 6 months after CAR22 infusion, at which time they were in remission (appendix p 21).

Response rates were similar between dose level 1 and dose level 2 (figure 2A). The median duration of response was 27·8 months (5·1–not evaluable [NE]; figure 2B). The overall response rate was 68% (95% CI 51–83) and the complete response rate was 53% (36–69). The median progression-free survival for all patients was 3·0 months (1·8–NE) and the median overall survival for all patients was 14·1 months (9·1–NE; figure 2C, D). At dose level 1, the maximum tolerated dose (1 million CAR T cells per kg) and recommended phase 2 dose, the median progression-free survival for all patients was 3·0 months (1·6–NE) and overall survival for all patients was not reached (9·1–NE; appendix p 27). The

	Dose level 1		Dose level 2		All	
	Any grade	Grade ≥3	Any grade	Grade ≥3	Any grade	Grade ≥3
Adverse event						
All adverse events	29 (100%)	29 (100%)	9 (100%)	9 (100%)	38 (100%)	38 (100%)
Neutropenia	29 (100%)	29 (100%)	9 (100%)	9 (100%)	38 (100%)	38 (100%)
Pyrexia	27 (93%)	1 (3%)	9 (100%)	3 (33%)	36 (95%)	4 (11%)
Anaemia	25 (86%)	15 (52%)	9 (100%)	8 (89%)	34 (89%)	23 (61%)
Thrombocytopenia	25 (86%)	15 (52%)	9 (100%)	9 (100%)	34 (89%)	24 (63%)
Nausea	18 (62%)	0	4 (44%)	1 (11%)	22 (58%)	1 (3%)
Fatigue	15 (52%)	0	7 (78%)	0	22 (58%)	0
Headache	11 (38%)	0	3 (33%)	0	14 (37%)	0
Hypotension	10 (34%)	0	7 (78%)	1 (11%)	17 (45%)	1 (3%)
Hypoxia	8 (28%)	1 (3%)	7 (78%)	5 (56%)	15 (39%)	6 (16%)
Diarrhoea	8 (28%)	0	5 (56%)	0	13 (34%)	0
Alanine transaminase increase	6 (21%)	2 (7%)	5 (56%)	2 (22%)	11 (29%)	4 (11%)
Aspartate aminotransferase increase	6 (21%)	2 (7%)	4 (44%)	3 (33%)	10 (26%)	5 (13%)
Anorexia	6 (21%)	0	1 (11%)	0	7 (18%)	0
Sinus tachycardia	5 (17%)	0	1 (11%)	0	6 (16%)	0
Chills	4 (14%)	0	2 (22%)	0	6 (16%)	0
Dyspnoea	3 (10%)	0	1 (11%)	0	4 (11%)	0
Myalgia	3 (10%)	0	2 (22%)	0	5 (13%)	0
Sepsis	2 (7%)	2 (7%)	2 (22%)	2 (22%)	4 (11%)	4 (11%)
Alkaline phosphatase increase	1 (3%)	0	3 (33%)	0	4 (11%)	0
Constipation	1 (3%)	0	3 (33%)	0	4 (11%)	0
Cytokine release syndrome	27 (93%)	0	9 (100%)	1 (11%)	36 (95%)	1 (3%)
ICANS	3 (10%)	0	1 (11%)	0	4 (11%)	0
IEC-HS	2 (7%)	0	3 (33%)	1 (11%)	5 (13%)	1 (3%)
Deaths*						
All deaths	0	0	0	3 (33%)	0	3 (8%)
Therapy-related myelodysplastic syndrome or acute myeloid leukaemia	0	0	0	2 (22%)	0	2 (5%)
Septicaemia	0	0	0	1 (11%)	0	1 (3%)

Data are n (%). Shown are any adverse events of any grade, as well as events associated with cytokine release syndrome and neurological events (ICANS) that occurred in at least 10% of patients. Cytokine release syndrome and ICANS were graded as per American Society for Transplantation and Cellular Therapy consensus criteria. Individual signs or symptoms of cytokine release syndrome or ICANS were graded as per National Cancer Institute's Common Terminology Criteria for Adverse Events version 5.0. IEC-HS was retrospectively graded as per American Society for Transplantation and Cellular Therapy consensus criteria.[17] ICANS=immune effector cell-associated neurotoxicity syndrome. IEC-HS=immune effector cell-associated HLH-like syndrome. *Possibly related to treatment.

Table 2: **Adverse events, cytokine release syndrome, and neurological events associated with treatment**

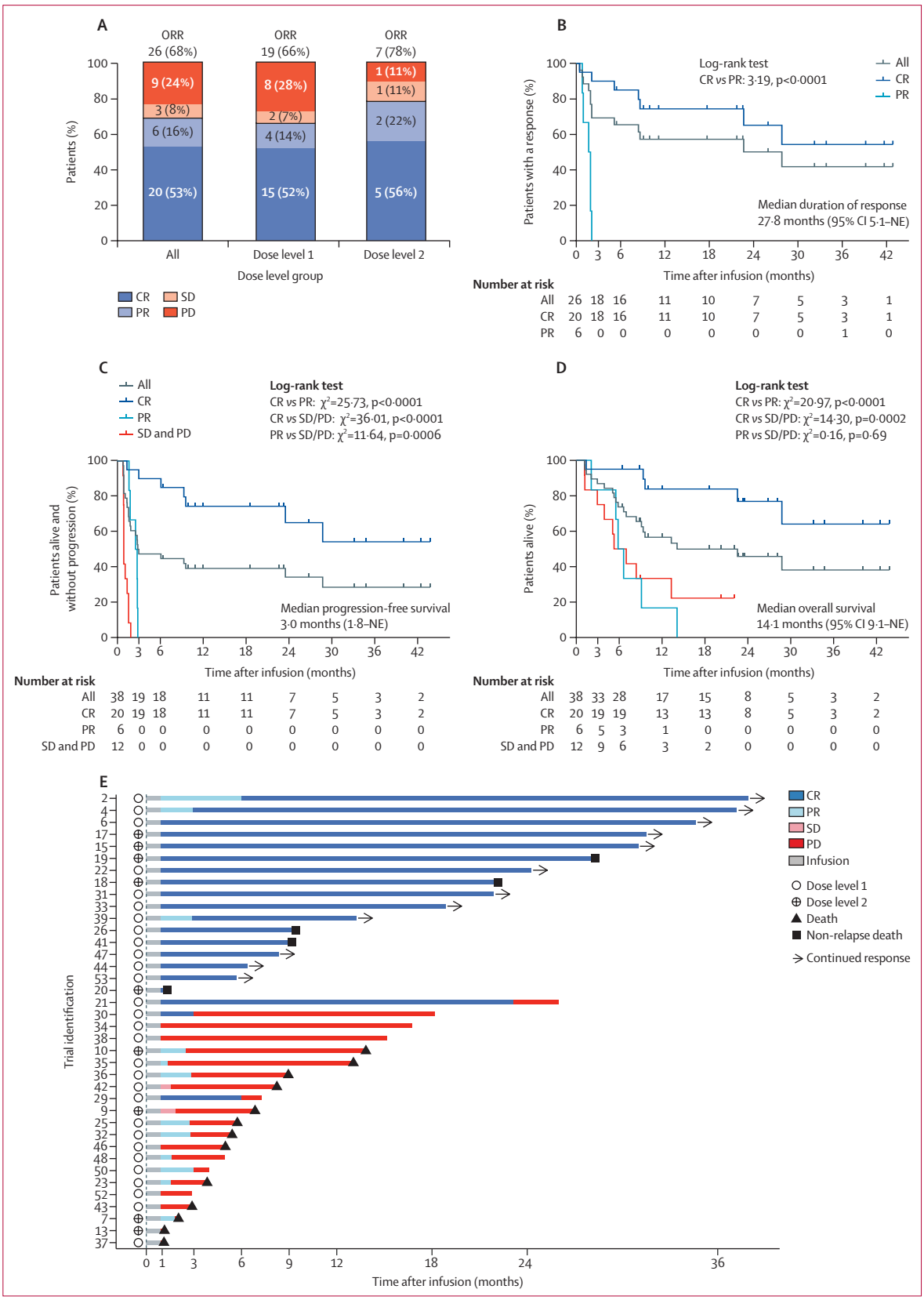


***Figure 2*: ORR, duration of response, progression-free survival, and overall survival**
(A) Best response among 38 patients treated, 29 in dose level 1 and 9 in dose level 2. Percentages are rounded and so may not add up to exactly 100%. (B) Kaplan–Meier estimate of duration of response in patients who had a complete response or partial response as a best response. (C) Kaplan–Meier estimate of progression-free survival in all patients, subdivided by those who had a CR, PR, or no response (SD and PD) as their best response. (D) Kaplan–Meier estimate of overall survival in all patients, subdivided by those who had a CR, PR, or no response as their best response. (E) Swimmer plot showing the duration of response and survival after infusion for all treated patients (N=38). CR=complete response. NE=not evaluable. ORR=overall response rate. PD=progressive disease. PR=partial response. SD=stable disease.

estimated 1-year survival at dose level 1 was 57% (95% CI 40–77%) and 2-year survival was 52% (36–74%). Of the 20 patients with a complete response after receiving CAR22, only three (15%) patients relapsed (one each at 3, 6, and 23 months; figure 2E) and the median progression-free survival and overall survival were not reached. There were no significant differences in survival or duration of response among different

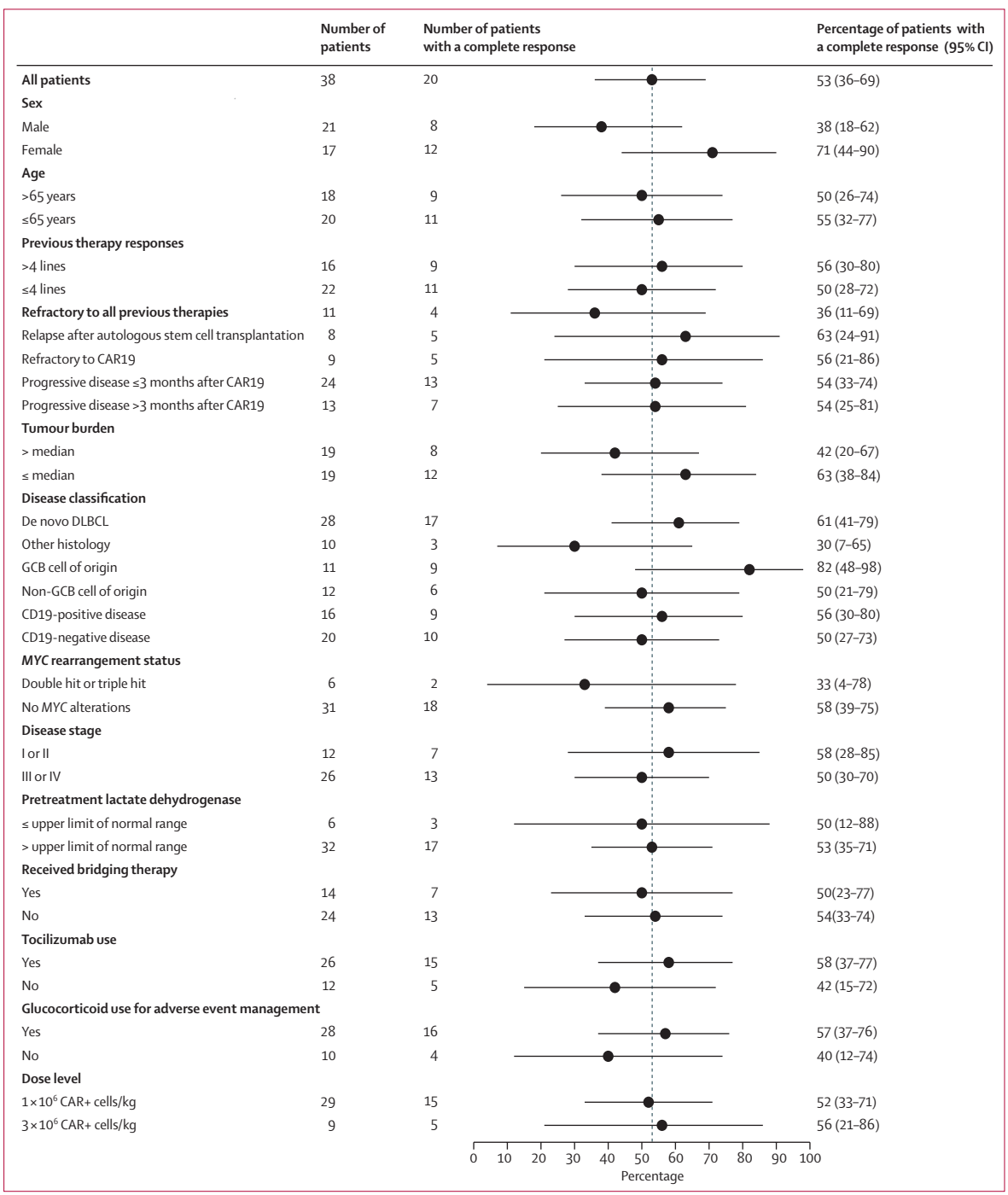
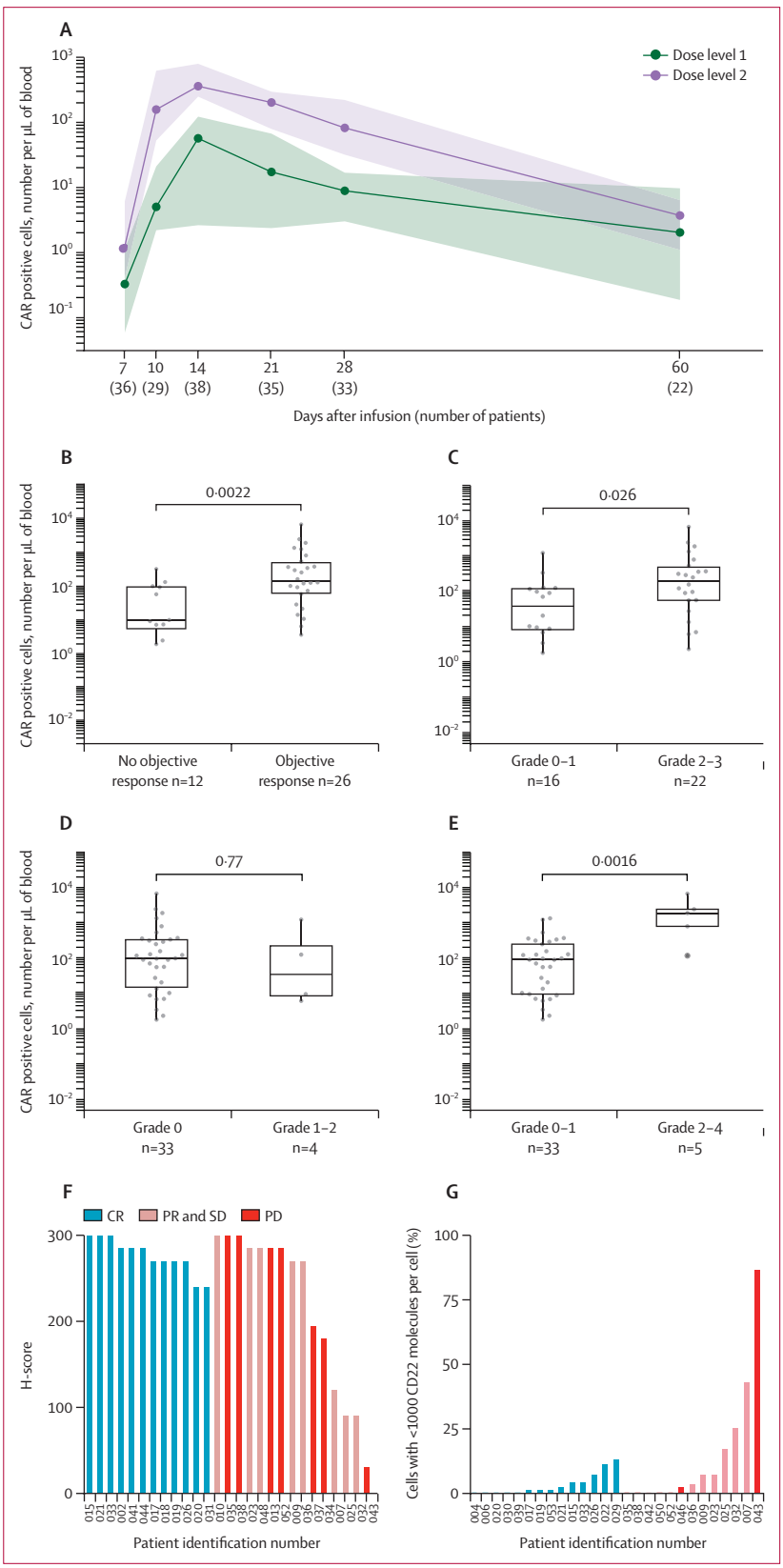


Figure 3: **Subgroup analysis of complete response**
Shown is the analysis of complete responses according to key baseline and clinical covariates. The Clopper–Pearson method was used to calculate the 95% CI, which is not adjusted for multiplicity. Tumour burden was assessed as the sum of the product diameters. CD19-negative disease was defined as an H-score of less than 150 (corresponding to ≤50% of cells staining by immunohistochemistry), or undetectable surface CD19 by flow cytometry, or both. CAR19=CD19-directed chimeric antigen receptor T-cell therapy. DLBCL=diffuse large B-cell lymphoma. GCB=germinal centre B-cell-like.



disease groups by previous CAR19 response or time until relapse to CAR19 (appendix pp 28–29). The overall response rate and complete response rate were not significantly different across all subgroups, including age, sex, previous therapy responses, tumour burden, disease classification or stage, cell-of-origin subtype, and the use of tocilizumab or glucocorticoids (figure 3; appendix p 30). New hypermetabolic lesions, ultimately identified to be pseudo-progression, were observed on the day 28 PET-CT scan of four patients (appendix p 31). An additional patient had a new hypermetabolic lesion adjacent to the initial lymphoma lesion observed 1 year after CAR22, ultimately biopsied and identified as a granuloma. For all five patients, these lesions resolved without intervention, and they were in remission at the time of last follow-up.

CAR+ T cells in the peripheral blood peaked at a median of 14 days (range 7–22) after infusion at a median of 71 CAR+ T cells per mm^3 at dose level 1 and 360 CAR+ T cells per mm^3 at dose level 2 (figure 4A; appendix p 22). CAR22 expansion measured by flow cytometry and quantitative PCR positively correlated with each other (appendix p 33). Higher circulating CAR22 T cells at peak and higher cumulative concentrations of CAR+ T cells measured by area under the curve over the first 28 days were observed in patients with a response compared with those without a response (median 141 *vs* 9·2; p value 0·0022); in patients with grade 2–3 CRS compared with those with grade 0–1 CRS (median 203 *vs* 46; p value 0·026); and in patients who required treatment for IEC-HS compared with those who did not (median 1884 *vs* 90; p value 0·0016; figure 4B–E; appendix pp 23, 34–35). There was no significant association between circulating CAR+ T-cell

Figure 4: **CAR T-cell expansion and correlation with response and adverse events**

(A) CAR+ cellular kinetic profile, illustrating the in vivo expansion and persistence of CAR22 cells in patients' peripheral blood, stratified by dose levels. Median values are shown by the interconnected dots, and IQRs are represented by the shaded regions. (B) Association between peak CAR22 expansion and the best overall response (median 129 for complete or partial response *vs* 8·8 for stable or progressive disease; p value 0·0022). (C) Association between peak CAR22 expansion and the maximum grade cytokine release syndrome (median 203 for grade 2–3 *vs* 46 for grade 0–1; p value 0·026). (D) Association between peak CAR22 expansion and the maximum ICANS grade (median 68 for grade 1–2 *vs* 98 for none; p value 0·77, not significant). (E) Association between peak CAR22 expansion and the maximum IEC-HS grade (median 2378 for grade 2–4 *vs* 189 for grade 0–1; p value 0·0016). (F) Baseline tumour CD22 expression by semiquantitative H-scoring of immunohistochemistry for all available patients (n=27) including those who did (n=12) or did not (n=15) have a complete response. H-score was calculated as the percentage of cells with positive staining multiplied by the intensity of staining on a scale from 0 to 3+. No patients (n=6) whose tumour sample had an H-score of less than 200 had a complete response (p value 0·02). (G) Proportion of baseline tumour surface CD22 expression at low or absent numbers by quantitative flow cytometry for all available patients (n=27) including those who did (n=14) or did not (n=13) have a complete response. CAR=chimeric antigen receptor. CAR22=CD22-directed chimeric antigen receptor T-cell therapy. CR=complete response. ICANS=immune effector cell-associated neurotoxicity syndrome. IEC-HS=immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome. PD=progressive disease. PR=partial response. SD=stable disease.

concentrations at peak or by area under the curve with baseline tumour burden (R^2=0·0034; p value 0·73). Circulating CAR+ T cells were detectable by both flow cytometry and quantitative PCR in most patients at 6 months after infusion (appendix p 36).

The baseline antigen expression and density of surface CD22 or CD19 expression as measured by immunohistochemistry or quantitative flow cytometry on tumour samples did not show any correlation with CAR22 expansion or response (appendix pp 37–40). However, all six patients with baseline CD22 H-scores of less than 200 had progression, and all four patients who had at least 15% of tumour cells with low or absent CD22 surface antigen density (<1000 molecules per cell) also progressed (figure 4F, G). In five of eight (63%) paired tumour biopsies collected at baseline and disease progression, there was a marked reduction in surface CD22 expression at relapse, with median antigen density dropping to 46% (range 10–204) of baseline expression (appendix pp 37–40).

A serial analysis of patient serum showed that the initial CAR22 expansion was accompanied by significant elevations in inflammatory and effector cytokines including interleukin (IL)-10, IL-15, interferon γ, IL-2, IL-6, and tumour necrosis factor α, with peak increases of more than 50% at day 7; peak concentrations were significantly higher in patients at dose level 2 than those at dose level 1 (appendix p 41).

Discussion

A high unmet medical need continues to exist for patients with relapsed or refractory large B-cell lymphoma who progress after CAR19 therapy. Reinfusion with a CAR19 product has not produced meaningful durable responses in a substantial number of patients.[19] Therapies such as polatuzumab, bendamustine, and rituximab; tafasitamab and lenalidomide; loncastuximab tesirine; and selinexor are unlikely to be curative in this setting.[20–24] Bispecific antibodies have shown the potential for durable remission in a subset of patients after CAR19 therapy,[25–28] but are unlikely curative for the vast majority of patients. In this study, CAR22 was a highly active therapy in patients with highly refractory disease who progressed after CAR19 therapy. Patients who had a complete response frequently had durable remissions. We observed similar clinical efficacy between the two tested dose levels. However, because of the observed increase in toxicity at the higher dose level 2, the recommended phase 2 dose was reduced back to 1×10^6 CAR+ T cells per kg. At the maximum tolerated dose (ie, the recommended phase 2 dose), the estimated 2-year survival was 52%, in stark contrast to the median overall survival of 6 months observed in patients who relapsed after CAR19.[5] However, those who did not have a complete response after CAR22 had poor overall survival rates, echoing outcomes seen after CAR19 relapse. The toxicity profile of CAR22 is favourable compared with other approved CAR T-cell therapies for relapsed or refractory large B-cell lymphoma. Notably, at the maximum tolerated dose, no patient had grade 3 or higher CRS, ICANS, or IEC-HS. Moreover, ICANS incidence was rare, a stark contrast from the outcomes of those with CAR19, with most cases resolving typically within a day. Markedly high CAR22 expansion was associated with the development of higher grade adverse events and increased non-relapse mortality. In particular, among patients needing treatment for IEC-HS, four of five individuals were in the highest quartile for peak expansion (appendix p 23).

Notably, we observed five cases of new hypermetabolic lesions after CAR22 infusion, which is concerning for radiographic progression of disease, that all resolved without intervention. Cases of so-called pseudo-progression similar to this have been described after CAR19.[29–31] Biopsies could not be safely obtained in four patients and therefore the causes of this occurrence need to be explored in future trials. For the fifth case, which occurred 14 months after infusion, non-caseating granulomatous inflammation was observed in the biopsy.

Haematological toxicity and infections after CAR22 therapy were expected, with observed rates similar to CAR19 therapies despite more extensive previous therapies.[32–34] Additionally, two patients at dose level 2 developed therapy-related myeloid neoplasms, a well documented complication in heavily pretreated patients. The precise incidence rates of therapy-related myeloid neoplasms subsequent to CAR T-cell therapy require further investigation.[35–37]

An exploratory analysis indicates an association between progression and patients having a lower CD22 H-score or a higher percentage of cells expressing CD22 at low levels. Notably, a decline in the median CD22 antigen density was noted in five of eight patients who relapsed, suggesting that antigen escape might be a shared mechanism of therapeutic resistance as previously noted in B-ALL.[13,38] The extended duration of response observed in this study, in contrast to the response duration observed in patients with B-ALL, suggests the presence of inherent biological disparities between precursor and mature B cells in their capacity to modulate CD22 expression. This modulation and the relationship between baseline CD22 antigen concentrations and response should be explored in a lineage-specific manner in future studies. Notably, CAR22 expansion was associated with an improved overall response rate and complete response rate. Interestingly, the manufacturing approach resulted in CAR22 products with a predominance of CD4+ T cells, in line with previous reports using a CD22.BB.z construct (appendix pp 42–45).[38] Despite this, CD8+ T cells represented most of the cells in in vivo CAR+ T-cell expansion. Further studies will evaluate the clonal dynamics of the CAR22 products to better understand T-cell subset contribution to response and toxicity.

This study has a number of limitations. A small proportion of patients received bridging therapy, which might have provided additional benefits beyond CAR22.

This study is unable to address the expression concentrations of CD22 on CAR19-refractory large B-cell lymphoma, which will likely affect the generalisability of these results to all patients who relapsed after CAR19. Furthermore, the exploratory analysis is limited by a small number of patients.

The emergence of CD19 downregulation after CAR19 therapy in relapsed or refractory large B-cell lymphoma has led to the investigation of immunotherapies that target alternative antigens; our results support the notion that CD22 is an effective target for relapsed or refractory large B-cell lymphoma, particularly after CAR19. These findings show that even in cases of early relapse after initial CAR T-cell therapy, patients can have positive treatment outcomes after subsequent autologous CAR T-cell therapy. However, there are multiple unanswered questions, including the role of CAR22 in other lymphoma subtypes, the response rate of CAR22 in patients who are CAR T-naive, and the role of co-administering adoptive cellular therapies that target multiple antigens simultaneously. On the basis of these results, a multicentre study investigating CAR22 for patients with large B-cell lymphoma who have relapsed after CAR19 is actively enrolling (NCT05972720). Furthermore, ongoing investigations are exploring the effect of residual CAR19 on CAR22 production and patient outcomes, along with CAR19 kinetics in these patients after CAR22 infusion, with findings expected to be reported in the near future.

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Contributors
LM, MJF, CLM, and DBM designed the study. JHB, MJF, and AMK interpreted the data. All authors contributed to data collection. JHB, AMK, and MJF analysed the data and created the figures. JHB, MJF, AMK, DBM, and CLM drafted the manuscript. All authors contributed to the writing and revision of the manuscript and approved the final version. MJF and AMK accessed and verified the data.

Declaration of interests
ARR received research support from Pharmacyclics and AbbVie; one-time ad hoc scientific advisory board work for Nohla Therapeutics and Kaleido; and expert witness work for the US Department of Justice. CLM is the founder, has equity in, consults for, and is a Director of Cargo Therapeutics and Link Cell Therapies; has equity in Lyell Immunopharma; and receives royalties from the National Institutes of Health for CAR22 consulting for Immatics, Ensoma, Mammoth, Adaptimmune, and Bristol Myers Squibb. DBM consults for Kite Pharma-Gilead, Juno Therapeutics-Celgene, Novartis, Janssen, and Pharmacyclics; and receives research support from Kite Pharma-Gilead, Allogene, Cargo Therapeutics, Pharmacyclics, Miltenyi Biotec, and Adaptive Biotechnologies. JHB consults for Kite Pharma-Gilead; and receives research support from Kite Pharma-Gilead, Genentech-Roche, Regeneron Pharmaceuticals, and Cellular Biomedicine Group. MJF consults for Kite Pharma-Gilead, Adaptative Biotechnologies, and Cargo Therapeutics; receives research support from Kite-Pharma-Gilead, Allogene Therapeutics, Cargo Therapeutics, and Adaptative Biotechnologies; and is on the data safety monitoring board for Fate Therapeutics. LM receives research support from Adaptive Biotechnologies and Servier Laboratories; and consults for Amgen and Pfizer. JYS consults for Kite Pharma-Gilead and ImmPACT Bio. PS receives research support from Kite Pharma-Gilead. SS consults for Janssen. SAF consults for Lonza PerMed, Gradalis, Obsidian, and Samsara BioCapital. MPH is on the Kite Pharma-Gilead advisory board. All other authors declare no competing interests.

Data sharing
De-identified individual participant data that underlie the reported results will be made available for approved use by the study authors. Proposals for access should be sent to mjfrank@stanford.edu. Complete trial cohort-level data will be published on ClinicalTrials.gov at the conclusion of the trial.

Acknowledgments
We acknowledge the staff and faculty of the Stanford University Blood and Marrow Transplantation Program, the Cellular Therapy Facility, and the Stanford Center for Cancer Cell Therapy for their tireless work caring for the patients involved in this study. We specifically thank Nimfa Fajardo, Carmela Baron, and Janette Kimes. We also acknowledge Nirali N Shah (Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA) for the provision of additional vector used to support the conduct of the study and insight into CD22 CAR T-cell targeting. This study was funded by the National Cancer Institute (grant number 2P01CA049605–29A1 to CLM and DBM, and Robert S Negrin of the CARdinal-22 Investigator group; and 5P30CA124435 to CLM), and by the Virginia and DK Ludwig Fund for Cancer Research. CLM is a member of the Parker Institute for Cancer Immunotherapy, which supports the Stanford University Cancer Immunotherapy Program. Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under award number K08CA248968 (to MJF). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This project was supported by a research grant award via the European Hematology Association (to AMK) and the Lymph & Co Foundation project (grant number PFvV-002 to AMK). MPH is a fellow of the Leukemia and Lymphoma Society. Further support was provided by the National Gene Vector Biorepository at Indiana University, which is funded through the National Heart, Lung, and Blood Institute (contracts 75N92019D00018 and HSN261201500003I) and the National Cancer Institute (task order number HHSN26100077).

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Frank MJ, Baird JH, Kramer AM, et al. CD22-directed CAR T-cell therapy for large B-cell lymphomas progressing after CD19-directed CAR T-cell therapy: a dose-finding phase 1 study. *Lancet* 2024; published online July 9. https://doi.org/10.1016/S0140-6736(24)00746-3.

Supplementary Appendix

Table of Contents

Supplementary Methods

Eligibility Criteria

1. Must have histologically confirmed disease as defined by WHO 2008:
    - DLBCL not otherwise specified; T cell/histiocyte rich large B cell lymphoma; DLBCL associated with chronic inflammation; Epstein Barr
    - virus (EBV)+ DLBCL of the elderly; OR
    - Primary mediastinal (thymic) large B cell lymphoma, OR
    - transformation of follicular lymphoma, marginal zone lymphoma or chronic lymphocytic leukemia/small lymphocytic lymphoma to DLBCL
    - Follicular Lymphoma Grade 3B
    - Subjects with DLBCL, Follicular Lymphoma Grade 3B –or- subjects with transformed FL, MZL, or CLL/SLL who have not received chemotherapy prior to transformation: must have received an anthracycline regimen and an anti-CD20 monoclonal antibody (unless documented CD20-neg) and be relapsed/refractory after second line of DLBCL treatment. Subjects with PR to second line therapy must be ineligible for autologous transplant.
    - Subjects with transformed FL, MZL, or CLL/SLL who have received anthracycline-containing chemotherapy prior to transformation: must have progressed, had SD or recurred with transformed disease after initial treatment for DLBCL
2. Measurable Disease: Must have evaluable or measurable disease according to the revised IWG Response Criteria for Malignant Lymphoma. Lesions that have been previously irradiated will be considered measurable only if progression has been documented following completion of radiation therapy.
3. CD22 expression: CD22 expression at any level, including undetectable, will be acceptable and subjects must have archival tissue available for analysis of CD22 expression, or must be willing to undergo biopsy of easily accessible disease.
4. Subjects who have progressed or relapsed after prior autologous SCT must be at least 100 days post-transplant, have no evidence of GVHD, and have been without immunosuppressive drugs at least 30 days.
5. Subjects with prior CAR therapy must be at least 30 days post CAR infusion and have < 5% CD3+ cells express the previous CAR, if a validated assay is available.
6. Toxicities from prior therapy stable or resolved (except for clinically non-significant toxicity and cytopenias)
7. Age: ≥ 18 years of age.
8. Adequate performance status (ECOG 0, 1, or 2; or Karnofsky ≥ 60%
9. Adequate organ and marrow function as defined by:
    - ANC ≥ 750/uL *, platelet count ≥ 50,000/uL *, ALC ≥ 150/uL *
    - Creatinine ≤ 2 mg/dL OR Creatinine Clearance ≥ 60 mL/min

- Serum ALT/AST ≤ 10 times the ULN (institutional normal) [Elevations related to leukemia involvement of the liver will not disqualify a subject]
- Total bilirubin ≤ 1.5 mg/dL (except in subjects with Gilbert's disease)
- Cardiac ejection fraction (LVEF) ≥ 45% and no evidence of pericardial effusion
- No clinically significant ECG findings
- No clinically significant pleural effusion
- SaO2 > 92% on room air

10. Subjects with CNS involvement or a history of CNS involvement are eligible only in the absence of neurologic symptoms that may mask or interfere with neurological assessment of toxicity.
11. Females of child-bearing potential must have negative pregnancy test.
12. Females of child-bearing potential and males of child-fathering potential must be willing to practice birth control from time of enrollment and for 4 months post preparative lymphodepletion regimen.
13. Must be able to provide informed consent (LAR is permitted if subject able to provide verbal assent).

Additional exclusion criteria

1. History of other malignancies, apart from non-melanoma skin cancer or carcinoma in situ, unless disease free for at least 3 years, or in remission 1-2 years and Principal Investigator assesses other malignancy as unlikely to return within 1 year or interfere with CAR T cell safety.
2. Active fungal, bacterial, viral or other infection requiring intravenous antimicrobials. Simple UTI or uncomplicated bacterial pharyngitis is permitted if responding to active treatment.
3. Ongoing HIV, HBV or HCV infection. History of HBV or HCV is permitted if viral load is undetectable by qPCR and/or nucleic acid testing.
4. Active cerebrovascular ischemia/hemorrhage, dementia, cerebellar disease, or autoimmune disease with CNS involvement that in investigator's judgement impair ability to evaluate neurotoxicity.
5. History of MI, cardiac angioplasty or stenting, unstable angina or other clinically significant cardiac disease with 12 months of enrollment.
6. Severe, immediate hypersensitivity reaction attributed to compounds of similar chemical or biologic composition to any agents used in study.
7. May not be breastfeeding.
8. Primary immunodeficiency or history of autoimmune disease (e.g. Crohns, rheumatoid arthritis, systemic lupus) requiring systemic immunosuppression/systemic disease modifying agents within the last 2 years.
9. In investigator's judgment, any medical condition likely to interfere with assessment of safety or efficacy, or be unlikely to complete all protocol-required visits and procedures.

* A subject will not be excluded because of pancytopenia ≥ Grade 3 if it is felt by the investigator to be due to underlying leukemia/lymphoma.

Dose Escalation

There will be a Phase 1 dose-escalation design with three dose cohorts in subjects with aggressive B-cell NHL to determine the MTD/RP2D. Each dose cohort will initially include a minimum of 3 subjects. Treatment will be staggered as follows for each dose level: at least 21 days will elapse between infusion of each subject during dose escalation. The final subject with aggressive B-cell NHL in a dose cohort must complete the 28-day DLT observation period before the decision is made whether to treat additional subjects at the current dose level or to dose escalate to allow for safety assessment of DLTs. If Dose Level 3 is completed without DLTs, an MTD may not be determined. This will be considered the 'highest cell dose' studied and will be the dose level that will be studied further in the expansion cohort or recommended phase 2 dose (RP2D). Dose escalation will follow the rules outlined in the Table below.

Number of Subjects with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter up to 3 subjects at the next dose level. If 0 out of 3 occur, dose may escalate.
1 out of 3	If DLT develops in one of first 3 subjects at a Dose Level, the cohort will be expanded to 6 subjects. If no additional subjects develop DLT, MTD will not have been exceeded and the next dose level can be administered after the four week safety assessment period of the last subject at this dose level. If DLT develops in any subject at Dose Level -1, accrual will be temporarily stopped while consultation with the IRB and FDA occurs.
2 out of 3	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional subjects will be entered at the next lowest dose level if only 3 subjects were treated previously at that dose.
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the RP2D. At least 6 subjects must be entered at this dose level.

Definition of Maximal Tolerated Dose (MTD)

The maximum tolerated dose (MTD) will be evaluated and reported separately in each disease group (ALL and aggressive B-cell NHL). During dose escalation of subjects with aggressive B-cell NHL, MTD is defined as the dose level below that at which 2/6 subjects develop DLTs. Subjects with ALL treated at the established dose of 1 x 106 transduced T cells/kg and subjects with aggressive B-cell NHL treated at the MTD/RP2D, the maximum acceptable rate of DLTs for each group is 30%.

Definition of DLT

Adverse events that are at least possibly related to the treatment regimen (conditioning lymphodepletion chemotherapy regimen and/or CD22-CAR T cells) with onset within the first 28 days following CD22-CAR T cell infusion will be considered DLTs as follows:

- Grade 4 CRS (of any duration) or Grade 3 CRS that lasts greater than 7 days.
- Grade 4 neurotoxicity (of any duration) or Grade 3 neurotoxicity not improving within 72 hrs

- Grade 3 or greater infusion reactions lasting more than 24 hours despite standard supportive care.
- Grade 4 or greater tumor lysis syndrome including associated abnormalities (e.g., electrolytes, uric acid, renal function) lasting more than 7 days if accompanied by end organ damage.
- Grade 3 or greater fever lasting > 14 days.
- Grade 4 infection uncontrolled for > 7 days. Grade 3 infection is not a DLT.
- In patients with history of prior SCT, any histologically proven acute GVHD grade 3 or higher within 30 days of receiving the CD22-CAR T cells will be considered DLT.
- Grade 4 or less hematologic toxicity will not be considered DLTs, as these are common after CAR T therapy and have been successfully managed with standard supportive therapies. o Hematologic toxicity includes cytopenias such as anemia, thrombocytopenia, lymphopenia, neutropenia, and white blood cell decreased; as well as coagulation lab abnormalities such as fibrinogen and INR increased (in the absence of clinically significant bleeding)
- Any other Grade 3 or greater, non-hematological toxicity lasting longer than 72 hours will be considered a DLT, with the following exceptions:
    - Grade 3 diarrhea improving within 4 days;
    - Hepatic function test (e.g. transaminase, alkaline phosphatase, bilirubin or other liver function test) elevation to ≤ 10x ULN, provided there is resolution to ≤ Grade 2 or baseline within 14 days;
    - Grade 3 nausea, fatigue, anxiety and/or anorexia;
    - Grade 3 or greater isolated changes in laboratory values will not be considered DLT unless they result in any one of the following:
        - Discontinuation from the study therapy;
        - Is medically significant requiring hospitalization or prolongation of hospitalization;
        - Is judged by the Investigator to be of significant clinical impact.

Adverse events will be graded according to NCI's Common Terminology Criteria for Adverse Events (CTCAE v5.0). CRS and neurotoxicity (ICANS) will be graded according to a ASTCT consensus grading criteria[16]. Adverse events attributed to CRS or neurotoxicity will be mapped to the overall grading assessment for the determination of DLT.

Disease Assessment

Disease evaluation methods will be determined by the investigator based on subjects' location of disease; not all are required on all subjects, but methods should remain consistent while on study:

- Imaging Studies appropriate to sites relevant to subject's disease: subject with bulky disease will undergo PET/CT, other imaging studies (e.g. MRI of the brain) will be performed as determined by investigator
- Bone marrow aspirate: subjects with bone marrow involvement prior to therapy or if new abnormalities in the peripheral blood counts or blood smear cause suspicion of bone marrow involvement will undergo bone marrow aspirate, with biopsy if needed
- Lumbar puncture, for subjects with known or suspected CNS involvement only
- Lymph node biopsy in subjects with lymphoma only, if feasible, once between Day 7 and Day 28 (during peak CAR activity) for correlative studies

Biomarker Analysis

Surface Antigen Binding Capacity per Cell Quantification

Peripheral blood, cerebrospinal fluid, or fine needle aspirate tissue specimens were processed within 24 hours of collection (mean +/- hours) and stained using the antibody combination listed **(Table M1)** and analyzed on the BD FACSLyric system. Median fluorescence intensity (MFI) for CD19, CD20, and CD22 were determined under saturating conditions and the antigen bound per cell (a.k.a. antigen density) calculated by calibration with BD Quantibrite beads for PE, APC, and BV421 (BD Biosciences, San Jose, CA) were used to determine the median equivalent saturating fluorescence.

Table M1: Quantibrite antibody panel

Antibody	Fluorochrome	Manufacturer	Part Number
Lambda	FITC	BD Biosciences	346586
Kappa	BV605	BD Biosciences	663192
CD22	PE	BD Biosciences	340708
CD34	PERCP	BD Biosciences	340666
CD10	PE-Cy7	BD Biosciences	341102
CD20	APC	BD Biosciences	340940
CD5	APC-R700	BD Biosciences	565121
CD38	APC-H7	BD Biosciences	653314
CD19	BV421	BD Biosciences	659477
CD45	V500-C	BD Biosciences	647450

CD22.BB.z-based Product Manufacturing

CAR22 products were manufactured in the automated closed-system Miltenyi CliniMACs Prodigy (Miltenyi Biotec) in a 7-12 day manufacturing process. All days provided in this CAR T production section are reflective of the manufacturing schema. Patient apheresis product was loaded on the Prodigy on manufacturing day 0. The apheresis product was enriched for CD4 and CD8 T-cells prior to T-cell activation with TransAct (Miltenyi Biotec). On manufacturing day 1, T-cells were transduced with CD22.BB.z lentiviral vector **(Supplementary Figure 1A)**. TransAct was subsequently washed out on manufacturing day 3, followed by a series of media exchanges. On day 7 (up to day 12), when target dose was achieved, the final product was harvested, sampled for QC testing, and cryopreserved. Product release criteria are listed below **(Table M2)**.

Table M2: CAR22 product release criteria

Product Rapid-Release for Infusion Criteria Table	
Release Test	*Acceptance Criteria*
Cell viability	≥ 70%
Cell number	± 20% of planned dose level
% CD3+ Cells	≥ 70%
% CAR+ cells	≥10%
Endotoxin	< 5 EU/kg body weight
Mycoplasma	Negative
Gram Stain	Negative
Preliminary Sterility (3-5 day)	Negative

Preliminary Fungal (3-5 day)	Negative
qPCR-based Replication Competent Lentivirus (RCL)	Negative
VCN	FIO
Product Phenotyping/Composition	FIO
Product Final Release and Lot Disposition Table	
Release Test	*Acceptance Criteria*
Sterility (14 days)	Negative
Fungal (42 days)	Negative
Cell-based RCL	Negative

Phenotyping of Manufacturing Samples at Apheresis, Enrichment, and Final Product Harvest

All samples were washed in FACS Buffer (1× PBS, 2% FBS) and stained for a minimum of 30 minutes at 4ºC, prior to additional washes and running on the flow cytometer.

Table M3: Immunophenotyping antibody panel.

Antibody	Fluorochrome	Supplier	Part Number
CD45	VioBlue-REA747	Miltenyi	130-110-637
CD3	FITC-REA613	Miltenyi	130-113-138
CD4	VioGreen-VIT4	Miltenyi	130-113-221
CD8	APC-Vio770-BW135/80	Miltenyi	130-113-155
CD56	PE-REA196	Miltenyi	130-113-312
CD16	PE-REA423	Miltenyi	130-113-393
CD14	APC-REA599	Miltenyi	130-110-520
CD20	PE-Vio770-LT20	Miltenyi	130-113-375

Flow Cytometry for Phenotyping and Exhaustion Profiling of CAR22 Products

All samples were washed in FACS Buffer (1x PBS, 3% FBS), stained for a minimum of 30 minutes at 4ºC, prior to additional washes and running flow cytometry. UltraComp ebeadsTM (Invitrogen, 01-2222-41) were used for compensation controls, stained with the respective antibody from the antibody index below. Samples were run on the CytoFLEX (Beckman) and stained using antibodies below **(Table M4)**.

Table M4: CAR22 product immunophenotyping antibody panel

Antigen	Fluorochrome	Supplier	Part Number
CD3	BUV496	BD Biosciences	612940
CD4	BUV737	BD Biosciences	612748
CD8	BUV805	BD Biosciences	612889
Recombinant Human Siglec-2/CD22β Fc Chimera	DyLight650	R&D Systems	1968-SL-050
CD45RO	PE-Cy7	Biolegend	304230
CD45RA	PerCP	Biolegend	304130
CCR7	FITC	Biolegend	353216
CD62L	BV605	BioLegend	304834

CD95	BV421	BioLegend	305624
CD39	FITC	BioLegend	328206
PD-1	PE-Cy7	BD Biosciences	561272
TIM3	PE	BioLegend	345006
LAG3	BV421	BioLegend	369314
Live-Dead	Zombie Aqua	BioLegend	423102

Lymphocyte Subset (CAR+ and CAR-) Quantification from Peripheral Blood

A High Dimensional (Hi-D) immuno-phenotyping flow cytometry panel was designed for tracking chimeric antigen receptor (CAR) positive and CAR negative T-cell lineage-specific surface antigens, as well as target B-cell lineage-specific surface antigens in patient samples in real time – referred to as CAR-FACS. Peripheral blood mononuclear cells (PBMCs) were isolated from fresh whole blood by density gradient centrifugation using the Ficoll-Paque Plus system (Sigma-Aldrich; St. Louis, MO). PBMCs (average yield: 2-5×10^6 cells) were then stained with Live/Dead Aqua Fixable Viability Stain (Thermo Fisher; Waltham, MA), then pre-incubated with Fc receptor blocking solution (Human TruStain FcX, BioLegend; San Diego, CA) for 5 minutes. After incubation, the cells were stained at room temperature (RT) in an 11-color, 13-parameter combination with fluorochrome-conjugated monoclonal antibodies (mAb) **(see Table M5)**. CD22.BB.z-transduced cells were used as a positive control and included in daily experiments. Stained and fixed cells were acquired on a BD LSRII analyzer using FACSDiva software (BD Biosciences) and analyzed with Cytobank software (Cytobank, Inc; Santa Clara, CA). The lower limit of quantification for the assay was 1 cell per 10,000 viable PBMCs (0.01%). B-cells were defined as live CD45+, CD3-, CD4-, CD8-, CD14-, and CD19+ and/or CD22+ cells. CD4+ T-cells were defined as live CAR+, CD45+, CD3+, CD4+, CD8-, CD14-, CD19- cells. CD8+ T-cells were defined as live CAR+, CD45+, CD3+, CD4-, CD8+, CD14-, CD19- cells.

Table M5: CAR-FACS antibody panel

Antigen	Fluorochrome	Clone	Supplier	Part Number
CD3	FITC	UCHT1	BioLegend	300406
CD19	PE	SJ25C1	BioLegend	363004
CD14	PE-Cy7	63D3	BioLegend	367112
CD8	PerCP-Cy5.5	SK1	BD Pharmingen	565310
Recombinant Human Siglec-2/CD22β Fc Chimera	DyLight650		R&D Systems	1968-SL-050
CD56	APC-Fire750	5.1H11	BioLegend	362554
Live/Dead-Viability	BV450	--	Thermo Fisher	L-34964
CD22	BV421	HIB22	BioLegend	302524
CD20	BV605	2H7	BioLegend	302334

CD4	BV711	RPA-T4	BioLegend	300558
CD45	BV785	2D1	BioLegend	368528

qPCR Measurement of CAR+ Cells from Peripheral Blood

DNA was extracted from PBMCs (average yield: 2-5×10^6 cells) using QIAmp DNA Mini Kit (Qiagen # 51306) at baseline, and Days 7, 14, 21, 28, 90, and 180 post-CAR22 infusion. CD22.BB.z presence was measured by quantitative polymerase chain reaction (qPCR) using the primer and probe sequences provided **(Table M6)**. For the standard curve, a custom Minigene® plasmid (IDT) was designed containing a partial CD22.BB.z sequence and a partial albumin sequence, which served as a control for normalization. The standard curve contained a ten-fold serial dilution of plasmid between 5×10^5 and 5 copies/µL. Both plasmid and patient DNA from each time point were run in triplicate, with each reaction containing 5 µL of DNA (50 ng total), 100 nM forward and reverse albumin primers (or 200 nM forward and 200 nM reverse CD22.BB.z primers), 150 nM probe suspended in 10 µL of TaqMan Fast Universal PCR Master Mix (2×), No AmpErase® UNG or equivalent (Thermo Fisher Scientific) and 5 µL of TE buffer (Invitrogen # AM9935). The Bio-Rad CFX96 Touch Real-Time PCR Detection System was used for qPCR with 20 µL per reaction. The quality metrics for all qPCR standard curve results were R^2 > 0.99, -3.46 > slope > -3.69 and efficiency > 87%.

Table M6: qPCR reagents for CAR22

Reagent	**Sequence**
Albumin FAM Probe	5’ - CCT GTC ATG CCC ACA CAA ATC TCT CC - 3’
Forward Primer Albumin	5’ - GCT GTC ATC TCT TGT GGG CTG T - 3’
Reverse Primer Albumin	5’ - ACT CAT GGG AGC TGC TGG TTC - 3’
CD22 FAM Probe	5’ - /56-FAM/CT GGC GTC G/ZEN/T GGT TGC GGC /3IABkFQ/ - 3’
Forward Primer CD22	5’ - GGA CCA AGC TGG AAA TCA AAG C - 3’
Reverse Primer CD22	5’ - CGC CGG TGT TGG TGG T - 3’
CD22 Albumin Minigene® Plasmid	AGC TAC AGC ATC CCC CAG ACC TTC GGC CAG GGG ACC AAG CTG GAA ATC AAA GCG GCC GCA ACC ACG ACG CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG CAG CCC CTG TCG CTG GCC TTT TGC TCA CAA GCT TGG GGT TGC TGT CAT CTC TTG TGG GCT GTA ATC ATC GTC TAG GCT TAA GAG TAA TAT TGC AAA ACC TGT CAT GCC CAC ACA AAT CTC TCC CTG GCA TTG TTG TCT TTG CAG ATG TCA GTG AAA GAG AAC CAG CAG CTC CCA TGA GTC CCA AGC TAT GTT CTT TCC TGC GTT

Area Under the Curve (AUC) Calculation D_0-D_{28}

Missing values were handled using a complete case design, which required at least three datapoints between day 0 and day 28 (Hughes RA, Heron J, Sterne JAC, et al. *International Journal of Epidemiology*. 2019;48(4):1294-304) . This design was implemented for AUC calculations because reduction in AUC values was associated with missing datapoints.

Cytokine Measurement from Patient Serum

Serum was isolated from peripheral blood by spinning at 1200×g for 10 minutes at room temperature, and subsequently aliquoted and frozen for future batched analysis. Frozen serum samples were thawed, centrifuged at 14,000 rpm and diluted 3× in 1× PBS, prior to running a 50-plex Luminex bead kit (Affimetrix). A set of 4 AssayChex QC beads were added to each well, each with a unique fluorescence, to provide quality assurance as follows: Chex1 (addition of biotinylated detector antibodies), Chex2 (addition of streptavidin-PE), Chex3 (instrument performance), and Chex4 (non-specific background fluorescence). Additionally, total bead count (>2000) and individual bead counts (>40) are verified and wells below threshold are flagged for possible exclusion. Serum and serial dilutions of cytokine standards were added to respective wells, and plates were incubated for 2 hours with shaking at room temperature, followed by an 18 hour incubation at 4ºC. Plates were then washed and developed according to the manufacturer protocol, and samples were acquired on the Luminex MAP200.

Stanford Post-CAR Toxicity Management, Supportive Care, and Antimicrobial Prophylaxis

CAR T-cell-associated toxicities were managed in accordance with the CARTOX working group recommendations [PMID: 28925994]. Treatment with tocilizumab (8 mg/kg IV per dose) and/or corticosteroids (dexamethasone 10 mg IV per dose or equivalent) was utilized for any patient who developed grade ≥2 CRS and/or neurotoxicity (ICANS), respectively.

Post-hospital discharge monitoring included visits at least weekly in the outpatient infusion center through day 28, followed by outpatient clinic visits and lab monitoring on post-infusion days 28, 60, 90, 180, 270, and 365. Granulocyte colony-stimulating factor (G-CSF) 5 µg/kg per day subcutaneously was administered after lymphodepletion until the absolute neutrophil count (ANC) was >1000 cells per µL, and repeated once daily for three days anytime the ANC was <1000 cells per µL. Initial institutional antimicrobial prophylaxis recommendations were modified from the post-autologous stem cell transplant setting based on best available data. All patients regardless of serologic status received acyclovir 800 mg twice a day starting with LD chemotherapy until at least 18 months after CAR-T infusion for herpes simplex (HSV) and varicella zoster virus (VZV) infection prophylaxis. Beginning on day 28 post-infusion, all patients without ongoing cytopenias received trimethoprim 80 mg/sulfamethoxazole 400 mg once daily (or atovaquone 1500 mg once daily if ongoing cytopenias were present) until at least 12 months after CAR-T infusion, or until recovering an absolute CD4+ T-cell count >200 cells per µL for *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis. Serum immunoglobulin G (IgG) levels were evaluated following CAR-T infusion, and IVIG 0.5 g/kg was recommended if recurrent or severe sinopulmonary infections developed with a serum IgG concentration <400 mg/dL.

Supplementary Table 1 | Deviation from the Protocol

Deviation	Rationale
De-escalation from DL2 to DL1	The statistical plan called for a 3+3 design for dose escalation. This approach was followed initially when moving from DL1 to DL2 when none of the first 3 patients at DL1 experience DLTs. Three patients were initially treated at DL2, and after no DLTs were observed 6 additional patients were treated in an expansion cohort. However, after these 9 patients were treated at DL2, the investigators found that this dose (DL2) was excessively toxic. The investigators observed 3 of the 9 patients required treatment for IEC-HS; 2 subjects developed DLTs, and patients had a higher rate of higher-grade CRS compared to DL1. Moreover, the 9th patient at the DL2 cohort experience a treatment-related death as a result of IEC-HS and multi-organ failure due to sepsis and DIC. Due to concerns about clinical safety at DL2, after review with the institutional safety board, the principal investigator decided to evaluate additional patients at DL1.

Supplementary Table 2 | Prior Therapy and Duration of Response, by subject

Subject ID	Line#	Regimen Name	Duration (m)
CCT5029-002	1	DA R-EPOCH	0.7
	1	R-CHOP	5.1
	2	R-DHAP	2.4
	3	axicabtagene ciloleucel	3.0
	4	R-lenalidomide	0.8
	5	R-GemOx	1.2
	6	CD22-CAR T	
CCT5029-004	1	R-CHOP	1.9
	2	R-ICE	1.0
	3	R-GemOx	2.4
	4	JCAR017 (CD19 CAR T)	3.3
	5	CC90002 (anti-CD47)/Rituxamab	0.0
	6	R-DHAOx/venetoclax	0.6
	7	lenalidomide	0.9
	8	R-bendamustine/polatuzumab	0.4
	9	CD22-CAR T	
CCT5029-006	1	CHOP + radiation	239.2
	2	R-GDP	0.0
	3	axicabtagene ciloleucel	3.1
	4	R-lenalidomide	0.8
	5	CD19-CD20 Bispecific CAR T	12.2
	6	R-GemOx	1.5
	7	CD22-CAR T	
CCT5029-007	1	R-CHOP	0.0
	2	R-GemOx	0.6
	3	axicabtagene ciloleucel	5.8
	4	lenalidomide	0.0
	5	CD22-CAR T	
CCT5029-009	1	R-CHOP	62.6
	2	R-ICE	0.0
	3	R-GDP	2.8
	4	Autologous stem cell transplant, BCNU/VP16/Cy	2.8
	5	axicabtagene ciloleucel	3.4
	6	CD22-CAR T	
CCT5029-010	1	R-CHOP	0.9
	2	R-lenalidomide	0.9
	3	R-GemOx	0.5

Subject ID	Line#	Regimen Name	Duration (m)
	4	R-ICE	1.3
	5	axicabtagene ciloleucel	2.7
	6	CD22-CAR T	
CCT5029-013	1	R-CHOP	2.9
	2	R-GDP	2.4
	3	Autologous stem cell transplant, BEAM	62.6
	4	R-GDP	0.1
	5	DHAP	0.8
	6	axicabtagene ciloleucel	1.0
	7	radiation	0.1
	8	CD22-CAR T	
CCT5029-015	1	R-CHOP	13.2
	2	R-GemOx	0.0
	3	R-ICE	2.5
	4	axicabtagene ciloleucel	1.1
	5	CD22-CAR T	
CCT5029-016	1	R-CHOP x 1, DA R-EPOCH x5	6.6
	2	R-GemOx	0.6
	3	axicabtagene ciloleucel	2.8
	4	R-DHAOx	0.7
	5	*withdrew from clinical trial*	
CCT5029-017	1	R-CHOP	11.7
	2	R-ICE	2.2
	3	Autologous stem cell transplant, BEAM	19.5
	4	axicabtagene ciloleucel	3.1
	5	CD22-CAR T	
CCT5029-018	1	R-bendamustine (for follicular lymphoma)	1.7
	2	R-CHOP	2.3
	3	R-ICE	2.3
	4	axicabtagene ciloleucel	1.0
	5	CD22-CAR T	
CCT5029-019	1	R-CHOP	0.5
	2	R-GDP	0.8
	3	axicabtagene ciloleucel	6.2
	4	radiation	0.8
	5	CD22-CAR T	

Subject ID	Line#	Regimen Name	Duration (m)
CCT5029-020	1	R-CHOP	23.6
	2	R-GDP	2.3
	3	Autologous stem cell transplant, BCNU/VP16/Cy	8.0
	4	axicabtagene ciloleucel	5.5
	5	CD22-CAR T	
CCT5029-021	1	R-CHOP	35.8
	2	R-GemOx	0.1
	3	radiation	3.7
	4	axicabtagene ciloleucel	28.2
	5	CD22-CAR T	
CCT5029-022	1	R-CHOP	1.4
	2	R-ICE	0.8
	3	axicabtagene ciloleucel	0.9
	4	radiation	2.3
	5	R-DHAP (bridging)	1.8
	6	CD22-CAR T	
CCT5029-023	1	R-CHOP	2.1
	2	VIPOR (NIH Clinical Trial)	0.2
	3	DA R-EPOCH + venetoclax	0.5
	4	axicabtagene ciloleucel	2.6
	5	R-bendamustine/polatuzumab (bridging)	1.4
	6	CD22-CAR T	
CCT5029-025	1	R-CHOP	23.4
	2	R-ICE	3.0
	3	Autologous stem cell transplant, BEAM	5.1
	4	Ibrutinib	0.0
	5	axicabtagene ciloleucel	3.0
	6	lenalidomide+ Ibrutinib	0.0
	7	CD22-CAR T	
CCT5029-026	1	MBACOD	[346-358]
	2	R-CEOP	0.0
	3	R-GemOx	1.5
	4	axicabtagene ciloleucel	2.8
	5	CD22-CAR T	
CCT5029-028	1	R-CHOP	0.9

Subject ID	Line#	Regimen Name	Duration (m)
[CCT5029-010]	2	R-lenalidomide	0.9
	3	R-GemOx	0.5
	4	R-ICE	0.3
	5	axicabtagene ciloleucel	2.7
	6	R-GemOx	0.9
	7	CD22-CAR T	2.6
	8	R-bendamustine/polatuzumab	2.9
re-enrolled	9	R-bendamustine/polatuzumab (bridging)	2.0
	10	CD22-CAR T	
CCT5029-029	1	R-EPOCH X 2, then 2 w/o anthracycline, followed by consolildative XRT to R arm	23.9
	2	radiation	11.1
	3	R-ICE	3.2
	4	Autologous stem cell transplant, BEAM	5.7
	5	CD19-CD22 Bispecific CAR T	5.6
	6	radiation	8.6
	7	R-GDP (bridging)	14.9
	8	CD22-CAR T	
CCT5029-030	1	DA R-EPCH (vincristine omitted)	13.2
	1	HD MTX	17.2
	2	R-ICE	2.6
	3	Autologous stem cell transplant, BCNU/VP16/Cy	15.5
	4	CD22-CAR T	3.0
CCT5029-031	1	Mosunetuzumab +CHOP; IT Cytabime and MTX	7.0
	2	radiation	0.0
	3	R-ICE	0.6
	4	R-GemOx (used as bridging)	0.0
	5	Tisagenlecleucel	2.8
	6	CD22-CAR T	
CCT5029-032	1	R-CHOP	3.7
	2	DA R-EPOCH	1.5
	3	JCAR017 (CD19 CAR T)	6.0
	4	CD22-CAR T	
CCT5029-033	1	R-CHOD x 6 with HD MTX x 6	9.4
	2	R-DHAP	1.6

Subject ID	Line#	Regimen Name	Duration (m)
	3	axicabtagene ciloleucel	5.2
	4	Tafasitamab monotherapy	0.5
	5	CD22-CAR T	
CCT5029-034	1	DA R-EPOCH + IT MTX	2.3
	2	R-ICE	0.7
	3	tisagenlecleucel	4.4
	4	CD22-CAR T	0.9
CCT5029-035	1	R-CHOP	30.3
	2	R-GemOx	7.5
	3	axicabtagene ciloleucel	5.3
	4	CD22-CAR T	
CCT5029-036	1	R-CHOP + IT MTX x 4 cycles	15.4
	2	radiation	12.6
	3	R-lenalidomide	0.1
	4	axicabtagene ciloleucel	3.7
	5	R-bendamustine/polatuzumab	2.8
	6	CD22-CAR T	
CCT5029-037	1	R-CHOP x 1, DA R-EPOCH x5	0.1
	2	R-GemOx	0.5
	3	radiation (bridging)	0.4
	4	axicabtagene ciloleucel	2.9
	5	CD22-CAR T	
CCT5029-038	1	R-CHOP	1.8
	2	radiation	9.3
	3	R-GDP	0.5
	4	radiation	3.1
	5	axicabtagene ciloleucel	3.0
	6	CD22-CAR T	
CCT5029-039	1	DA-R-EPOCH	0.5
	2	HiDAC alternating with R-ICE	0.6
	2	HD MTX	0.0
	3	R-lenalidomide	2.0
	4	tisagenlecleucel	37.7
	5	tisagenlecleucel	1.0
	6	CD22-CAR T	
CCT5029-041	1	R-mini-CHOP	6.2
	2	HD MTX	5.2
	3	R-GemOx	0.6

Subject ID	Line#	Regimen Name	Duration (m)
	4	radiation	0.2
	5	axicabtagene ciloleucel	2.4
	6	R-polatuzumab	0.6
	7	CD22-CAR T	
CCT5029-042	1	R-EPOCH + IT MTX x 6	5.1
	2	R-GDP x 2 cycle	
	3	20 Gy radiation (5 days)	
	4	20 Gy radiation (5 days)	
	5	axi-cel (yescarta)	5.9
	6	CD22-CAR T	
CCT5029-043	1	R-CHOP x 6	10.9
	2	Cytoxan 1000 mg/m2 x 1 due to AKI and hypercalcemia	
	3	R-GemOx x 3	.4
	4	Miltenyi CD19-CD20 CAR T	
	5	CD22-CAR T	
CCT5029-044	1	R-CHOP	1.7
	2	R-ICE	
	3	R-DHAP	
	4	Yescarta	
	5	revlimid/Ibrutinib	
	6	R-CHOP	
	7	CD22-CAR T	
CCT5029-046	1	ABVD	9.6
	2	R-CEOP	.1
	3	RGDP	.4
	4	Axi-cel	2.1
	5	CD22-CAR T	
CCT5029-047	1	DA-EPOCH-R x 4 + IT MTX	
	2	R-DHAX x 2	
	3	CAR T (1XX/CD19CAR)	3.5
	4	REGN 3767 1600mg, cemiplimab 350 mg, splene	
	5	splenectomy	
	6	ISRT 4500 cGY to L para-aortic	1.3
	7	ISRT to LLL lesion 3600 cgy	
	8	BV + Lenalidomide	.1
	9	CD22-CAR T	
CCT5029-048	1	DA-EPOCH-R	1.4

Subject ID	Line#	Regimen Name	Duration (m)
	2	R-ICE	
	4	Gem-Ox (bridging)	
	5	axi-cel	2.6
	6	Lenalidomide started empirically, Tafasitamab added	1.1
	3	15 fractions of radiation at UCSF	
	7	CD22-CAR T	
CCT5029-050	1	RCHOP	3.3
	2	R-DHAOx	.4
	3	Lisocabtagene Maraleucel	1
	4	Tafasitamab+Lenalidomide	2.5
	5	CD22-CAR T	
CCT5029-052	1	R-CHOP	2.5
	2	Revlimid-Rituxan	.2
	3	Yescarta	3
	4	CD22-CAR T	
CCT5029-053	1	R-CHOP	3.7
	1	IT MTX ppx	
	1	30 Gy RT to the left axilla	4.7
	2	Yescarta	3
	3	CD22-CAR T	

UN = day unknown; Unk = month unknown

A: Duration in months calculated as days / 30
B: Censored at date of new treatment
C: Censored at date deceased

Supplementary Table 3 | Infectious Adverse Events for All Patients on Trial

Infection, n (%)	Any Grade	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Dose Level
Any	16 (42)	3 (8)	11 (29)	1 (3)	0	1 (3)	1
Upper respiratory tract infection	3 (8)	0	3 (8)	0	0	0	1
Vaginal infection, yeast	2 (5)	0	2 (5)	0	0	0	1
Cytomegalovirus reactivation	2 (5)	2 (5)	0	0	0	0	1,2
Urinary tract infection	2 (5)	1 (3)	1 (3)	0	0	0	1
Pneumonia	2 (5)	0	2 (5)	0	0	0	2
Eye infection, Herpes Zoster	1 (3)	0	1 (3)	0	0	0	1
E. coli osteomyelitis	1 (3)	0	0	1 (3)	0	0	2
Lip infection, fungal	1 (3)	0	1 (3)	0	0	0	2
Skin infection	1 (3)	0	1 (3)	0	0	0	2
Bacteremia, *Klebsiella Pneumoniae*	1 (3)	0	0	0	0	1 (3)	2

* Numbers shown represent distinct infectious events.

Supplementary Table 4 | Toxicity Management

Cytokine Release Syndrome	
Grade 1	• Supportive Care
Grade 2	• Administer Tocilizumab 8 mg/kg intravenously once • Administer Dexamethasone 10mg intravenously once
Grade 3	• Transfer to Intensive Care Unit • Administer tocilizumab 8 mg/kg intravenously once • Administer dexamethasone 10 mg intravenously every 6 hours • Consider anakinra 100 mg subcutaneously every 6 hours until event is grade 1
Grade 4	• Transfer to Intensive Care Unit • Administer tocilizumab 8 mg/kg intravenously once • Administer methylprednisolone 1 gram intravenously daily for 3 days • Administer anakinra 100 mg subcutaneously every 6 hours until event is grade 1

Neurotoxicity	
Grade 1	• Supportive Care • Continue levetiracetam 500 mg twice daily prophylactically
Grade 2	• Consult Neurology • CT head if none post-CAR-T • Consider EEG • Consider increasing levetiracetam to 1000 mg twice daily • Administer tocilizumab 8 mg/kg intravenously once if concurrent CRS. Follow CRS pathway. • Administer dexamethasone 10 mg intravenously every 6 hours
Grade 3	• Consider transfer to Intensive Care Unit • Consult with Neurology and/or Neurocritical Care • Repeat neuroimaging (CT vs MRI) if neurologic worsening or focal neurologic signs • Consider electroencephalogram • Order neurological assessments every 2 hours; decrease frequency if stable • Increase levetiracetam to 1000 mg twice daily; consider lacosamide 100 mg twice daily • Administer tocilizumab 8 mg/kg intravenously once if concurrent CRS. Follow CRS pathway • Administer methylprednisolone 1 gram intravenously daily until improvement • Administer anakinra 100 mg subcutaneously every 6 hours until event is grade 1
Grade 4	• Transfer to Intensive Care Unit • Consult with Neurology and Neurocritical Care • Repeat neuroimaging (CT vs MRI) once clinically stable • Obtain continuous electroencephalogram • Order neurological assessments every hour; decrease frequency if stable • Continue levetiracetam 1000 mg twice daily; consider lacosamide 100 mg twice daily • Administer tocilizumab 8 mg/kg intravenously once if concurrent CRS. Follow CRS pathway. • Administer methylprednisolone 1 gram intravenously daily until improvement • Administer Anakinra 100 mg subcutaneously every 6 hours until event is grade 1

Supplementary Table 5 | Deaths on Trial

PATIENT ID	DOSE LEVEL	CAUSE OF DEATH	STUDY DAY	RELATED TO STUDY TREATMENT
007	DL2	Progression of disease	Day +62	No
009	DL2	Progression of disease	Day +209	No
010	DL2	Progression of disease	Day +422	No
013	DL2	Progression of disease	Day +48	No
018	DL2	tMDS/AML	Day +676	Possibly
019	DL2	tMDS/AML	Day +862	Possibly
020	DL2	Multi-Organ Failure secondary to Klebsiella pneumoniae septicemia with Disseminated Intravascular Coagulation (DIC).	Day +41	Possibly
023	DL1	Progression of disease	Day +117	No
025	DL1	Progression of disease	Day +175	No
026	DL1	Unknown, lost to follow up 6 months post-infusion	Day +288	No
032	DL1	Progression of disease	Day +165	No
035	DL1	Progression of disease	Day +398	No
036	DL1	Progression of disease	Day +273	No
037	DL1	Progression of disease	Day +34	No
041	DL1	Heart failure	Day +280	No
042	DL1	Progression of disease	Day +251	No
043	DL1	Progression of disease	Day +88	No
046	DL1	Progression of disease	Day +152	No

Supplementary Table 6 | CAR+ T Cell Absolute Numbers by Dose Level

CAR T-Cell Levels (cells/µL)	Dose Level 1	Dose Level 2
Treatment day 7, median (Q1, Q3)	0.32 (0.06, 1.13)	1.24 (0.40, 6.07)
Treatment day 10, median (Q1, Q3)	5.01 (2.19, 20.95)	157.25 (52.51, 621.42)
2 weeks post-treatment, median (Q1, Q3)	56.70 (2.62, 122.18)	359.76 (248.62, 795.12)
3 weeks post-treatment, median (Q1, Q3)	17.24 (2.36, 66.98)	201.9057 (79.19, 295.89)
4 weeks post-treatment, median (Q1, Q3)	8.84 (3.00, 16.78)	82.03 (31.77, 220.46)
2 months post-treatment, median (Q1, Q3)	2.02 (0.19, 9.66)	3.69 (1.09, 6.35)
3 months post-treatment, median (Q1, Q3)	2.69 (0.37, 3.98)	1.34 (0.76, 1.77)
6 months post-treatment, median (Q1, Q3)	0.39 (0.0, 9.00)	0.61 (0.48, 2.89)
9 months post-treatment, median (Q1, Q3)	0.20 (0.07, 1.27)	0.06 (0.03, 2.65)
12 months post-treatment, median (Q1, Q3)	0.32 (0.21, 0.90)	0.35 (0.23, 0.56)
18 months post-treatment, median (Q1, Q3)	3.82 (2.90, 4.74)	6.38 (6.38, 6.38)
24 months post-treatment, median (Q1, Q3)		2.16 (2.16, 2.16)
Peak, median (Q1,Q3)	70.73 (9.59, 218.13)	359.76 (289.92, 1310)
AUC0-28, cells/µl×days, median (Q1,Q3)	912.09 (68.23, 1300)	6929.83 (2993.11, 11690)

* Measured in circulating peripheral blood mononuclear cells (PBMC) sorted by multiparameter flow cytometry. Absolute numbers were calculated as (% of gated CAR+, CD45+, CD3+, CD4 or CD8+, CD14-, CD19- lymphocytes) x (ALC measured from CBC).

** AUC was calculated using the trapezoidal method.

Supplementary Table 7| Response, Toxicity and Non-Relapse Mortality Stratified by Peak CAR T Expansion

	ORR	CR	Grade ≥ 2 CRS	Treated for IEC-HS	NRM	DL1	DL2
Overall (N=38), %	68%	53%	58%	13%	13%		
CAR T peak							
Evaluable N	38	38	38	38	38	29	9
Min-Q1, % (0.001-11.2) n=10	30%	20%	30%	0%	0%	31%	11%
Q1-Median, % (11.2-97.8) n=9	67%	67%	67%	0%	11%	31%	0%
Median - Q3, % (97.8-331) n=9	78%	44%	56%	11%	0%	21%	33%
Q3-Max, % (331-6673) n=10	100%	80%	80%	40%	40%	17%	56%

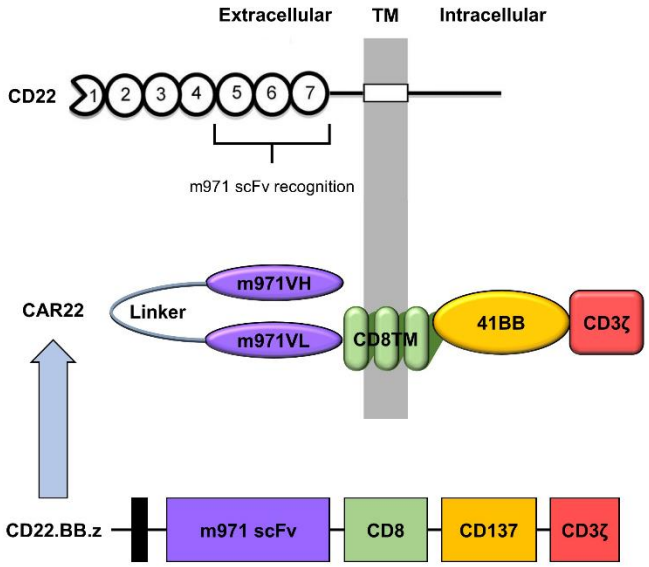
* Peak CAR+ cells/ul blood. Interquartile ranges based upon absolute CAR+ T cell numbers calculated from peripheral blood flow cytometry as shown in Supplementary Table 3 for all treated patients.

ORR=objective response rate; CR=complete response; CRS=cytokine release syndrome; IEC-HS=immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome; NRM=non-relapse mortality; DL1=dose level 1; DL2=dose level 2.

Supplementary Figure 1 | CAR22 Construct and Trial Schema

(A) The CD22.BB.z-CAR transcript contains a humanized CD22 scFv (m971), CD8α hinge and transmembrane domains, a 4-1BB costimulatory domain and a CD3ζ domain. (B) CAR T manufacturing and clinical trial schema, showing screening, lymphodepletion, CAR T cell infusion and post-infusion disease evaluation and DLT monitoring time points.

A



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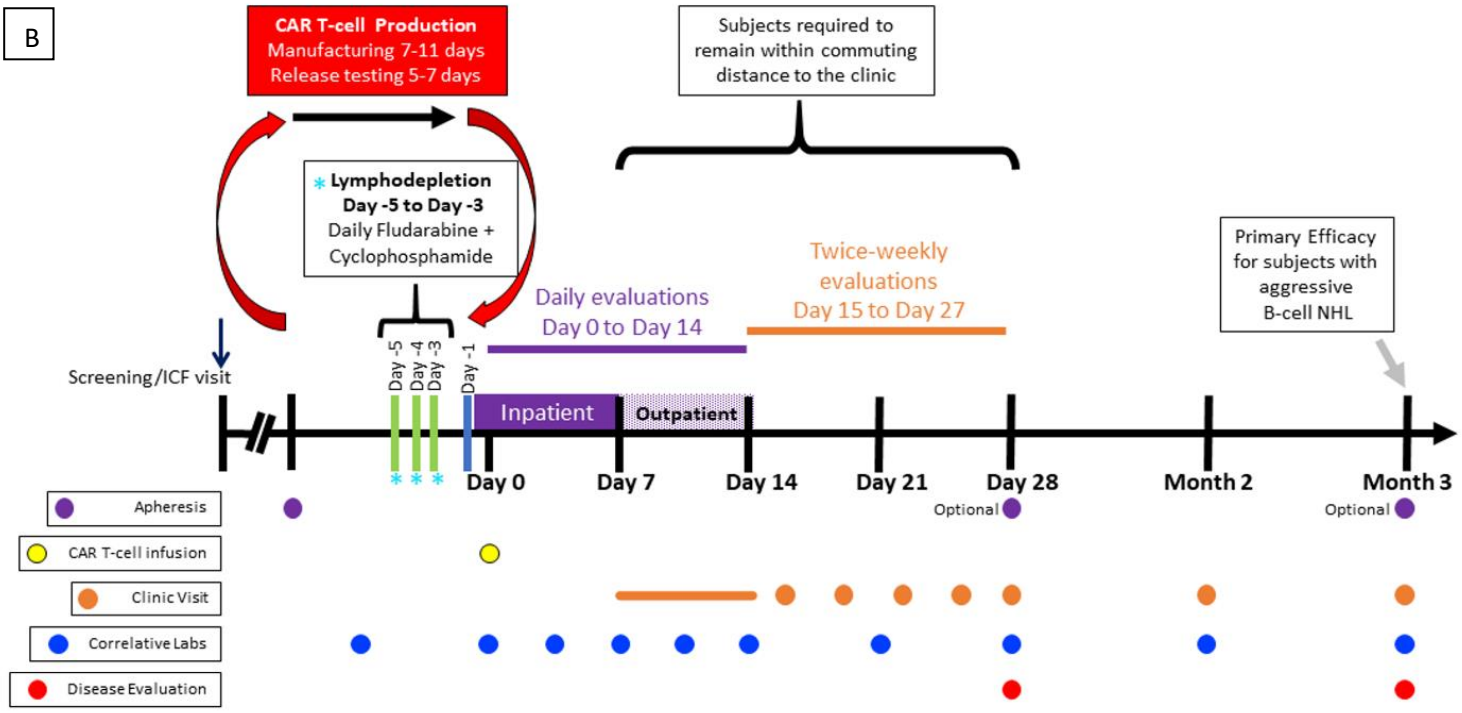
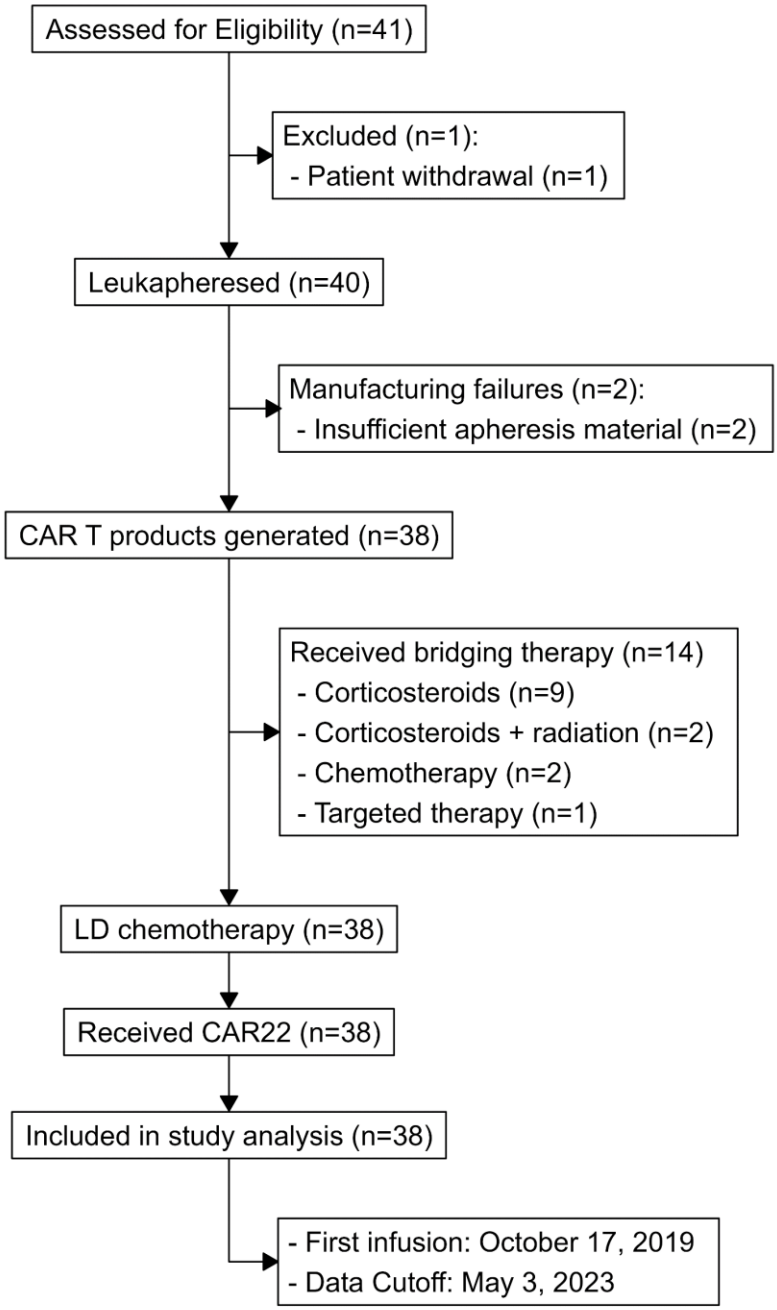


Supplementary Figure 2 | CONSORT Diagram

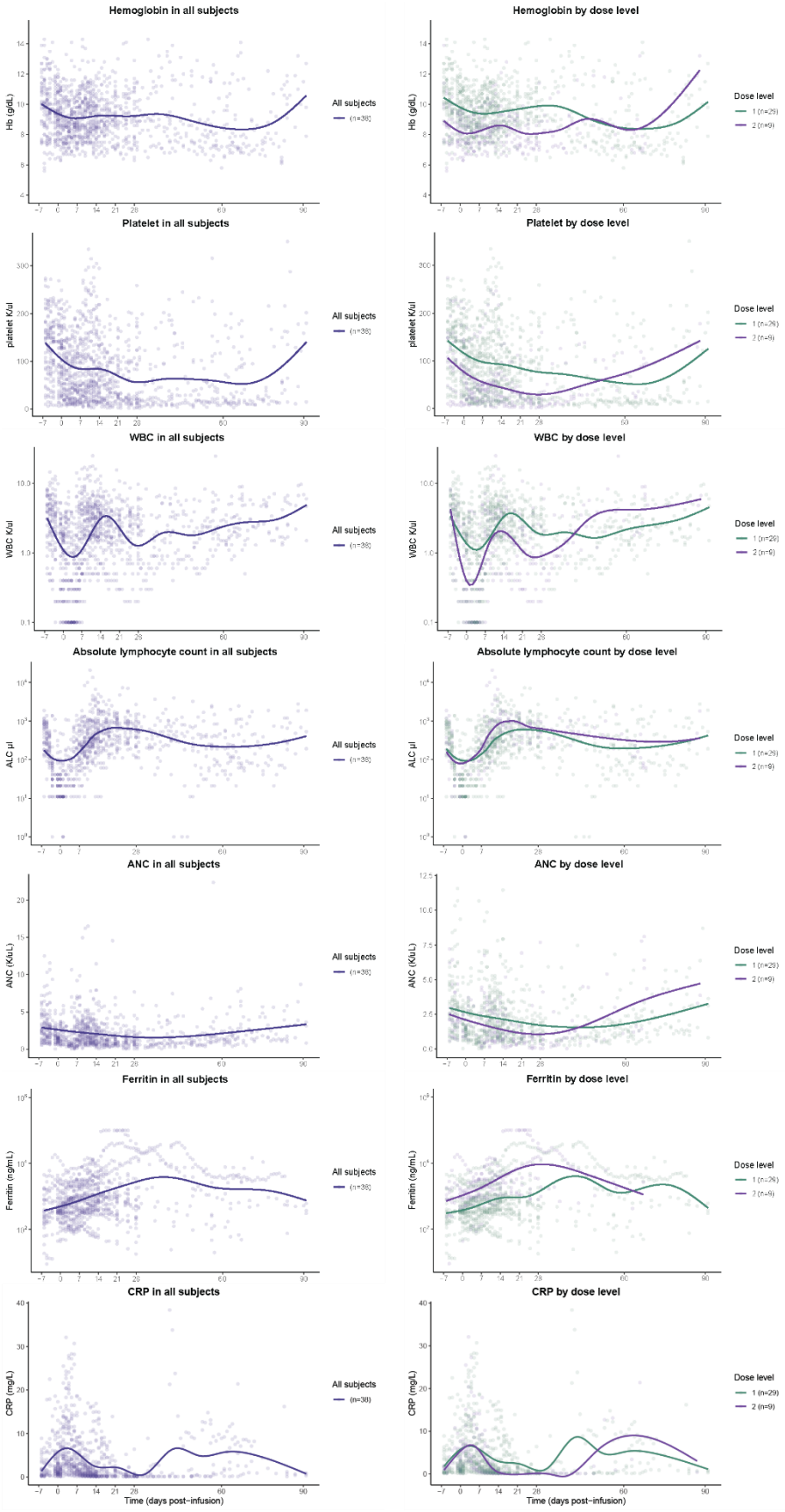
Consort diagram showing enrollment, treatment, and follow-up of patients on the CAR22 clinical trial.



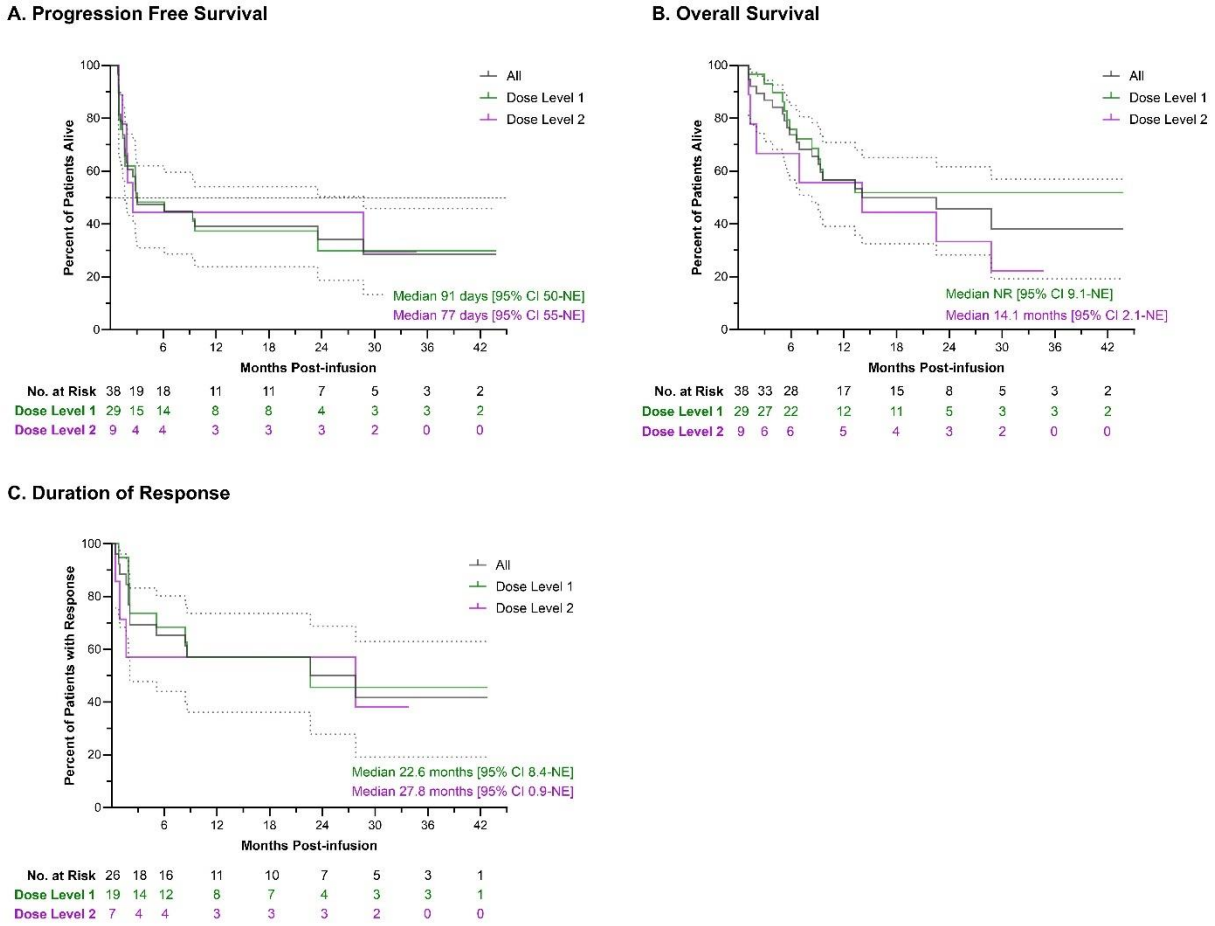
Supplementary Figure 3 | Changes in Blood Cell Counts Following CAR T Cell Infusion

Serial complete blood count measurements following CAR22 infusion. The y-axis represents the absolute blood cell count, while the x-axis indicates the time points post-infusion when the blood was drawn. To enhance the visualization of the data, a generalized additive model (GAM) for smoothing and predicting the data was employed. The GAM model allows for the representation of non-linear relationships.

Due to the nature of the study and selection bias, patients with relatively low blood cell counts were selected for multiple blood draws at shorter intervals; and those patients with disease control were selected for at long-term follow up timepoints, leading to a bias in the data. However, the graphs allow the identification of underlying trends and patterns and provide valuable information on the longitudinal changes in blood cell counts following CAR22 therapy in a heavily pre-treated patient population, furthering our understanding of the treatment's impact on hematologic toxicity.



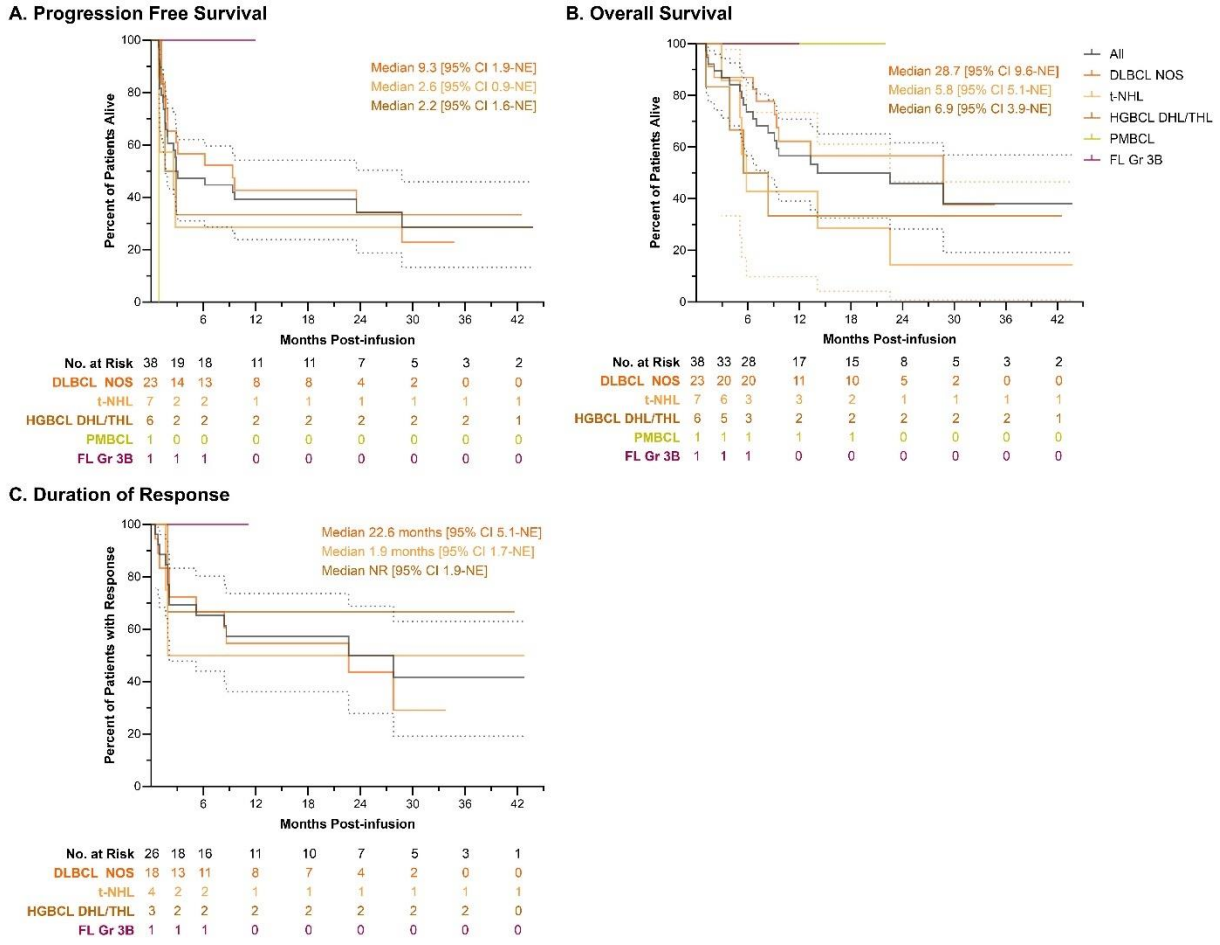
Supplementary Figure 4 | Progression-free Survival, Overall Survival, and Duration of Response Subdivided by CAR22 Dose Level



(A) Kaplan-Meier estimate of progression-free survival for patients treated at DL1 and DL2. (B) Kaplan-Meier estimate of overall survival for patients treated at DL1 and DL2. (C) Kaplan-Meier estimate of duration of response for patients treated at DL1 and DL2.

DL1= Dose Level 1; DL2= Dose Level 2; NE= not evaluable.

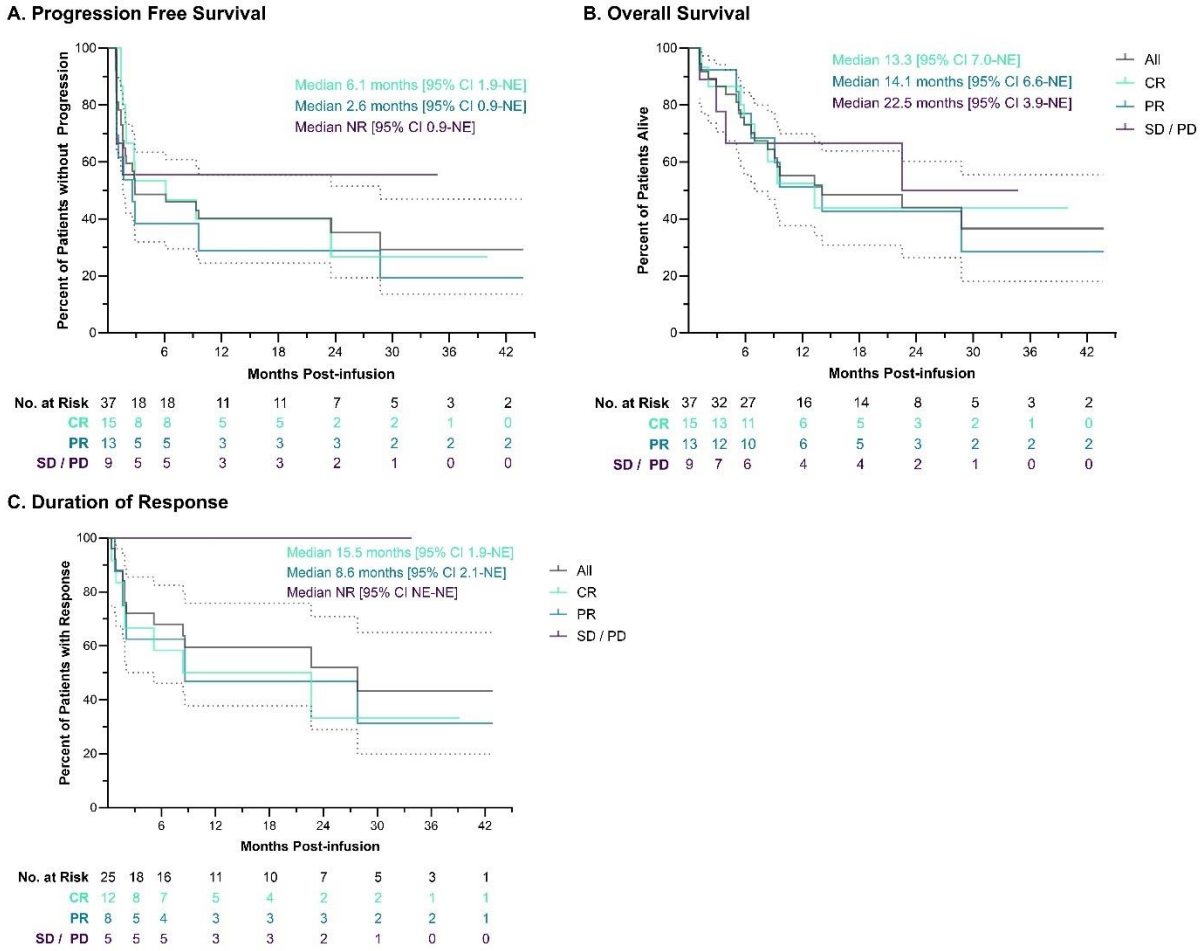
Supplementary Figure 5 | Progression-free Survival, Overall Survival, and Duration of Response Subdivided by Disease Histology



(A) Kaplan-Meier estimate of progression-free survival for patients subdivided by disease histology. (B) Kaplan-Meier estimate of overall survival for patients subdivided by disease histology. (C) Kaplan-Meier estimate of duration of response for patients subdivided by disease histology.

DLBCL NOS=diffuse large B-cell lymphoma, not otherwise specified; t-NHL=large cell transformation from indolent non-Hodgkin lymphoma; HGBCL DHL/THL= High-grade B-cell lymphoma, including rearrangement of MYC with BCL2 or BCL6 or both (a.k.a. Double- or triple-hit lymphoma); PMBCL=primary mediastinal B-cell lymphoma; FL Gr 3B=follicular lymphoma, grade 3B; NE= not evaluable.

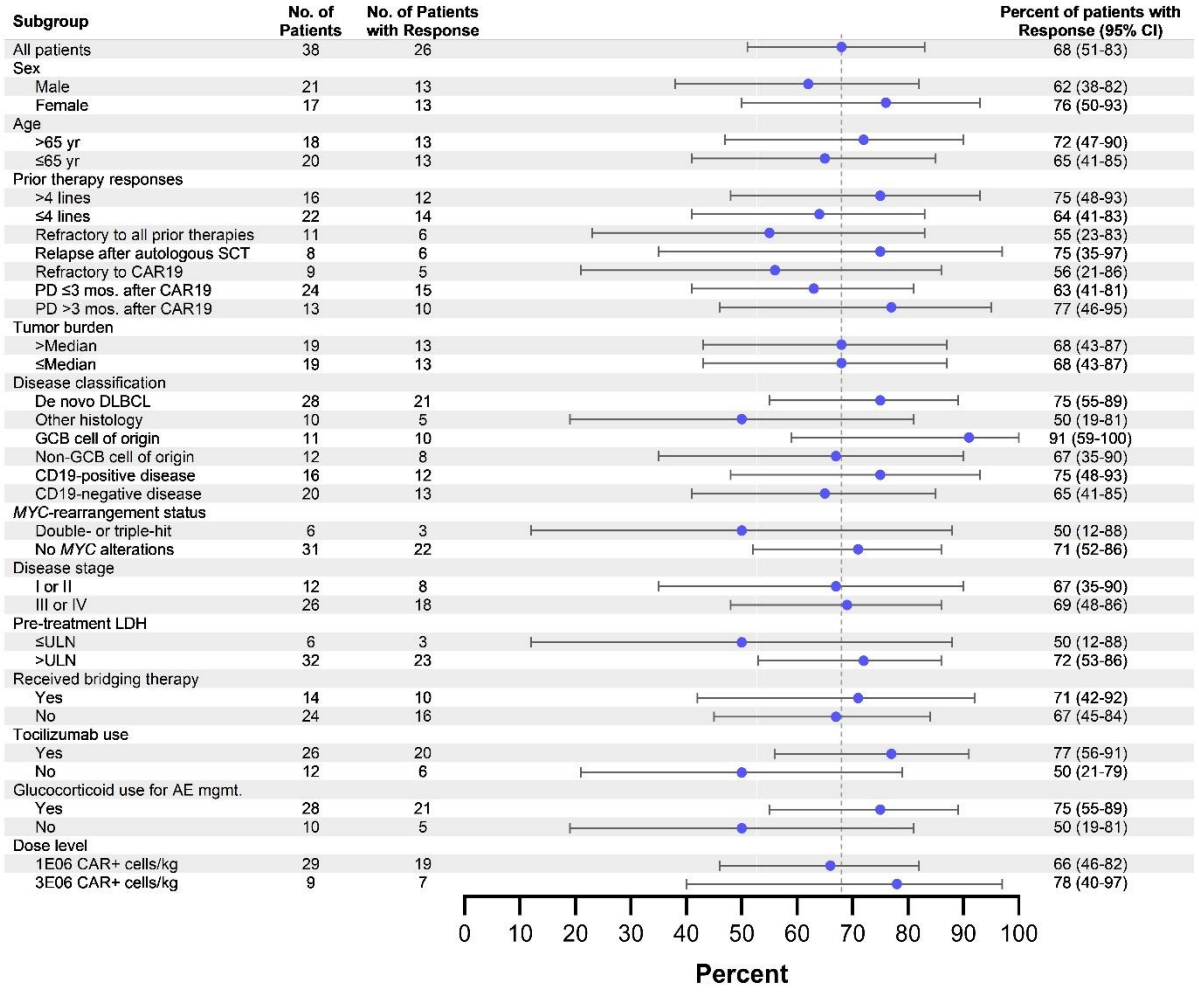
Supplementary Figure 6 | Progression-free Survival, Overall Survival, and Duration of Response Subdivided by Best Response to Prior CAR19 Therapy



(A) Kaplan-Meier estimate of progression-free survival for patients subdivided by best response achieved after CAR19 therapy. (B) Kaplan-Meier estimate of overall survival for patients subdivided by best response achieved after CAR19 therapy. (C) Kaplan-Meier estimate of duration of response for patients subdivided by best response achieved after CAR19 therapy.

CAR19=CD19-directed CAR T cell therapy; NE= not evaluable.

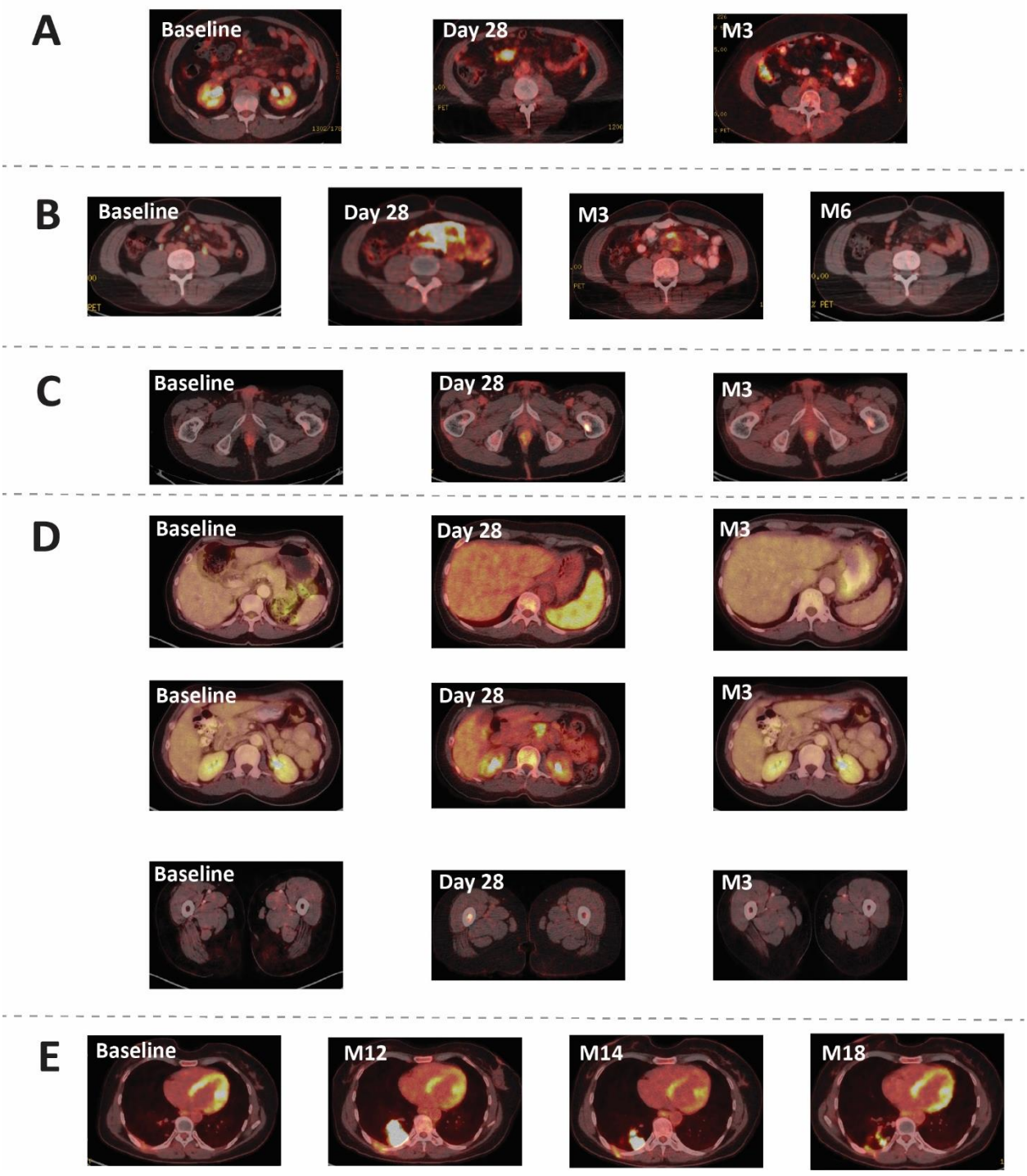
Supplementary Figure 7 | Subgroup Analysis of Overall Response



Shown is the analysis of objective response according to key baseline and clinical covariates. The Clopper–Pearson method was used to calculate the 95% confidence interval and are not adjusted for multiplicity. Tumor burden was assessed as the sum of the product diameters (SPD). CD19-negative disease was defined as an H-score <150 (corresponding to ≤50% staining by IHC), and/or undetectable surface CD19 by flow cytometry.

SCT=Stem Cell Transplantation; CAR19=CD19-directed chimeric antigen receptor T cell therapy; PD=progressive disease; DLBCL=Diffuse large B-cell lymphoma; GCB= Germinal Center B-cell-like; LDH=Lactate dehydrogenase; ULN=Upper limit of normal range; AE mgmt.=Adverse event management

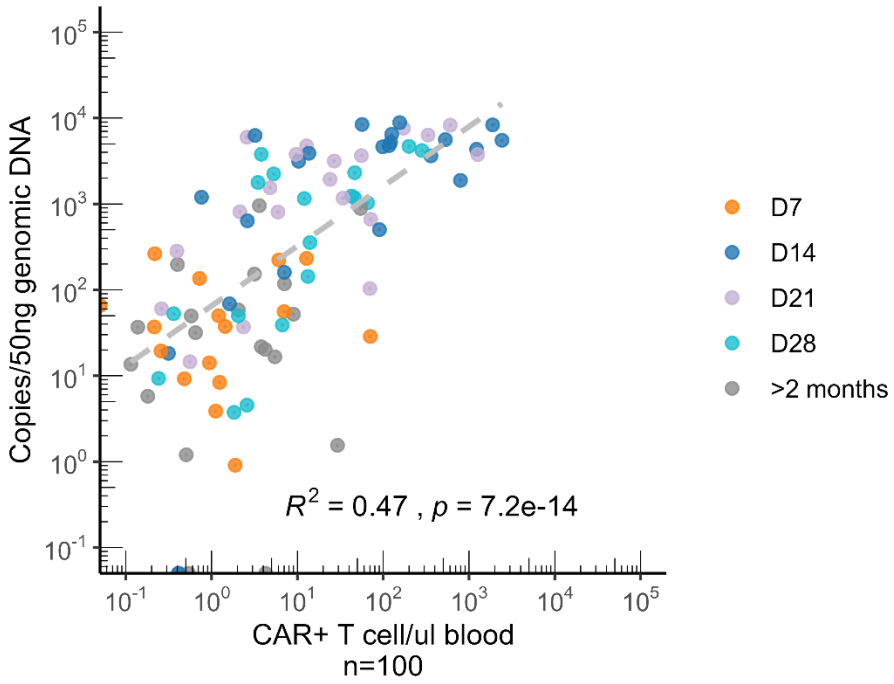
Supplementary Figure 8 | Serial PET-CT Imaging Before and After CAR22 Therapy Demonstrating Pseudoprogression



Five out of the twenty patients who achieved a CR as best response developed new hypermetabolic lesions post-infusion, concerning for radiographic progression of disease. In four patients, these new lesion occurred on the day 28 PET-CT scan, and for one subject this occurred on the 1 year PET-CT scan. Each of these lesions ultimately resolved without intervention. For the 4 subjects whose new lesions developed on the day 28 PET-CT scan, all previous index lesions responded, and no infections were present contemporaneously. Details regarding the

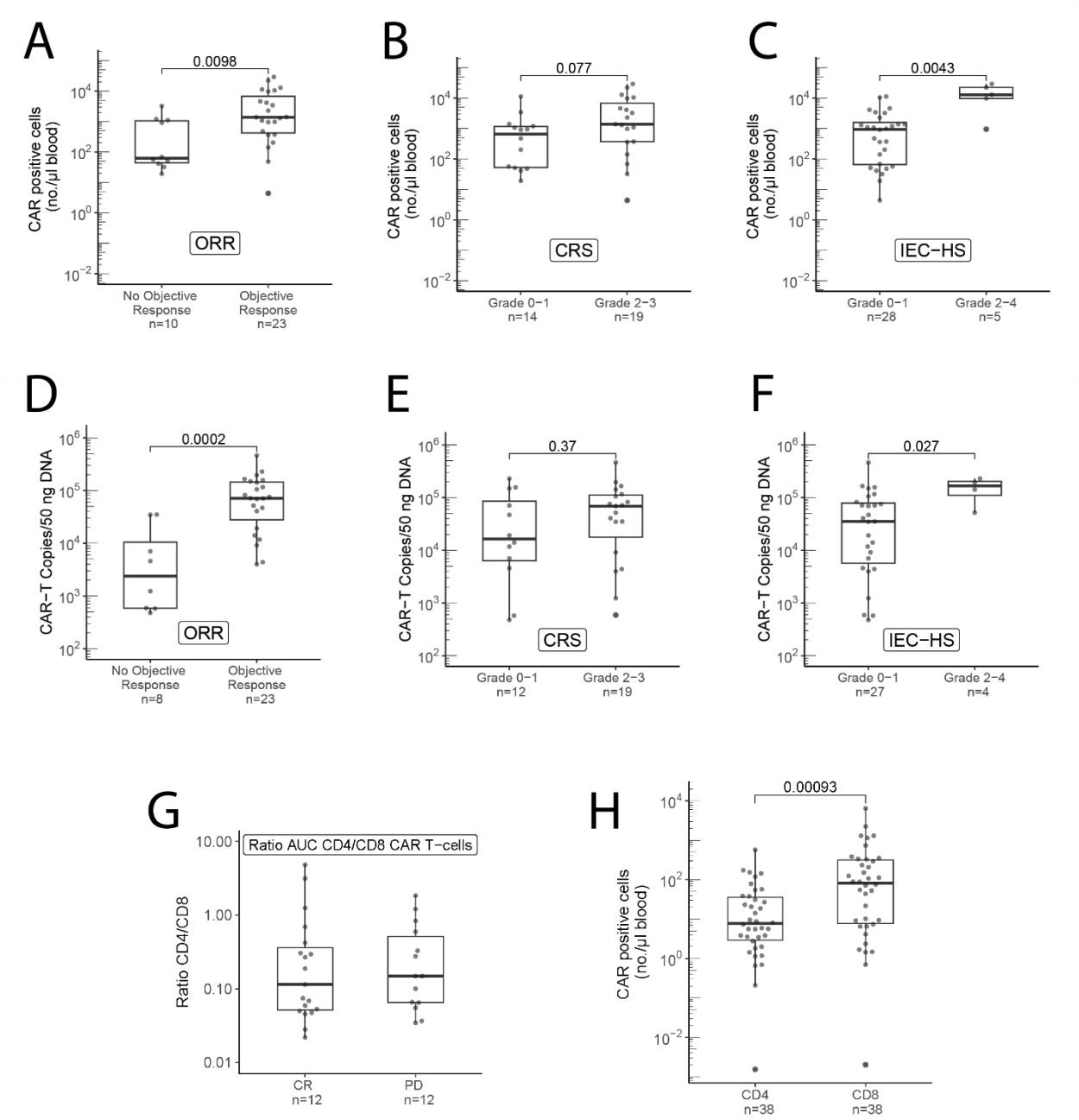
individual patients are as follows **(A)** A new 3.1 x 2.3 cm right mesenteric lesion with a Standard Uptake Value maximum (SUV max) of 6.5 was observed on the day 28 PET-CT scan. This site resolved on the subsequent month 3 PET-CT scan to a 3.1 x 2.3 cm, SUV 1.9 lesion (below liver FDG uptake, Deauville 3). **(B)** PET-CT scan at time of enrollment demonstrated a 1.6 x 1.6 cm, SUV max 5.7 mesenteric lesion, which was subsequently measured at 11.2 x 6.1 cm, SUV max 15.9 on the day 28 PET-CT scan. The patient specifically denied abdominal pain and B-symptoms. On the month 3 PET-CT scan, this mesenteric site measured 9.8 x 4.5, SUV max 10.4, and on the month 6 PET-CT scan, this site had resolved (below mediastinal blood pool uptake, Deauville 2). **(C)** PET-CT scan at day 28 demonstrated new focal hypermetabolic update in the left femoral neck, SUV max 6.7 that resolved on the subsequent month 3 scan (Deauville 2). **(D)** This subject developed a new 2.4 x 2.3, SUV max 6.0 mesenteric site, new diffuse splenic hypermetabolism (spleen size 10.5 cm), and new focal hypermetabolic update in the right proximal femur, SUV max 5.7. All of these new hypermetabolic sites resolved on the subsequent month 3 PET-CT scan. **(E)** After achieving a complete metabolic response on all prior post-infusion PET-CT scans, the 1 year PET-CT scan demonstrated a new 4.3 x 3.5 cm, SUV max 23.3 foci in the right lower lobe index lesion. 10 days after this PET-CT scan, a biopsy of this mass demonstrated non-caseating granulomatous inflammation with a small foci of necrosis. Stains for acid-fast bacilli and fungal organisms were negative. There was no morphologic or immunophenotypic support for involvement by lymphoma. At 14 months, a subsequent PET-CT scan demonstrated the mass was 3.3 x 2.4 cm with an SUV max 17.8 and by 18 months, the mass was 1.1 x 0.8 cm with an SUV max 8.3.

Supplementary Figure 9 | Correlation between CAR22 levels measured by qPCR and Flow Cytometry



Spearman's rank-order correlation method was applied to pooled serial samples from all treated patients. Color depicts time point of blood draw. Dashed line depicts linear regression fit.

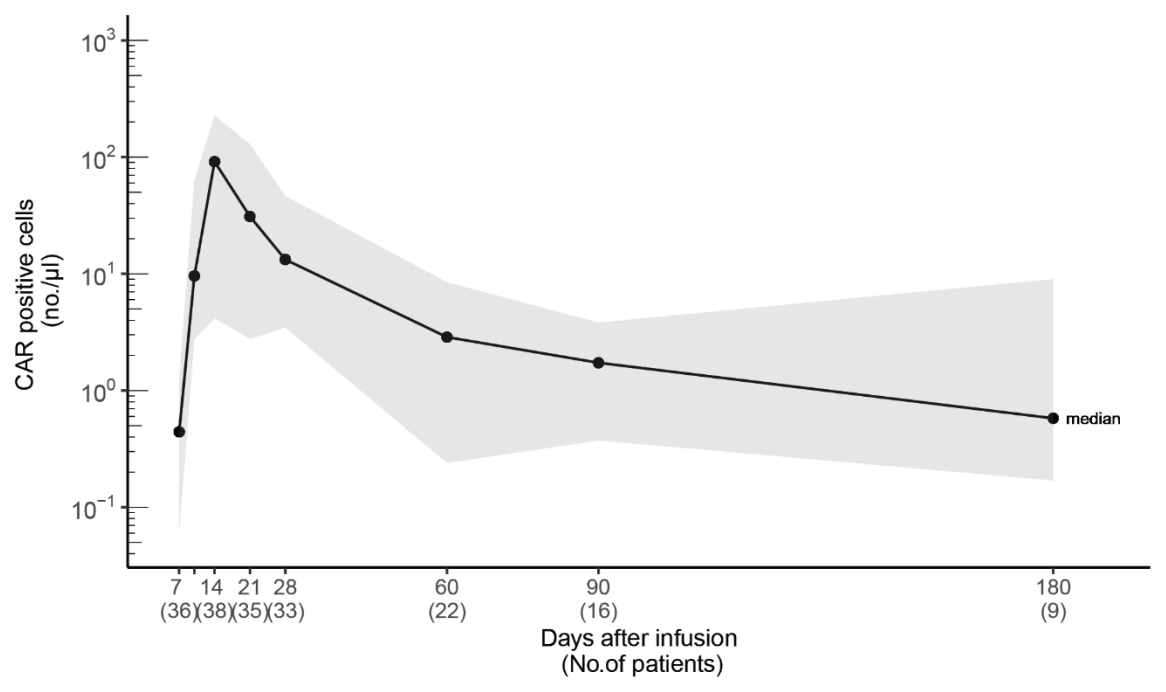
Supplementary Figure 10 | Response and Adverse Events Correlate with CAR22 Expansion Measured by Flow Cytometry and qPCR D_0-D_{28} Area Under the Curve (AUC)

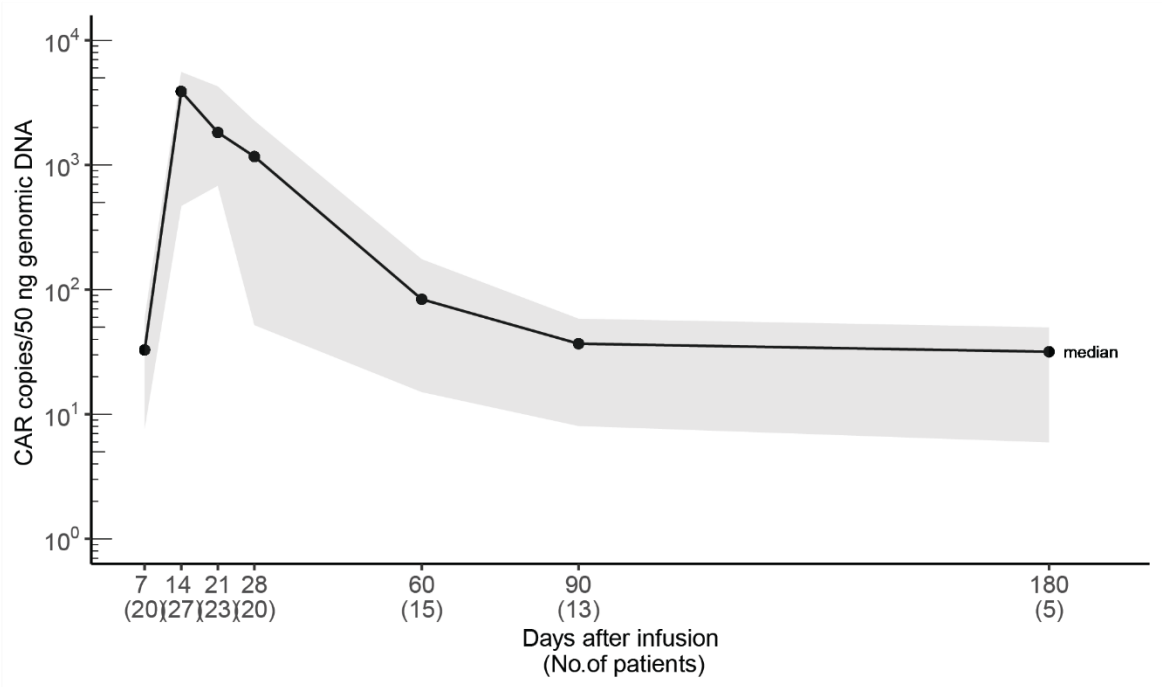


Association of CAR22 expansion by AUC as measured by flow cytometry with (A) objective response rate (ORR) (median 63 vs 1381, p-value=0.0098); (B) cytokine release syndrome (CRS) (median 693 vs 1381, p-value=0.077); and (C) IEC-HS (median 944 vs 12878, p-value=0.0043)). AUC is defined as cumulative levels of CAR+ cells/µL of

blood over the first 28 days post CAR22. Association of CAR22 expansion by AUC as measured by qPCR with (D) objective response rate (ORR) (median 2913 vs 71238, P=0.0002); (E) cytokine release syndrome (CRS) (median 16611 vs 69063, P=ns); and (F) IEC-HS (median 35079 vs 168840, P=0.027). (G) Ratio of CD4/CD8 AUCs does not dictate response (CR median 0.11 vs PD median 0.15, P=ns). (H) In vivo CAR T cell expansion at peak is CD8+ CAR+ T cell predominant (median CD8+ 82 vs CD4+ 7.7, P=0.00093). All P values were calculated by Wilcoxon rank-sum test.

Supplementary Figure 11| Kinetics of Peripheral Blood CAR22 T Cells for All Treated Patients

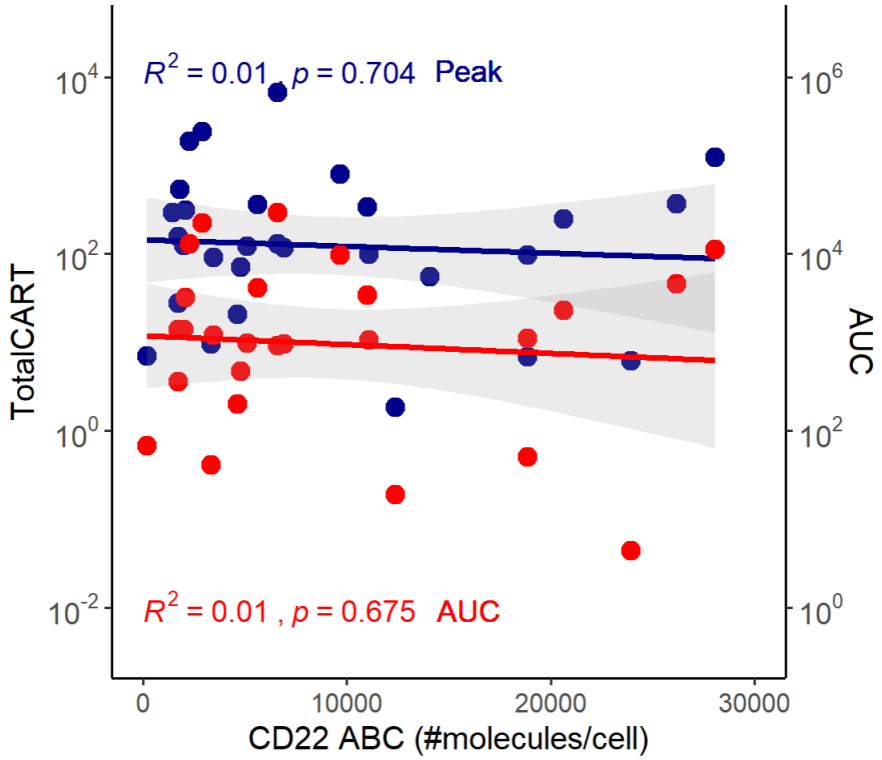




(A) Flow Cytometry and (B) quantitative PCR measurements of circulating CAR+ cells demonstrate exponential expansion and persistence of CAR22 cells in peripheral blood. Expansion occurred rapidly, with peak levels achieved within the first 14 days following CAR22 infusion. Nine patients with ongoing CR had detectable CAR+ T cells at 6 months post infusion.

Supplementary Figure 12 | Association of Baseline Surface CD19 and CD22 Expression Measured by Quantitative Flow Cytometry to CAR22 Kinetics, Response, and Toxicity.

A



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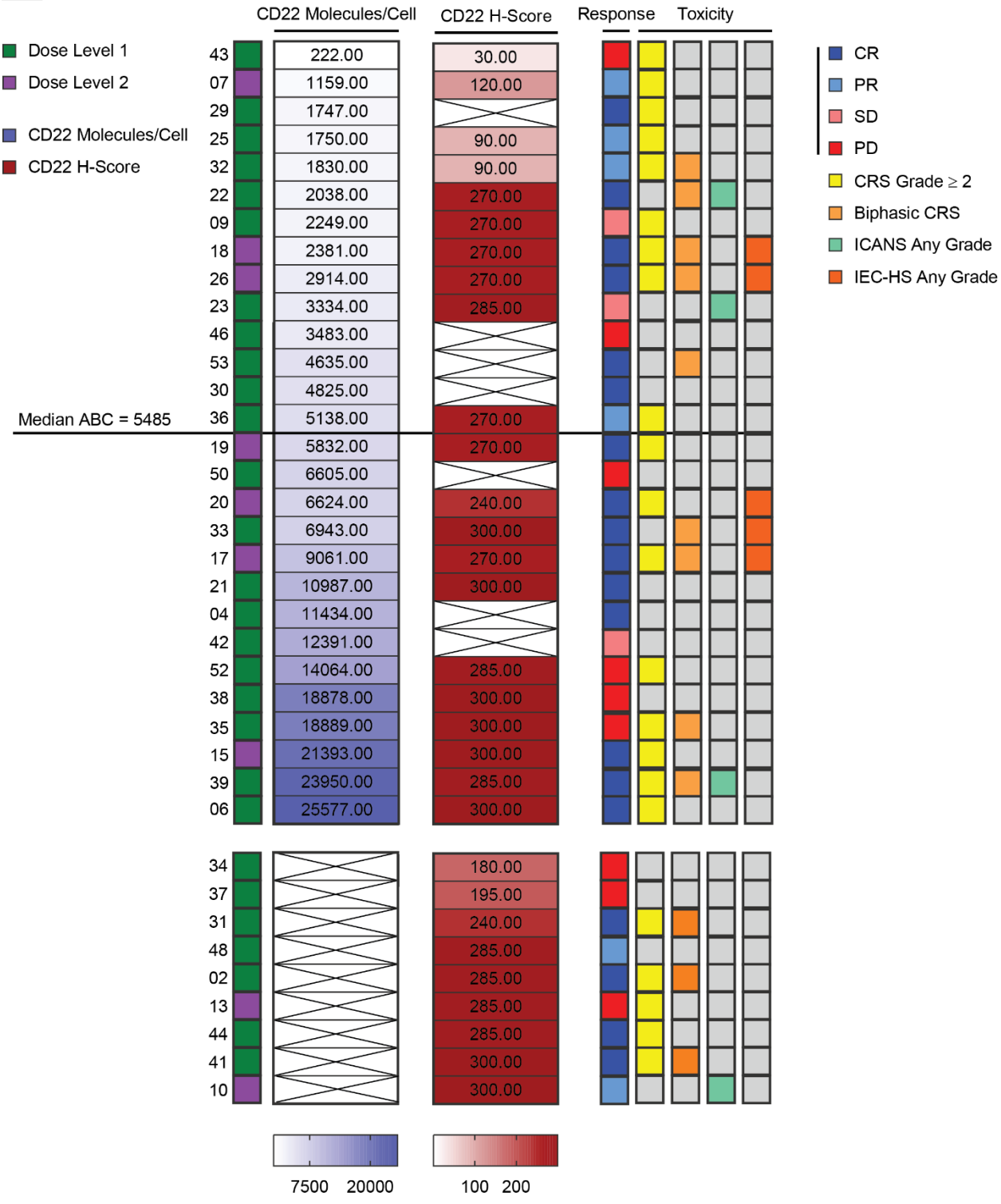
CD22 Molecules/Cell
CD22 H-Score
Response
Toxicity
Dose Level 1
Dose Level 2
CD22 Molecules/Cell
CD22 H-Score
CR
PR
SD
PD
CRS Grade ≥ 2
Biphasic CRS
ICANS Any Grade
IEC-HS Any Grade
Median ABC = 5485
43 222.00 30.00
07 1159.00 120.00
29 1747.00
25 1750.00 90.00
32 1830.00 90.00
22 2038.00 270.00
09 2249.00 270.00
18 2381.00 270.00
26 2914.00 270.00
23 3334.00 285.00
46 3483.00
53 4635.00
30 4825.00
36 5138.00 270.00
19 5832.00 270.00
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20 6624.00 240.00
33 6943.00 300.00
17 9061.00 270.00
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38 18878.00 300.00
35 18889.00 300.00
15 21393.00 300.00
39 23950.00 285.00
06 25577.00 300.00
34 180.00
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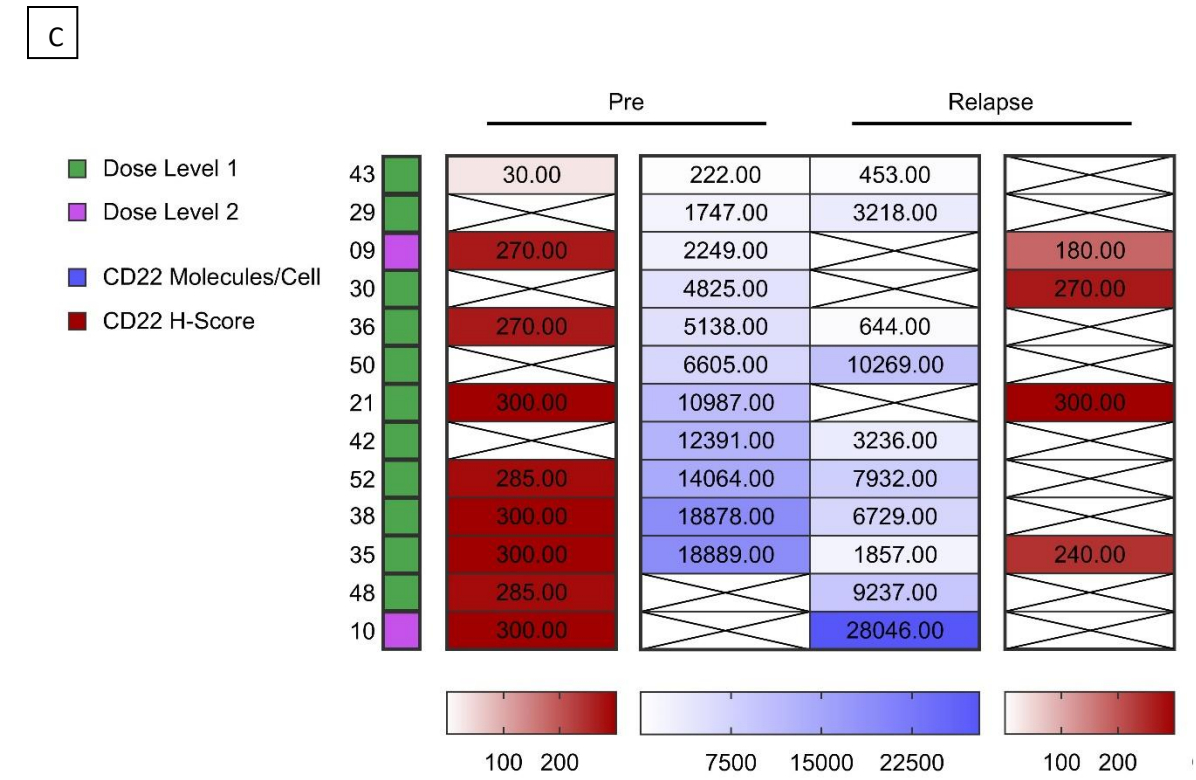
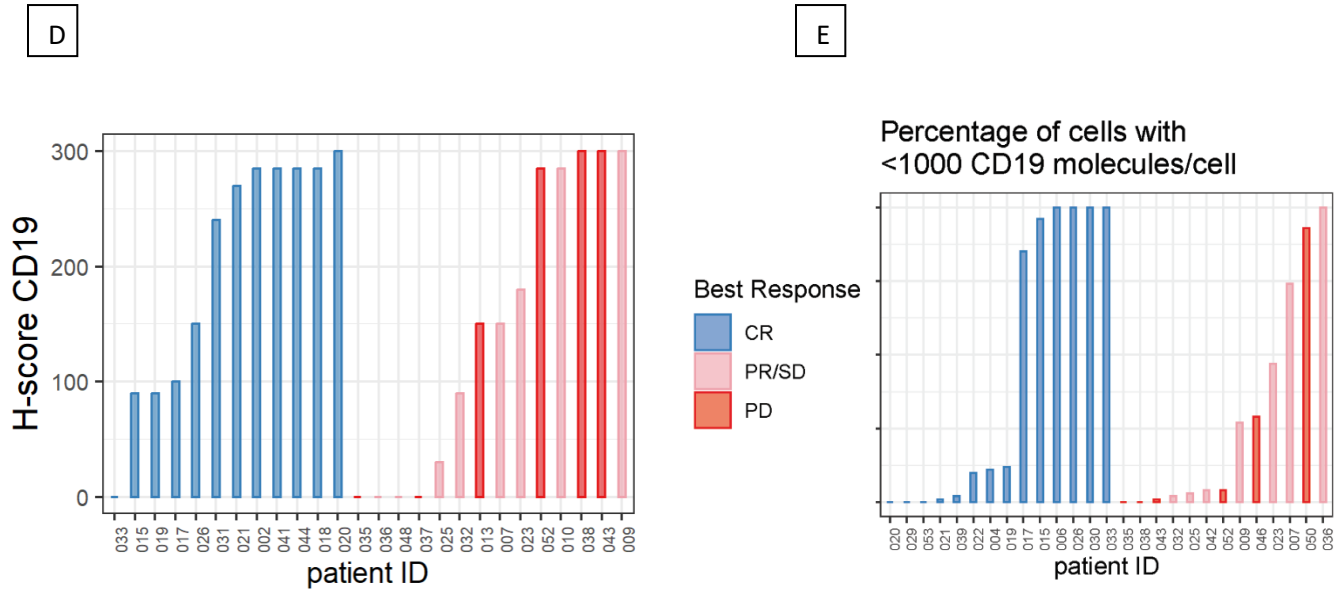
44 285.00
41 300.00
10 300.00
7500 20000
100 200

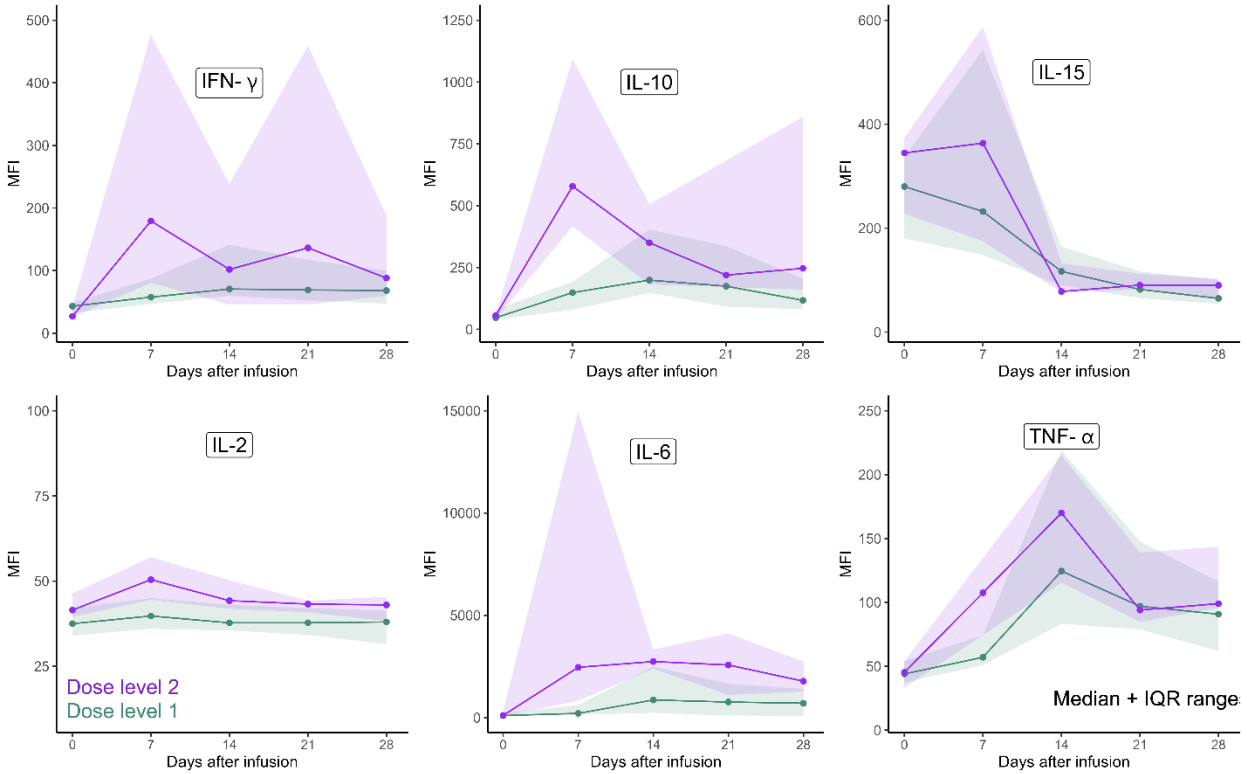




(A) Baseline CD22 antigen density (ABC) measured by quantitative flow cytometry did not correlate with CAR22 expansion at peak or over the first 28 days by AUC as measured by Flow Cytometry (B) Heat map of baseline median CD22 ABC by quantitative flow cytometry organized from highest (dark blue) to lowest (white) antigen density in 29 treated patients. There were no significant differences in patients above or below the median CD22

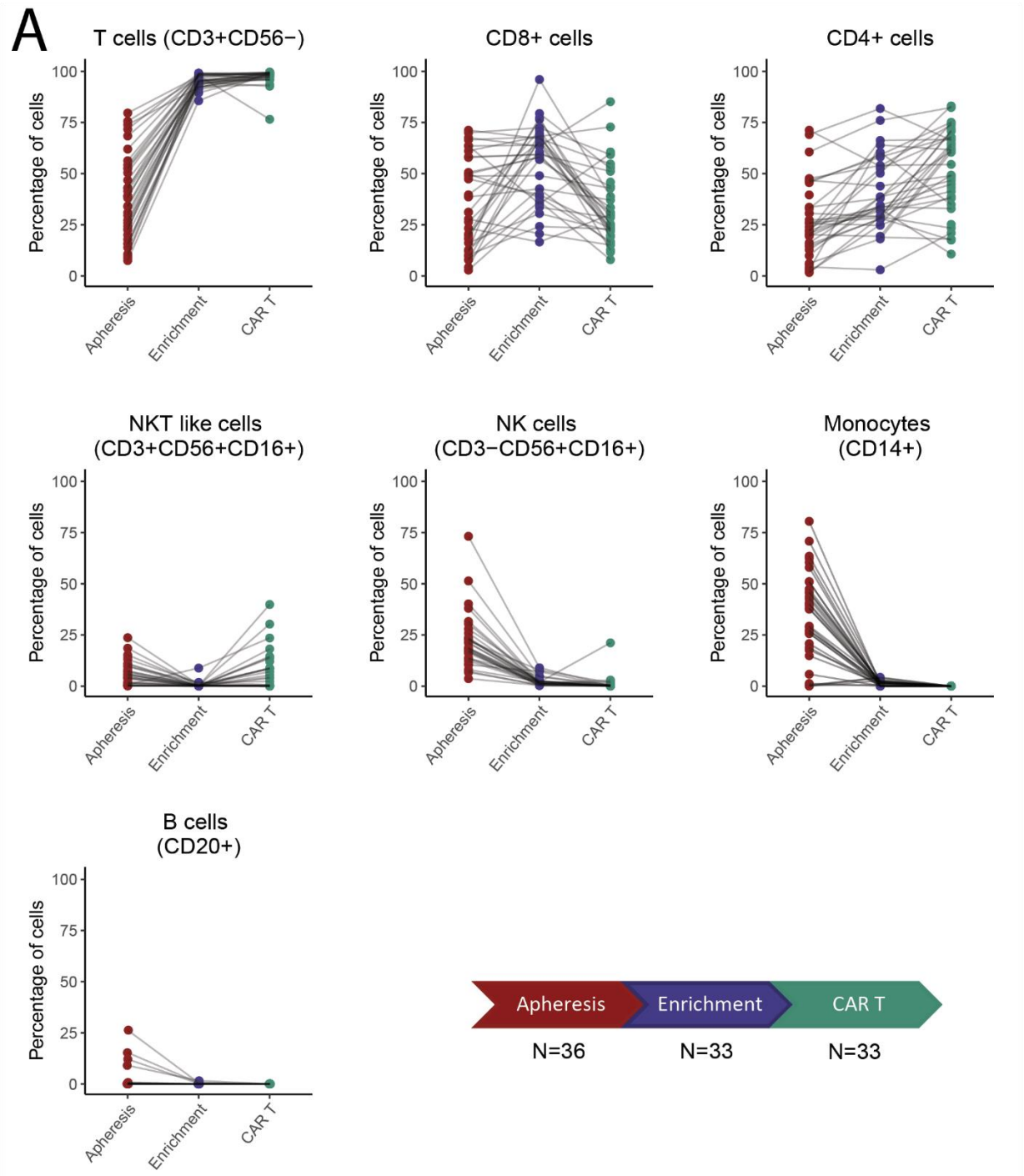
ABC in the risk of relapse or the development of any severe toxicity. (C) Paired CD22 ABC assessments at baseline and at the time of relapse were available in 8 patients. CD22 ABC showed a marked reduction at the time of relapse in 5 out of 8 (63%) of patients. (D) Baseline tumor CD19 expression by semiquantitative H-scoring of immunohistochemistry for all available patients (n=26) including those who did (n=12) or did not (n=14) achieve a CR. H-score was calculated as the percentage of cells with positive staining multiplied by the intensity of staining on a scale from 0 to 3+. Proportion of baseline tumor surface CD19 expression at low or absent levels by quantitative flow cytometry for all available patients (n=27) including those who did (n=14) or did not (n=13) achieve a CR.

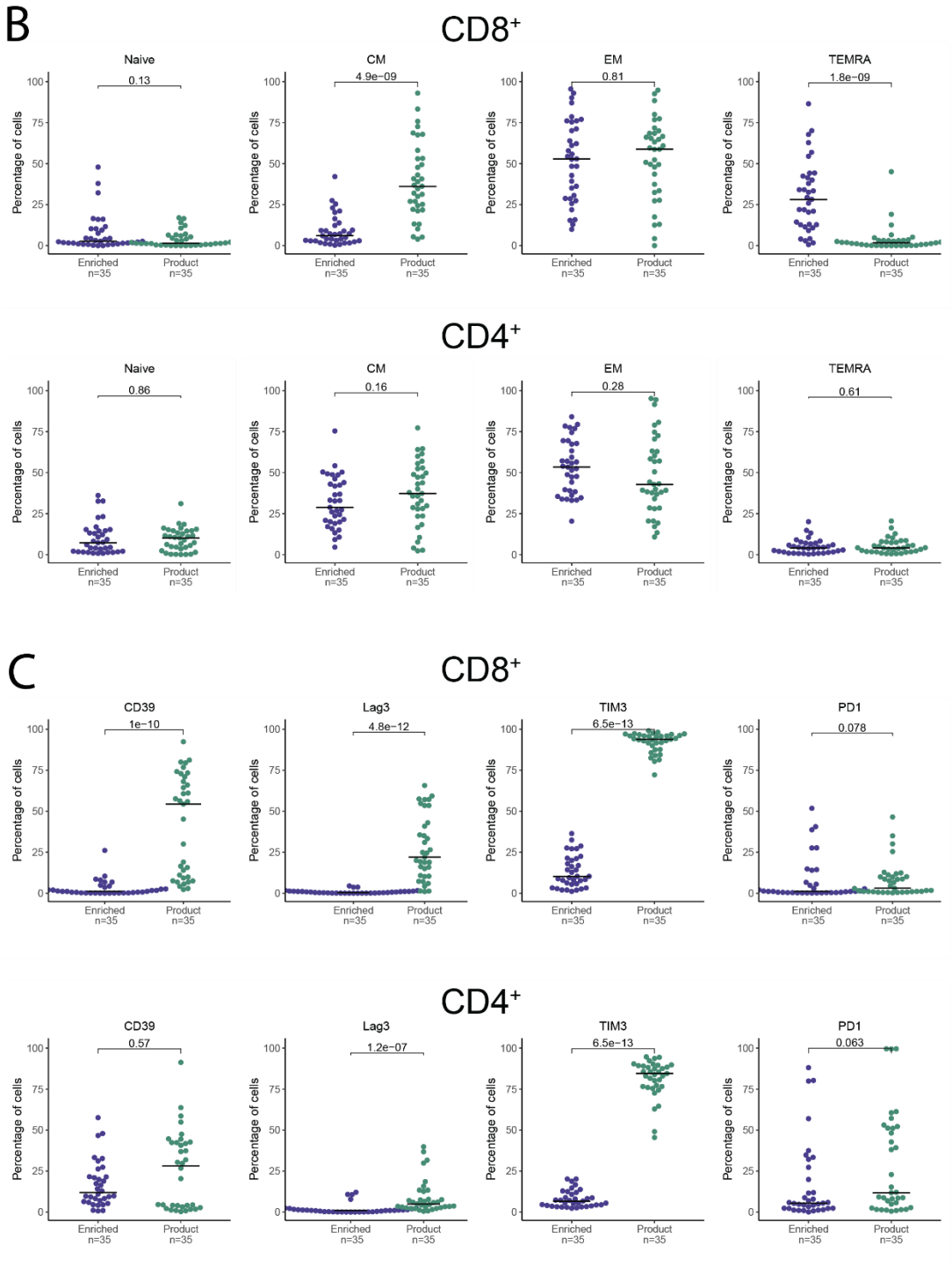
Supplementary Figure 13 | Serum Cytokines Show an Association with Dose Level



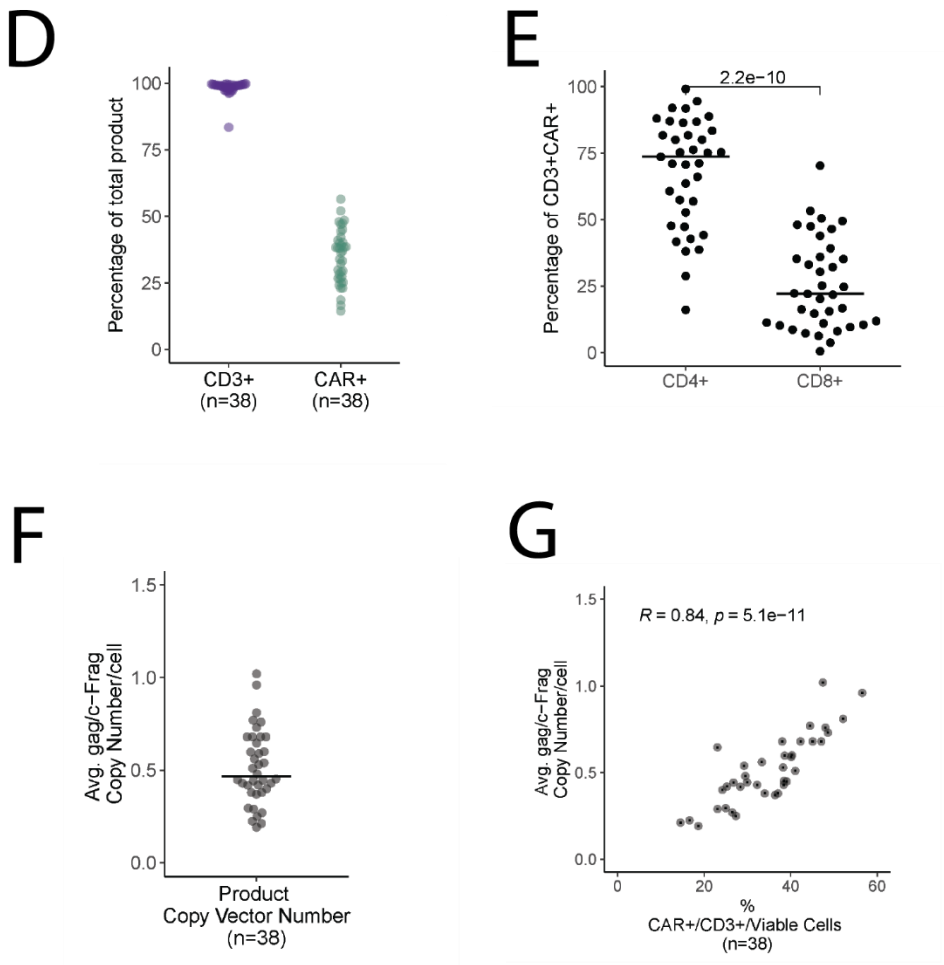
Serial analysis of patient serum was performed following CAR22 infusion. The expansion of CAR22 cells was accompanied by induction and elevation of a range of cytokines that regulate proliferation, activation, and effector function. Early induction of IL-10, IL-15, IFN-gamma, IL-2, IL-6 and TNF-alpha occurred around day 7, with patients at DL2 demonstrating a biphasic peak in IFN-gamma and IL-10 around day 21 or later.

Supplementary Figure 14 | Immunophenotypic Characterization of CAR22 Products and Manufacturing Process




B
CD8+
Naive
0.13
CM
4.9e−09
EM
0.81
TEMRA
1.8e−09
CD4+
Naive
0.86
CM
0.16
EM
0.28
TEMRA
0.61
C
CD8+
CD39
1e−10
Lag3
4.8e−12
TIM3
6.5e−13
PD1
0.078
CD4+
CD39
0.57
Lag3
1.2e−07
TIM3
6.5e−13
PD1
0.063
Percentage of cells
Enriched n=35
Product n=35

514


D
Percentage of total product
100
75
50
25
0
CD3+
(n=38)
CAR+
(n=38)
E
Percentage of CD3+CAR+
2.2e−10
100
75
50
25
0
CD4+
CD8+
F
Avg. gag/c−Frag
Copy Number/cell
1.5
1.0
0.5
0.0
Product
Copy Vector Number
(n=38)
G
Avg. gag/c−Frag
Copy Number/cell
R = 0.84, p = 5.1e−11
1.5
1.0
0.5
0.0
0
20
40
60
%
CAR+/CD3+/Viable Cells
(n=38)

515
516
517

(A) Composition of immune subsets in apheresis, CD4/8-enriched, and CAR22 products over the course of manufacturing, including T cell (CD3+ CD56−), CD8+ T cell, CD4+ T cell, NKT-like cell (CD3+ CD56+ CD16+), NK cell (CD3- CD56+ CD16+), monocyte (CD14+), and B cell (CD20+) subsets. Apheresis products reflected diversity among patients enrolled in the study. (B) Phenotyping of T cell memory subsets revealed an enrichment in CD8 TCM ($P < 0.0001$) cell subsets and a depletion of the CD8 TEMRA ($P < 0.0001$) subset. There was no significant change in CD4 memory subsets or CD8 TN or TEM cell subsets between enrichment and CAR22 product. (C) Evaluation of exhaustion markers in T cell subsets revealed significant increases in CD39+, LAG3+, and TIM3+ CD8+ T cells and LAG3+, TIM3+ CD4+ T cells from CD4/8 enriched to final CAR T product ($P < 0.0001$). There was no difference observed in PD1 expression in any subset. (D) Purity and transduction efficiency of all manufactured products (N=38; median transduction efficiency 36.7%, range 14.5 to 56.5). (E) Immunophenotyping of CD3+ CAR+ T cells in the final product revealed a skewing toward CD4+ cells (N=37; median CD4+ 73.6% vs CD8+ 22.1%, $P < 0.0001$). (F) Average product vector copy number (VCN) in all manufactured products (N=38; median 0.47, range 0.19 to 1.02). (G) Correlation between vector copy number and transduction efficiency. Spearman's rank-order correlation method was applied to pooled samples from all manufactured products (N=38; $P < 0.0001$). All P values were calculated by Wilcoxon rank-sum test except where noted.