Supplemental Data

Inclusion and exclusion criteria (NCT02706405)

Patients must meet all the following criteria to be enrolled in this study:

Inclusion criteria:

1) Male or female ≥ 18 years of age at the time of screening consent.
2) Relapsed or refractory, PET-positive DLBCL not otherwise specified (NOS), high grade B cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements, PMBCL, or DLBCL transformed from indolent histology with one of the following:
    a) Persistent disease after first-line chemo-immunotherapy.
    b) Relapse after first-line chemo-immunotherapy and not eligible for autologous hematopoietic stem cell transplant (HCT).
    c) Relapse or persistent disease after at least two lines of therapy or after autologous HCT.
3) Evidence of CD19 expression on any prior or current tumor specimen or a high likelihood of CD19 expression based on disease histology.
4) Karnofsky performance status ≥ 60%.
5) Adequate organ function, defined as:
    a) Assessed by the Investigator to have adequate bone marrow function to receive lymphodepleting conditioning chemotherapy.
    b) Serum creatinine < 1.5 x age-adjusted upper limit of normal (ULN).
    c) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) < 3 x ULN and total bilirubin ≤ 2 x ULN.
    d) Adequate pulmonary function, defined as CTCAE Grade ≤ 1 dyspnea and SaO2 ≥ 92% on room air. Patients with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing and must have a forced expiratory volume in 1 second (FEV1) of ≥ 50% of predicted value or diffusing capacity of the lung for carbon monoxide (DLCO; corrected) ≥ 40% of predicted value.
    e) Adequate cardiac function, defined as left ventricular ejection fraction (LVEF) ≥ 35% as assessed by echocardiogram or multiple uptake gated acquisition (MUGA).

6) Women of reproductive potential (defined as all women physiologically capable of becoming pregnant) must agree to use suitable methods of contraception for 90 days after the last dose of study therapy (durvalumab or JCAR014 infusion).
7) Males who have partners of reproductive potential must agree to use an effective barrier contraceptive method for 90 days after the last dose of study therapy (durvalumab or JCAR014).
8) Ability to understand and provide informed consent.

Exclusion criteria:

1) Subjects with known active central nervous system (CNS) involvement by malignancy. Subjects with prior CNS disease that has been effectively treated will be eligible if treatment was completed at least 3 months prior to enrollment and there is no evidence of disease or stable abnormalities on repeat imaging.
2) Planned use of corticosteroids (> 10 mg/day prednisone or equivalent) or other systemic immunosuppression within 4 days prior to leukapheresis or within 72 hours prior to JCAR014 infusion. Topical and/or inhaled steroids are permitted.
3) Prior treatment with any CD19 CAR T-cell therapy.
4) Prior allogeneic HCT.
5) Known active hepatitis B, hepatitis C, or human immunodeficiency virus (HIV) infection.
6) Pregnant or breastfeeding women.
7) Known exclusion criteria for leukapheresis, JCAR014, or durvalumab therapy.
8) Prior treatment with PD-1, PD-L1, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) targeted therapy, or tumor necrosis factor receptor superfamily (TNFRSF) agonists including CD134 (OX40), CD27, CD137 (4-1BB), and CD357 (glucocorticoid-induced tumor necrosis factor receptor family-related protein [GITR]).
9) Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease [e.g., ulcerative colitis, Crohn's disease], celiac disease, or other serious chronic gastrointestinal conditions associated with diarrhea, autoimmune vasculitis, systemic lupus erythematosus, Wegener syndrome [granulomatosis with polyangitis], myasthenia gravis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc.) within 3 years prior to the planned start of treatment. The following are exceptions to this criterion:
    a) Vitiligo.

b) Alopecia.

c) Hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement.

d) Psoriasis not requiring systemic treatment.

e) Other conditions considered to be low risk of serious deterioration by the Principal Investigator (PI).

9) History of any one of the following cardiovascular conditions within the past 6 months: Class III or IV heart failure as defined by the New York Heart Association (NYHA), cardiac angioplasty or stenting, myocardial infarction, or unstable angina. History of other clinically significant cardiac disease that, in the opinion of the PI or designee, is a contraindication to lymphodepleting chemotherapy, JCAR014 infusion, or durvalumab infusion is also excluded.

10) History or presence of clinically relevant CNS pathology, such as epilepsy, seizure, paresis, aphasia, stroke, severe brain injuries, dementia, Parkinson's disease, cerebellar disease, or psychosis. History of other organic brain syndrome that, in the opinion of the PI or designee, is a contraindication to lymphodepleting chemotherapy, JCAR014 infusion, or durvalumab infusion is also excluded.

11) History of solid organ transplantation.

12) Uncontrolled infection.

13) Receipt of live, attenuated vaccine within 28 days prior to the first dose of durvalumab. Note: enrolled patients should not receive live vaccine during the study and for 180 days after the last dose of durvalumab.

Dose-limiting toxicity (DLT) definition

The following treatment-related (JCAR014 or durvalumab) events will be considered DLTs:

1) Death within 4 weeks of the study treatment.

2) Grade ≥ 3 neurotoxicity of greater than 7 days duration.

3) Grade ≥ 3 neurotoxicity that does not revert to Grade 1 or baseline within 28 days.

4) Grade ≥ 3 seizures that do not resolve to grade ≤ 2 within 3 days.

5) Grade ≥ 4 cytokine release syndrome.

6) Grade 3 cytokine release syndrome that does not resolve to grade ≤ 2 within 7 days.

7) Grade ≥ 3 non-infectious colitis or non-infectious pneumonitis.

8) Grade ≥ 3 irAE or other grade ≥ 3 autoimmune toxicity (excluding B-cell aplasia).

9) Any increase in AST or ALT > 3 x ULN and concurrent increase in total bilirubin > 2 x ULN that is unrelated to CRS and has no other probable reason to explain the combination of increases.
10) Grade ≥ 3 allergic reaction to the JCAR014 infusion.
11) Any Grade 3 or 4 event deemed unexpected by the Investigator and considered a DLT upon evaluation by the SC.

Supplemental Methods

Immunohistochemistry of patients biopsies

CLIA/CAP validated single-marker immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded (FFPE) tumor sample sections (4 µm thickness) after antigen retrieval. CD19 was stained with antibody clone BT51E on the Leica Bond III and PAX5 was stained with antibody clone SP34 on Ventana Benchmark Ultra according to manufacturer recommendations and clinically validated protocols.

Flow cytometry of patients CAR-T cells

For analysis of the immunophenotype of CAR-T cells, aliquots of the infusion products and peripheral blood at different time points after JCAR014 infusion were stained with Live/Dead Fixable Blue stain followed by fluorochrome-conjugated antibodies to CD45, CD3, CD4, CD8, EGFRt, CD127, CD69, 2B4, CD160, KLRG1, LAG-3, PD-1, TIGIT, TIM-3, and PD-L1. For intracellular staining, cells were fixed and permeabilized using BD Transcription Factor Buffer set and stained with TCF-1 and active caspase-3 antibodies. Data were acquired using a BD FACSymphony (BD Biosciences) and analyzed using FlowJo software (BD Life Sciences).

Healthy donor CAR-T cell generation

CAR-T cells were generated from healthy donor leukaphereses as previously described.[1]

Flow cytometry of healthy donor CAR-T cells

Healthy donor CAR-T cells were cocultured with irradiated (12,000 cGy) K562 cells stably expressing CD19 (K562-CD19^{+}) and different concentrations of soluble PD-L1 (sPD-L1; PeproTech). Cells were washed and stained with Live/Dead Fixable Violet stain followed by fluorochrome-conjugated antibodies to CD45, CD3, CD8, CD4, and EGFRt. For intracellular staining, cells were fixed and permeabilized using BD Cytofix/Cytoperm Solution Kit and stained with interferon-γ (IFN-γ), tumor necrosis factor receptor-α (TNF-α), and interleukin-2 (IL-2)

antibodies. Data were acquired using a BD FACSymphony (BD Biosciences) and analyzed using FlowJo software (BD Life Sciences).

Statistical analyses

A time-dependent Cox regression was fit to assess the association between patients who did or did not achieve CR and PFS/OS. Achievement of CR was considered as a time-dependent binary covariate because response status can change after CAR-T cell infusion. Based on the time-dependent Cox model, a novel survival curve (Smith-Zee plot) was generated, which represents the trajectory of a hypothetical patient with a prespecified status change at a specific time, such as achievement or failure to achieve CR at day 28 after CAR-T infusion.

Table S1. Durvalumab dose levels

Dose level	Group 1	Group 2
-1	75 mg	-
1	225 mg	7.5 mg
2	750 mg	22.5 mg
3	-	75 mg
4	-	225 mg
5	-	750 mg

Table S2. Treatment-emergent adverse events in ≥ 10% of patients or ≥ grade 3 by Group and time

Adverse event	Any grade	Grade ≥ 3
	Number of patients (%)	
Group 1 – lymphodepletion to the day of the first post-JCAR014 durvalumab infusion		
Hypotension	3 (27)	1 (9)
Sinus tachycardia	3 (27)	1 (9)
Fatigue	3 (27)	0
Hypogammaglobulinemia	1 (9)	0
Neutropenia	1 (9)	1 (9)
AST increased	1 (9)	1 (9)
Hypofibrinogenemia	1 (9)	1 (9)
Thrombocytopenia	1 (9)	1 (9)
Acute kidney injury	1 (9)	1 (9)
Atrial fibrillation	1 (9)	1 (9)
Hyperglycemia	1 (9)	0
Hypophosphatemia	1 (9)	1 (9)
Cytokine release syndrome (CRS)	4 (36)	1 (9)
Neurotoxicity	3 (27)	1 (9)
Group 1 – after the first post-JCAR014 durvalumab infusion		
Fatigue	2 (18)	0
Headache	2 (18)	0
Hypotension	1 (9)	0
Sinus tachycardia	1 (9)	0
Hypogammaglobulinemia	1 (9)	0
Neutropenia	1 (9)	1 (9)
Bacteremia	1 (9)	1 (9)
Joint infection	1 (9)	1 (9)
Cytokine release syndrome (CRS)	1 (9)	0
Neurotoxicity	0	0
Group 2 – lymphodepletion to the day of first post-JCAR014 durvalumab infusion		
Hypotension	6 (33)	0
Sinus tachycardia	4 (22)	0
Fatigue	2 (11)	0

Neutropenia	2 (11)	2 (11)
Hypogammaglobulinemia	1 (6)	0
AST increased	1 (6)	1 (6)
Hypofibrinogenemia	1 (6)	1 (6)
Thrombocytopenia	1 (6)	1 (6)
Acute kidney injury	1 (6)	0
Atrial fibrillation	1 (6)	0
Hyperglycemia	1 (6)	1 (6)
Hypertension	1 (6)	1 (6)
Hypokalemia	1 (6)	1 (6)
Hypoxia	1 (6)	1 (6)
Cytokine release syndrome (CRS)	7 (39)	1 (6)
Neurotoxicity	2 (11)	1 (6)
Group 2 – after the first post-JCAR014 durvalumab infusion		
Neutropenia	6 (33)	6 (33)
Hypotension	3 (17)	0
Hypogammaglobulinemia	2 (11)	1 (6)
Fatigue	1 (6)	0
AST increased	1 (6)	1 (6)
Bacteremia	1 (6)	1 (6)
Cytokine release syndrome (CRS)	0	0
Neurotoxicity	0	0

Adverse events (AEs) in ≥ 10% of all patients and all grade ≥ 3 are reported (all 29 treated patients were evaluable for safety with 1 patient who did not receive durvalumab infusion and 1 patient who received an out-of-specification JCAR014 product not evaluable for DLT). AEs were graded using the CTCAE v4.03, except for CRS, which was graded according to the Lee criteria (Lee, *et al*. *Blood*. 2014). Fever and encephalopathy as symptoms of CRS and neurotoxicity, respectively, were not reported as separate AEs.

Table S3. Patient characteristics in JCAR014 in combination with durvalumab (NCT02706405) and JCAR014 alone cohorts (NCT01865617)

Characteristic	Group 1 (n = 9)	Group 2 (n = 17)	JCAR014 alone (n = 12)	*P* value
Age				
Median (interquartile range) – years	65 (56-68)	58 (51-68)	61 (54-66)	.57
≥ 65 years – number (n; %)	5 (56)	6 (35)	3 (25)	.35
Male sex – number (%)	6 (67)	10 (59)	6 (50)	.74
ECOG performance score ≥ 1 – n (%)	6 (67)	6 (35)	3 (25)	.14
Disease histology – n (%)				
Diffuse large B-cell lymphoma, NOS	4 (44)	7 (41)	5 (42)	.99
DLBCL transformed from indolent histology	4 (44)	4 (24)	5 (42)	.45
High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements	1 (11)	4 (24)	1 (8)	.49
Other	0	2* (12)	1† (8)	.57
Cell of origin (Hans algorithm) – n (%)				
Germinal-center B-cell phenotype	7 (78)	8 (47)	7 (58)	.33
Non-germinal center B-cell phenotype	2 (22)	8 (47)	3 (25)	.33
Missing	0	1 (6)	2 (17)	.34
Ann Arbor stage III or IV – n (%)	8 (89)	13 (76)	12 (100)	.18
Extranodal disease – n (%)	4 (44)	12 (71)	11 (92)	.06
Lactate dehydrogenase (LDH)				
Median (interquartile range) – U/L	227 (176-442)	171 (148-359)	252 (139-371)	.67
Elevated – n (%)	6 (67)	6 (35)	7 (58)	.25
International Prognostic Index (IPI) score – n (%)‡				
0-2	4 (44)	10 (59)	6 (50)	.76
≥ 3	5 (56)	7 (41)	6 (50)	.76
Tumor cross-sectional area – median (interquartile range) – mm^2§	4758 (3159-5514)	3422 (1315-6320)	3240 (1643-7017)	.72
Number of prior therapies – median (range)	3 (2-7)	2 (1-9)	6 (2-11)	.01
JCAR014 dose level – n (%)				
2 (2.0 x 10^6/kg)	9 (100)	17 (100)	12 (100)	
Lymphodepletion regimen – n (%)				
Cy 300 mg/m^2 x 3 + Flu 30 mg/m^2 x 3	9 (100)	17 (100)	12 (100)	

Cy, cyclophosphamide; ECOG, Eastern Cooperative Oncology Group; Flu, fludarabine. *P* values per Kruskal-Wallis test or Chi-square test, as appropriate. * One patient with primary mediastinal B-cell lymphoma and one patient with Richter's transformation. † One patient with primary cutaneous DLBCL, leg type. ‡ Scores on the IPI include low risk (0 or 1 point), low-intermediate risk (2 points), high-intermediate risk (3 points), and high risk (4 or 5 points). § Sum of the product of the perpendicular diameters of up to 6 target measurable nodes and extranodal sites.

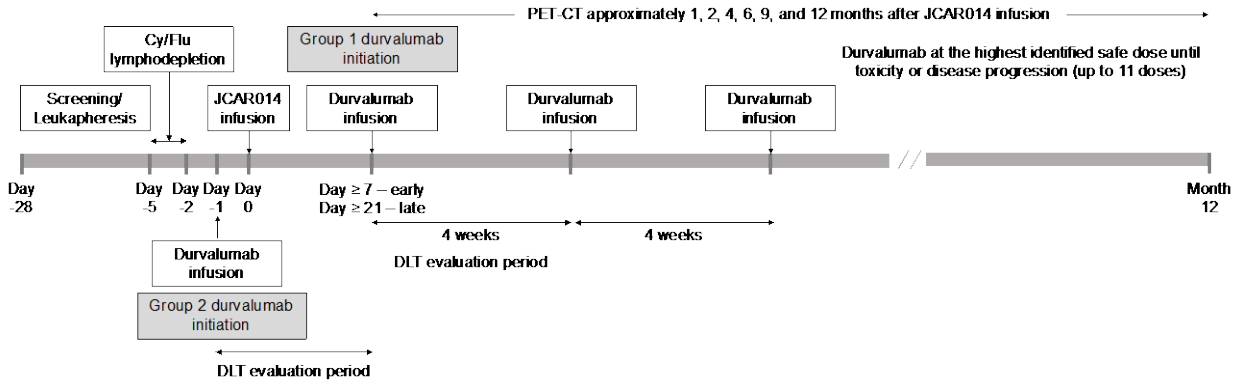

Figure S1. Treatment regimen/groups (NCT02706405). Cy, cyclophosphamide; DLT, dose-limiting toxicity; Flu, fludarabine.

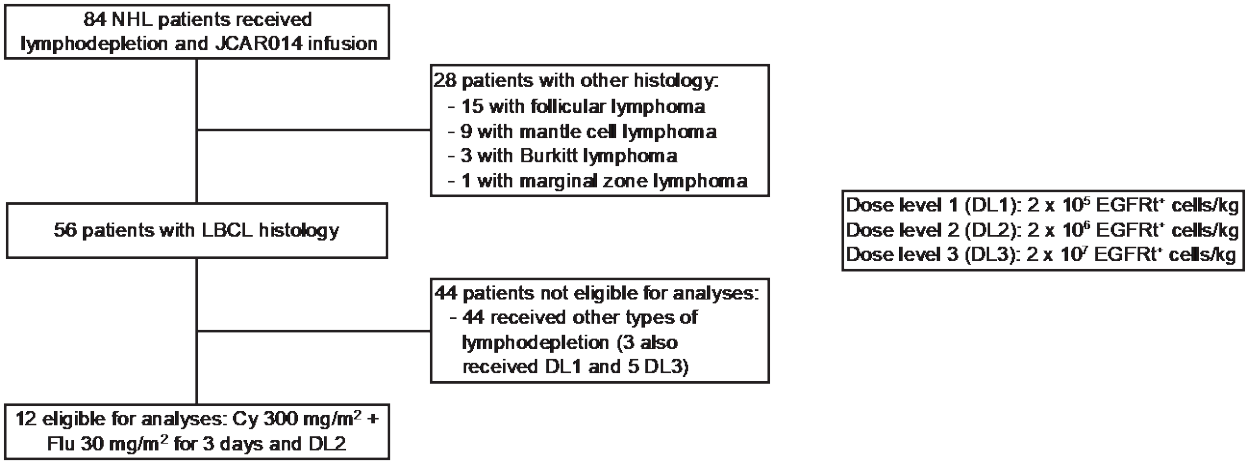

Figure S2. Flow chart of patients treated with JCAR014 alone (NCT01865617) who were identified for retrospective comparative analyses with patients treated with JCAR014 in combination with durvalumab (NCT02706405). Cy, cyclophosphamide; DL, dose level; EGFRt, truncated human epidermal growth factor receptor (marker of expression of the transgene that included the CAR); Flu, fludarabine; LBCL, large B-cell lymphoma; NHL, non-Hodgkin lymphoma.

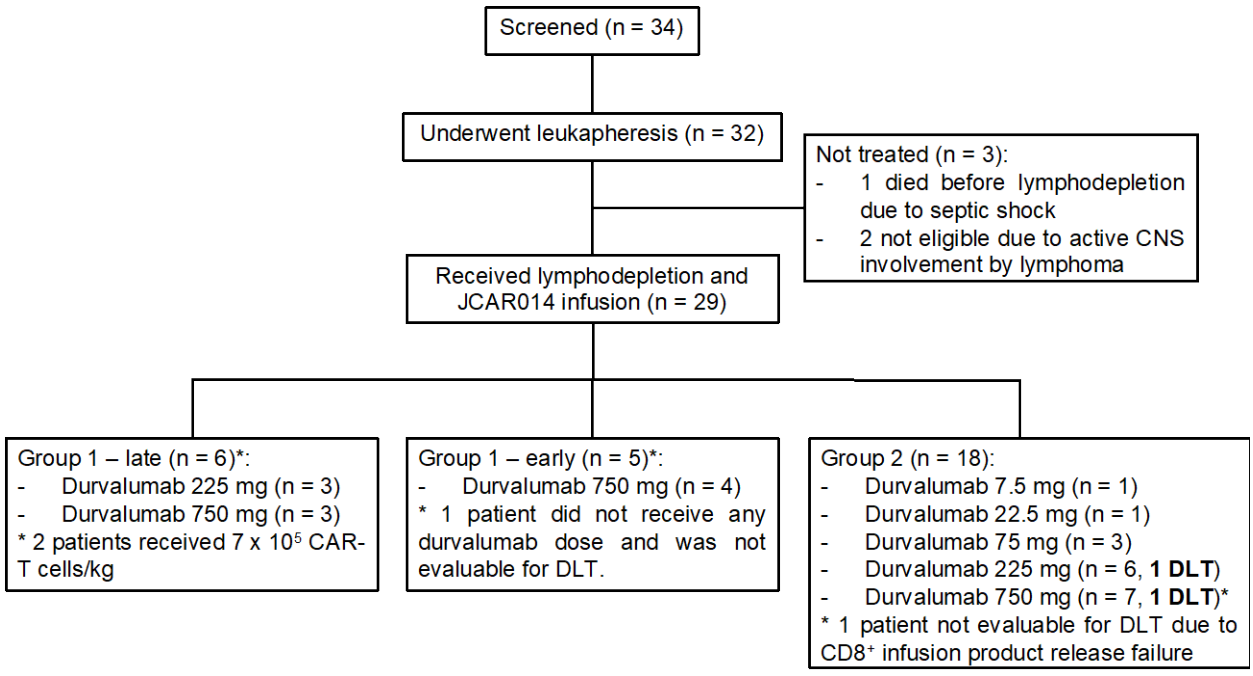

Figure S3. Flow chart of patient enrollment and eligibility for safety and efficacy analyses. CAR-T cells, chimeric antigen receptor T cells; CNS, central nervous system; DLT, dose-limiting toxicity.

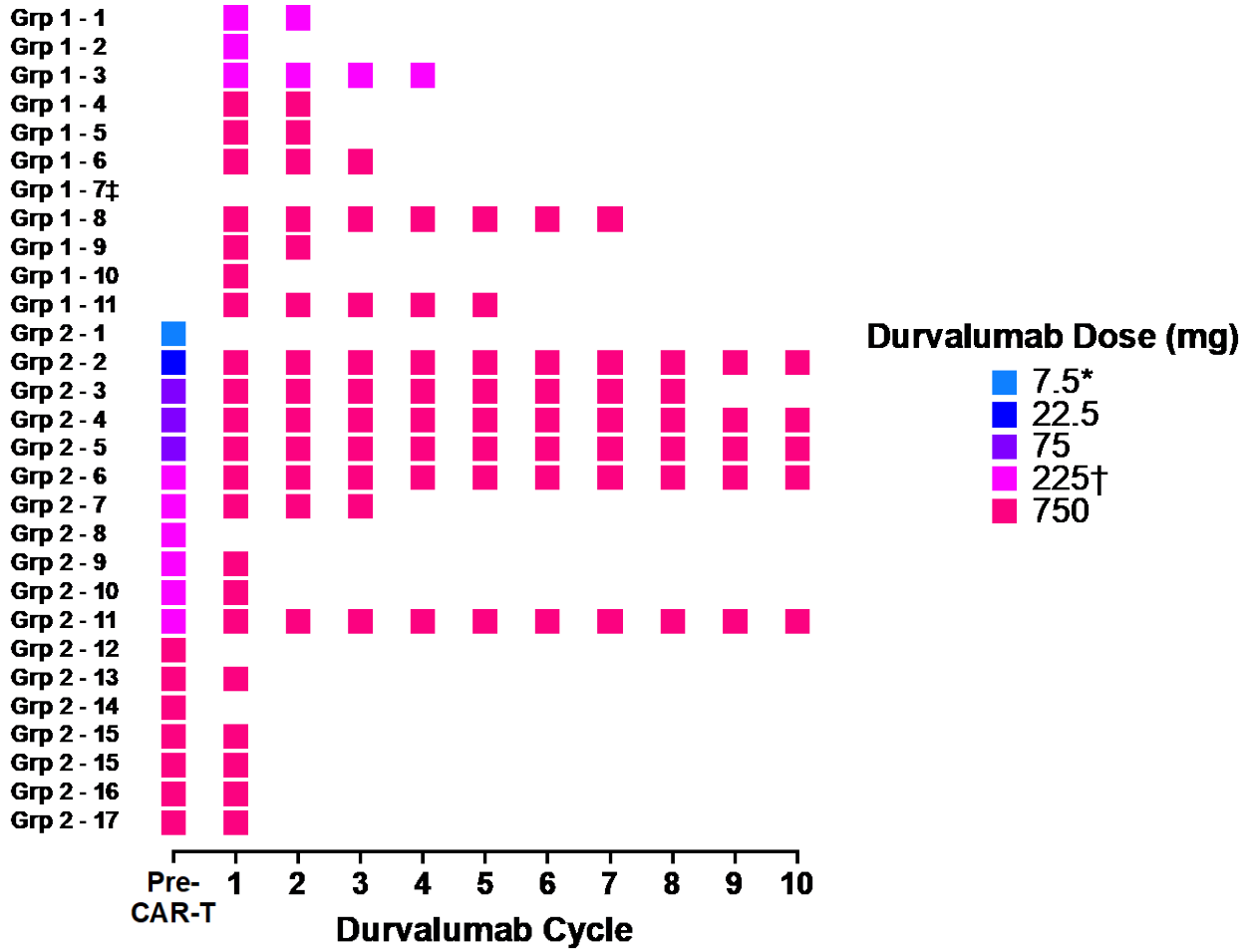

Figure S4. Durvalumab cycles and doses administered. Grp 1, Group 1; Grp 2, Group 2; Pre-CAR-T, one day prior to CAR-T cell infusion. * Starting dose level in group 2. † Starting dose level in group 1. ‡ One patient did not receive durvalumab. Colored squares depict the durvalumab dose.

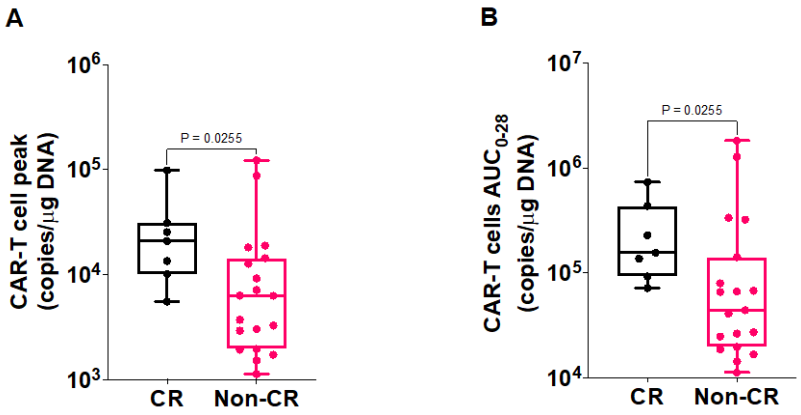

Figure S5. CAR-T cell kinetics in patients who did or did not achieve CR after JCAR014 in combination with durvalumab. CAR-T cell peak (A) and area under the curve of CAR-T cell counts in blood by qPCR from day 0 to 28 (AUC_{0-28}) after infusion (B) in patients who did or did not achieve complete response (CR) after JCAR014 in combination with durvalumab. Mann-Whitney tests were used to compare differences between groups.

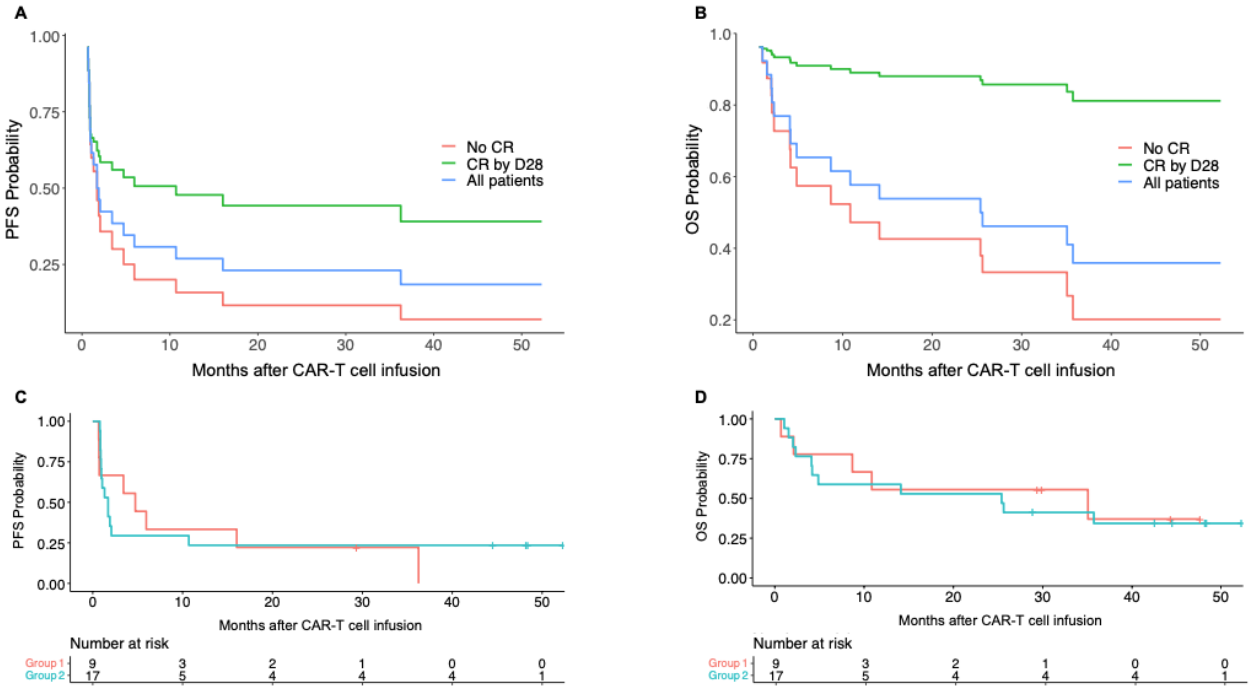

Figure S6. Progression-free (PFS) and overall survival (OS) in patients with LBCL treated with JCAR014 in combination with durvalumab. (A-B) Smith-Zee estimates of PFS (A) and OS (B) according to the probability of achieving complete response (CR) by day 28 (D28) after CAR-T cell infusion and in all patients. (C-D) Kaplan-Meier estimates of PFS (C) and OS (D) according to treatment group. The numbers of patients at risk at 10-month intervals are indicated.

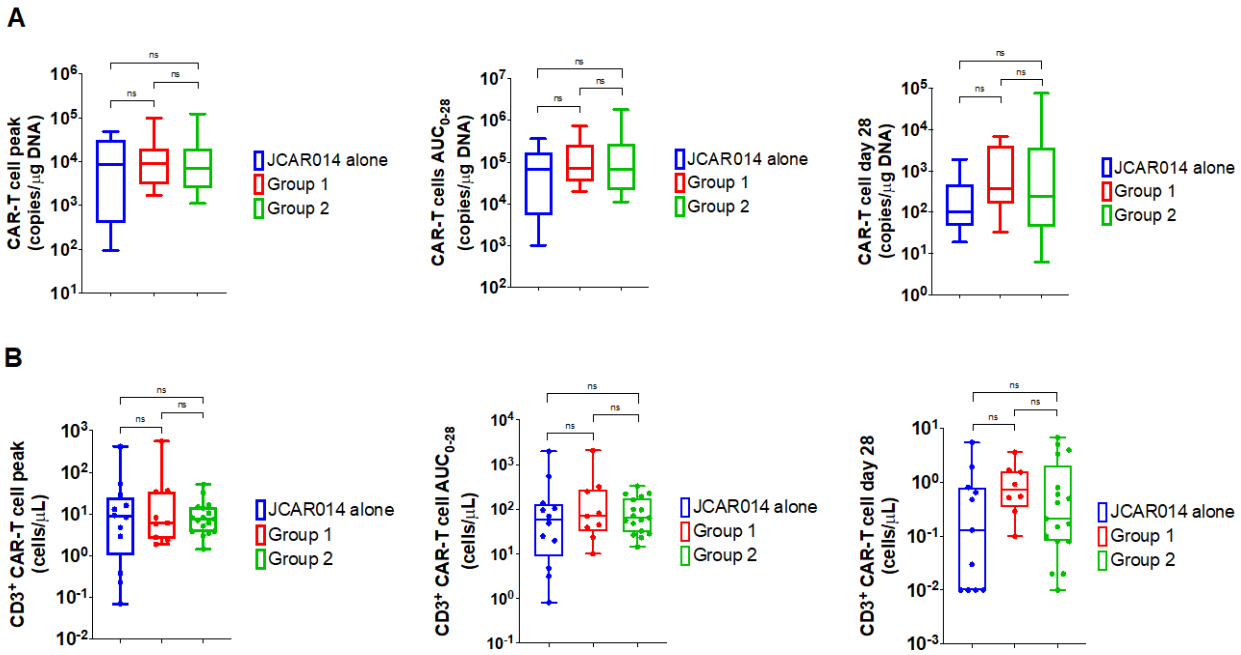

Figure S7. CAR-T cell kinetics in patients treated with JCAR014 with and without durvalumab. CAR-T cell peak (left), area under the curve of CAR-T cell counts from day 0 to 28 (AUC_{0-28}) after infusion (middle), and day 28 counts in blood by qPCR (A) and flow cytometry (B) in the JCAR014 in combination with durvalumab (Groups 1 and 2; NCT02706405) and JCAR014 alone cohorts (NCT01865617). Mann-Whitney tests were used to compare differences between groups. ns, not significant.

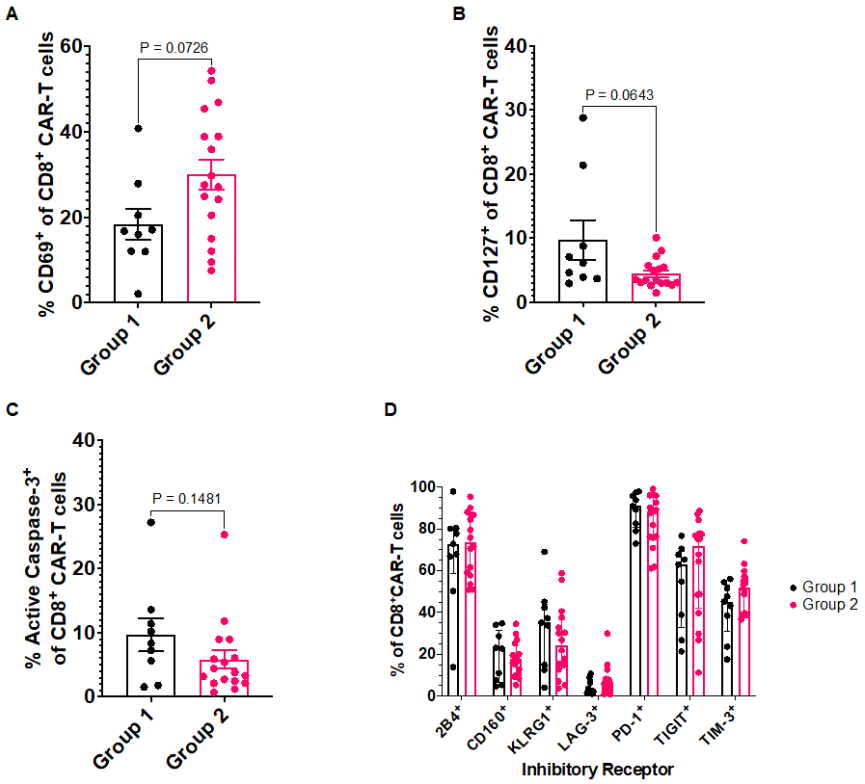

Figure S8. CD8⁺ CAR-T cell immunophenotype in peripheral blood at expansion phase according to treatment group. Percentage of CD69 (A), CD127 (B), active caspase-3 (D), and inhibitory receptor (D) positive CD8⁺ CAR-T cells. Figures show mean +/- standard error of the mean (SEM). Mann-Whitney tests were used to compare differences between groups.

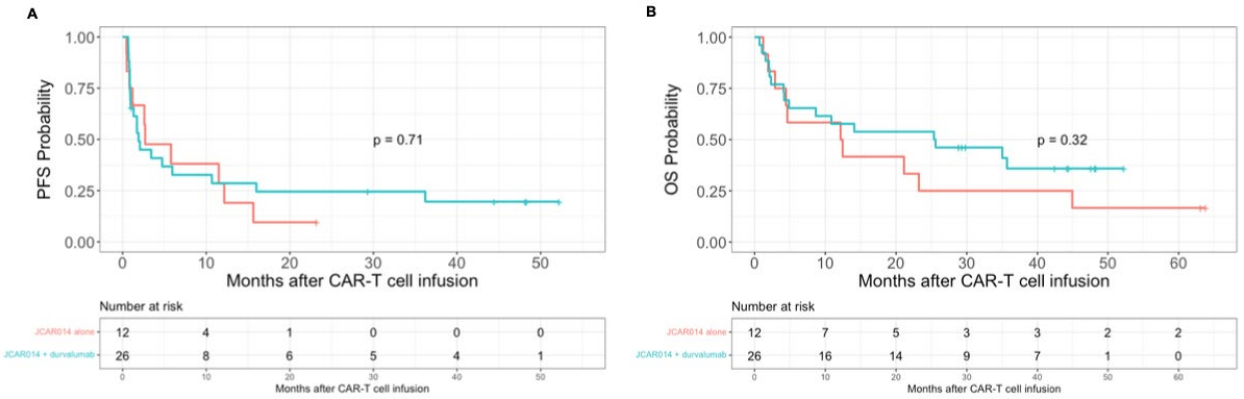

Figure S9. Progression-free (PFS) and overall survival (OS) in patients treated with JCAR014 with and without durvalumab. Kaplan-Meier estimates of PFS (A) and OS (B) according to treatment cohort. *P* value per log-rank test. The numbers of patients at risk at 10-month intervals are indicated.

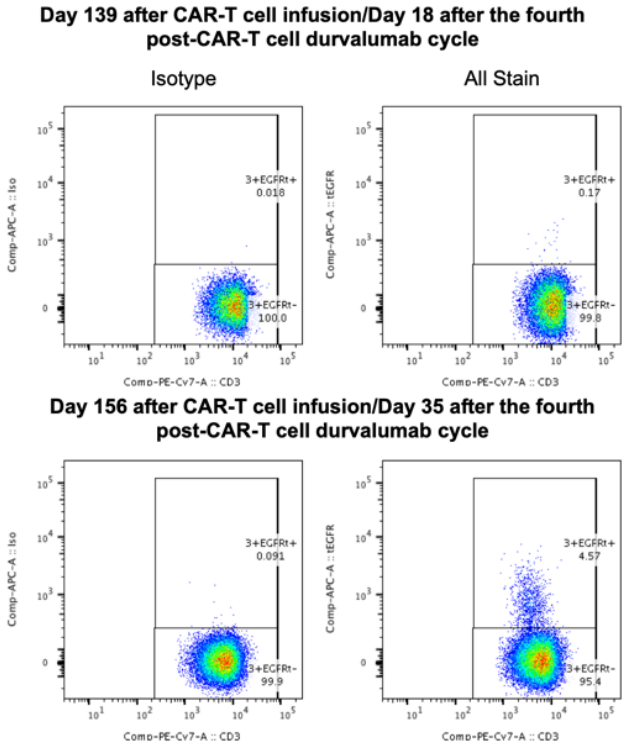

Figure S10. CAR-T cell *in vivo* re-expansion in the blood after durvalumab. Representative graphs gated on $CD3^+$ T cells showing an increase in the frequency of CAR-T cells detected by flow cytometry.

References

1. Hirayama AV, Chou CK, Miyazaki T, et al. A novel polymer-conjugated human IL-15 improves efficacy of CD19-targeted CAR-T cell immunotherapy. Blood Adv 2022. DOI: 10.1182/bloodadvances.2022008697.