SUPPLEMENTAL MATERIAL

Early quantification of anti-CD19 CAR T-cells by flow cytometry predicts response in r/r DLBCL

Short title: Immune monitoring of CAR T-cell kinetics in DLBCL

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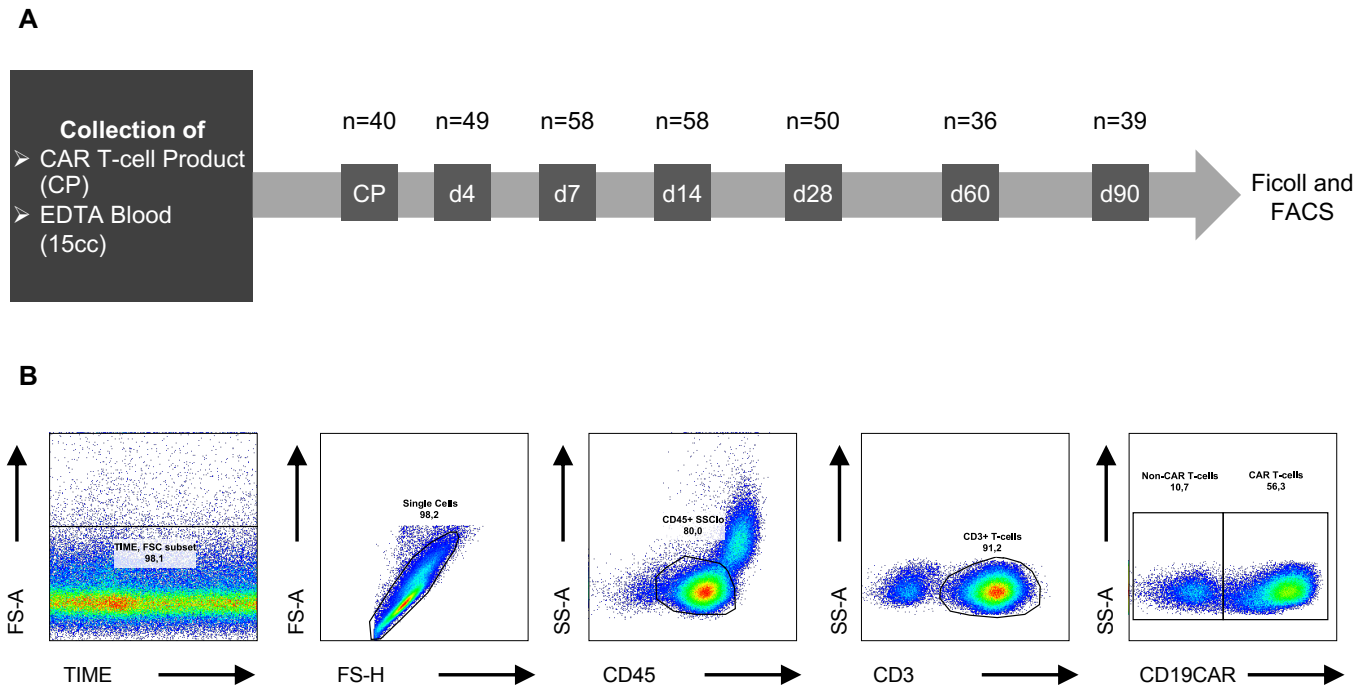
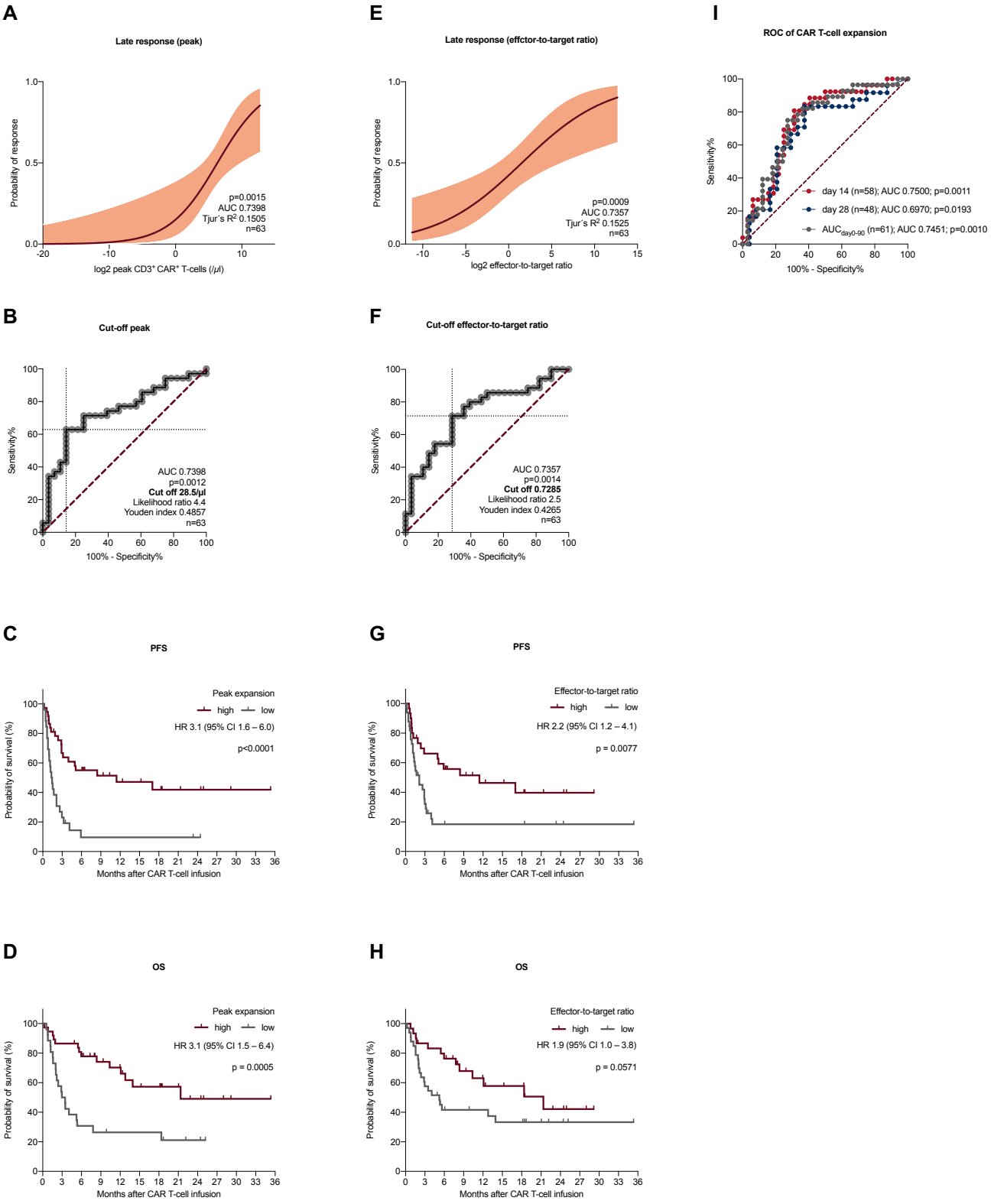
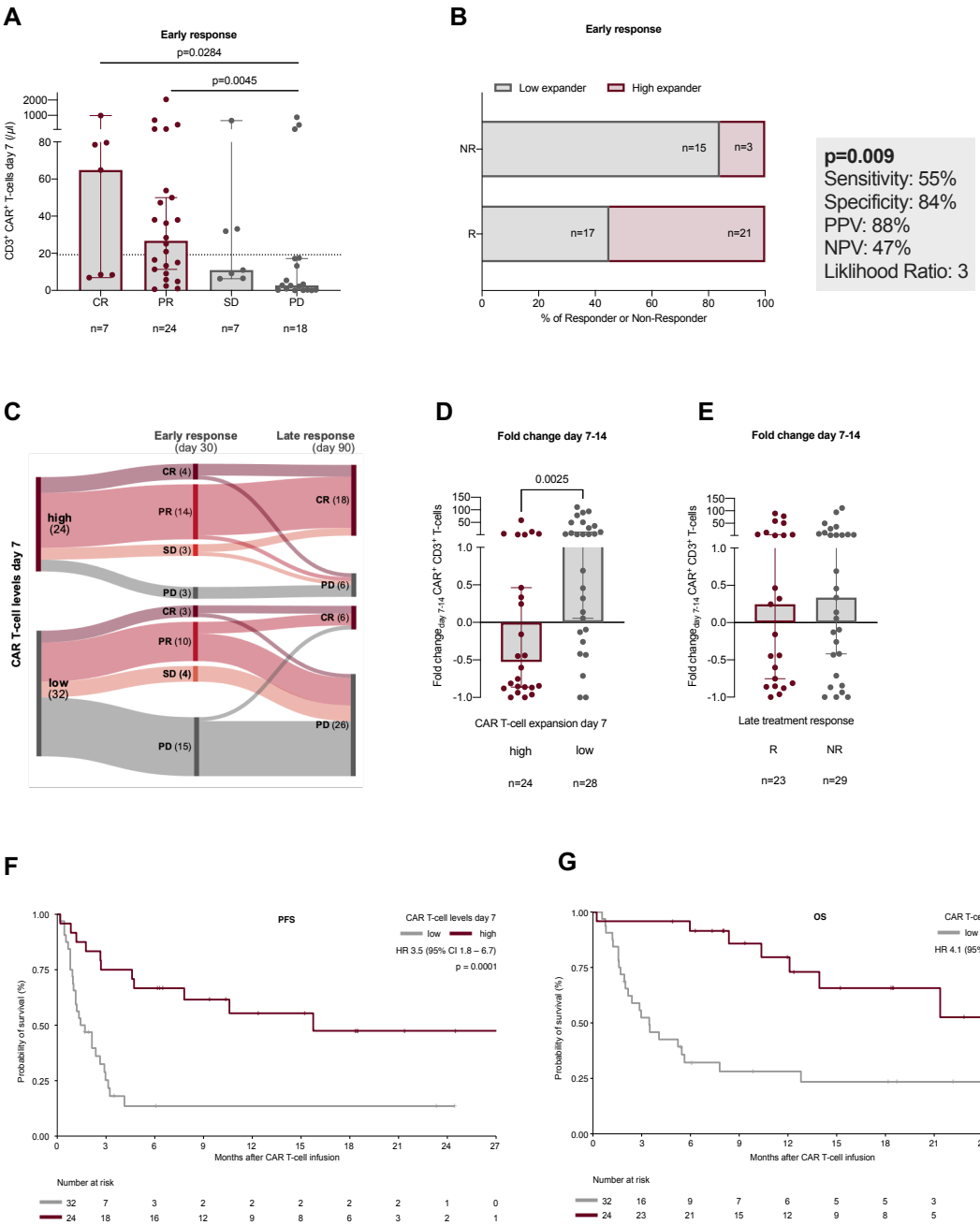


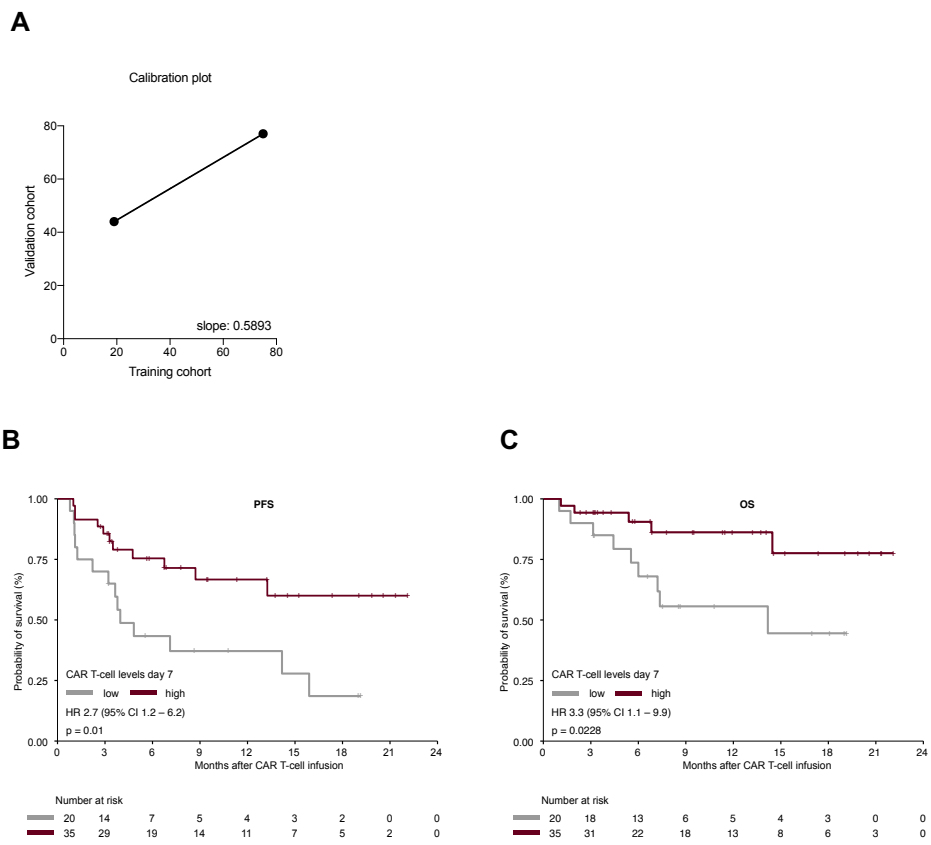
Supplemental Figure 1. Sample collection and gating strategy. (A) Sequential sample collection and longitudinal monitoring of CAR T-cells at two treatment sites. Range of time points: Apheresis product day -102 to day -28, day 2 to day 5, day 6 to day 9, day 12 to day 21, day 25 to day 34, day 46 to day 72, day 80 to day 105. (B) Gating strategy of anti-CD19 CAR^+ T-cells. Mononuclear cells were isolated from peripheral blood by Ficoll density gradient centrifugation. Cell surface antigens have been stained with fluorescence-conjugated monoclonal antibodies. CAR T-cells were detected with a sensitivity of 1:2000 (minimum required number of events: 100 000, minimum number of CAR^+ events: 0.05% or at least 20 events). To estimate the number of CAR^+ T-cells / µl blood we multiplied the relative frequency of CAR^+ T-cells of all $CD3^+$ T-cells with the number of peripheral lymphocytes / µl blood obtained from each patient´s differential blood test. All samples were analyzed using Navios (Beckman Coulter, LMU) and LSR Fortessa (BD Biosciences, Erlangen) and Navios EX (Beckman Coulter, Barcelona) instruments and FlowJo software (version 10.6.2.). Data was analyzed using Prism (version 9, GraphPad Software), R (version 4.1.3) and RStudio (version 1.4.1106).



Supplemental Figure 2. CAR T peak expansion and effector:target ratio are associated with treatment response and survival. Association of peak expansion (A) and E:T (E) with treatment response. *P* values from logistic regression analysis are shown. Absolute CAR T peak expansion (B) and E:T (F) according to treatment response. *P* values from receiving operator characteristics are shown. Association of peak expansion (C,D) and E:T (G, H) with PFS and OS recorded from day of CAR T-cell infusion. *P* values from log-rank tests are shown. Association of CAR T-cell levels at day 14 and day 28 and area under the curve of CAR T-cells from day 0 to day 90 according to treatment response (I). *P* values from receiving operator characteristics are shown.



Supplemental Figure 3. Predictive value of CAR T-cell levels at day 7 for early and late treatment response. Association of number of CAR T-cells at day 7 with remissions status at day 30 (A). CR denotes for complete remission, PR for partial remission, SD for stable disease, and PD for progressive disease. Only significant *P* values from Mann Whitney U tests are shown. Association of absolute number of CAR T-cells at day 7 with early treatment response at day 30 (B). Relative frequency of patients and the *P* value from Fisher's exact test are shown. (C) Association of high or low absolute CAR T-cell expansion at day 7 with remission status at days 30 and 90. The numbers of patients are given in parentheses. Median fold change of absolute CAR T frequencies between day 7 and day 14 comparing patients with high vs low CAR T-cell expansion at day 7 (D) and comparing R vs NR (E). *P* value from Mann-Whitney U test is shown. (F–G) Association of absolute number of CAR T-cells at day 7 with PFS (F) and OS (G) recorded from day of CAR T-cell infusion. *P* values from log-rank tests are shown. Time recorded from day of CAR T-cell infusion (day 0).



Supplemental Figure 4. CAR T-cell levels of > 19 cells/µl blood at day 7 post-infusion predicted treatment response in the validation cohort. (A) Calibration plot showing positive predictive values and 100 - negative predictive values on day 7 CAR T-cell levels of training and validation cohort (B–C) Association of absolute number of CAR T-cells at day 7 with PFS (B) and OS (C) recorded from day of CAR T-cell infusion. *P* values from log-rank tests are shown. Time recorded from day of CAR T-cell infusion (day 0).

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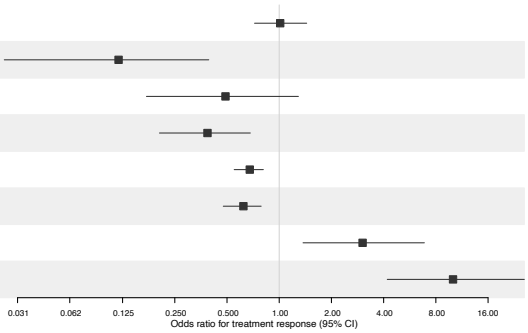
Variable	Odds ratio	P–value	
Prior therapies (median number)	1.01 (0.72–1.44)	0.9395	
ECOG (0–1 vs 2–4)	0.12 (0.03–0.30)	0.0002	***
Ann Arbor (I–II vs III–IV)	0.49 (0.17–1.29)	0.1510	
LDH (U/l)	0.39 (0.20–0.66)	0.0008	***
CRP (mg/dl)	0.68 (0.55–0.81)	0.0001	****
Ferritin (ng/ml)	0.62 (0.48–0.79)	0.0001	****
CAR T–cell product (Axi–cel vs Tisa–cel)	3.03 (1.37–6.87)	0.0058	**
CAR T–cell levels day 7 (≥ vs < 19/ul)	10.05 (4.20–25.89)	0.0001	****



Supplemental Figure 5. CAR T-cell expansion was not associated with CAR T-cell dose. (A) Univariate analysis of clinical characteristics of advanced-stage disease and inflammation at baseline obtained on the day of lymphodepletion as well as administered CAR T-cell product and CAR T-cell levels at day 7 with treatment response; n=104. The odds ratio, 95% confidence intervals (CI), and *P* values from a logistic regression analysis are shown.

SUPPLEMENTAL TABLES

	Munich - LMU				Erlangen			
Antigen	**Fluorochrome**	**Volume (µl)**	**Clone**	**Manufacturer**	**Fluorochrome**	**Volume (µl)**	**Clone**	**Manufacturer**
CD45	KrOrange	2.5	J33	Beckman Coulter	APC-H7	5	2D1	Becton Dickinson
CD3	FITC	10	UCHT1	Beckman Coulter	BUV737	3.5	UCHT1	Becton Dickinson
rCD19 / anti Biotin	PE	5 / 1	REA746	Miltenyi Biotec	PE	2 / 1	REA746	Miltenyi Biotec

Supplemental Table 1. Harmonized FACS panel between two treatment sites.

Variable		Training cohort (n = 63)	Validation cohort (n = 55)	Univariate
Age	Median (range in years)	64 (19–83)	60 (25–82)	0.23
Sex	Male, n (%)	38 (60)	31 (56)	0.71
ECOG	0–1, n (%)	45 (71)	52 (95)	0.001
Treatment history	Prior therapies, median number (range)	3 (2–9)	2 (2-4)	<0.0001
Tumor burden	Ann-Arbor III–IV, n (%)	49 (78)	42 (76)	0.99
	LDH > ULN prior to lymphodepletion, n (%)	39 (62)	31 (56)	0.58
CAR product	Tisa-cel, n (%)	40 (64)	18 (33)	0.001
	Axi-cel, n (%)	23 (36)	37 (67)	
CRS	Grade ≥ II, n (%)	25 (40)	20 (36)	0.62
ICANS	Grade ≥ II, n (%)	12 (19)	16 (30)	0.20
Survival	PFS, median (range in months)	3.0 (0.6–35.3)	14.2 (0.9–22.2)	0.01
	OS, median range in months)	12.1 (0.2–35.3)	Not reached (1–22.1)	0.01

Supplemental Table 2. A comparison of the training and validation cohorts- Key baseline characteristics, toxicity, and survival. Axi-cel, axicabtagene ciloleucel; CRS, cytokine release syndrome; ECOG, Eastern Cooperative Oncology Group; ICANS, immune-effector cell neurotoxicity syndrome; LDH, lactic acid dehydrogenase; OS, overall survival; PFS, progression-free survival; Tisa-cel, tisagenlecleucel. Response was assessed by PET/CT scan three months after infusion. Toxicity was graded according to the ASTCT consensus grading of Lee et al., 2019[56]. *P* values were calculated using the Mann–Whitney *U* test for continuous or Fisher's exact test for categorical variables and log-rank testing for survival data.