



La leucaferesi

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Terapia Cellulare e CAR-T
Humanitas Cancer Center
Rozzano (MI)

Hospital apheresis unit/cell lab provides:

Cryopreserved leukapheresis material

Novartis provides:

Applicable agreements needed with apheresis centers related to the collection, cryopreservation, and supply of autologous leukapheresis material

HOSPITAL



Supplier

Customer



NOVARTIS
MANUFACTURING

Hospital provides:

- Product request form
- Ordering process

Novartis provides:

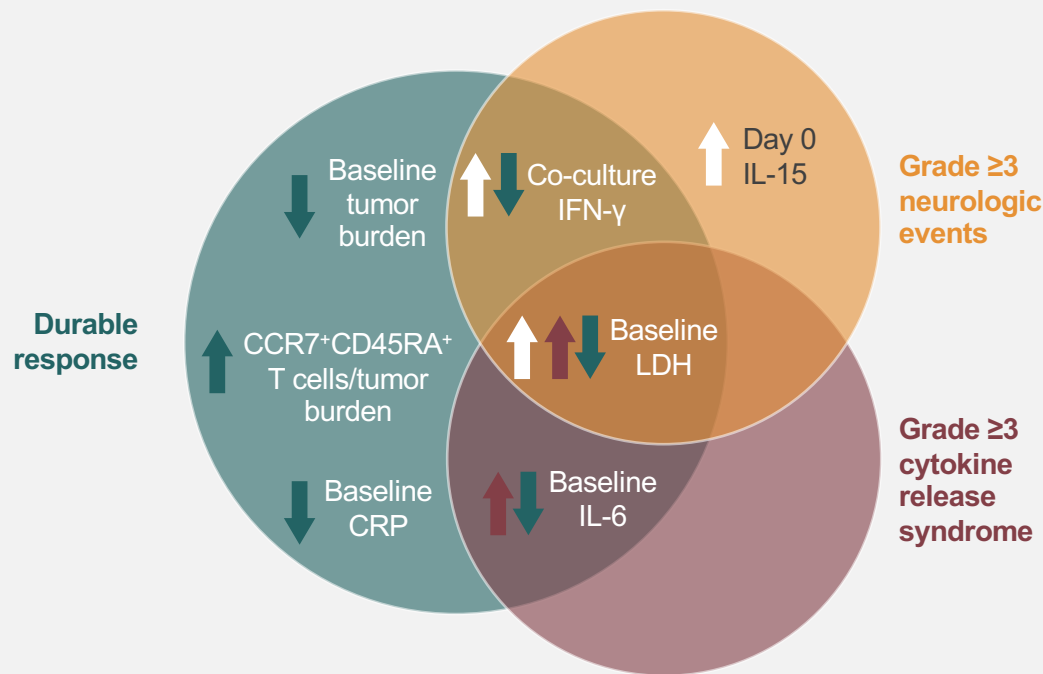
Finished product

Key points

- Axi-cel durable responses were associated with low baseline tumor burden, low systemic inflammation, and high product CCR7⁺ CD45RA⁺ T cells
- Distinct sets of factors associated with durable response, grade ≥3 cytokine release syndrome, and grade ≥3 neurologic events

Baseline systemic inflammation is negatively associated with both CAR-T cell expansion relative to pretreatment TB and the rate of durable responses

Distinct sets of factors associated with durable response and key grade ≥3 adverse events after axi-cel CAR-T cell therapy

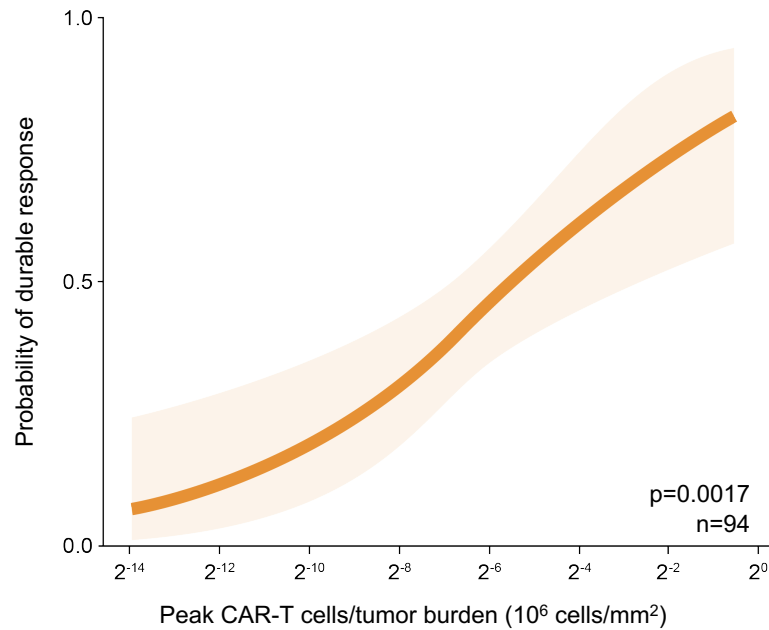


Arrows indicate directionality of association. For example, lower baseline tumor burden was positively associated with durable response

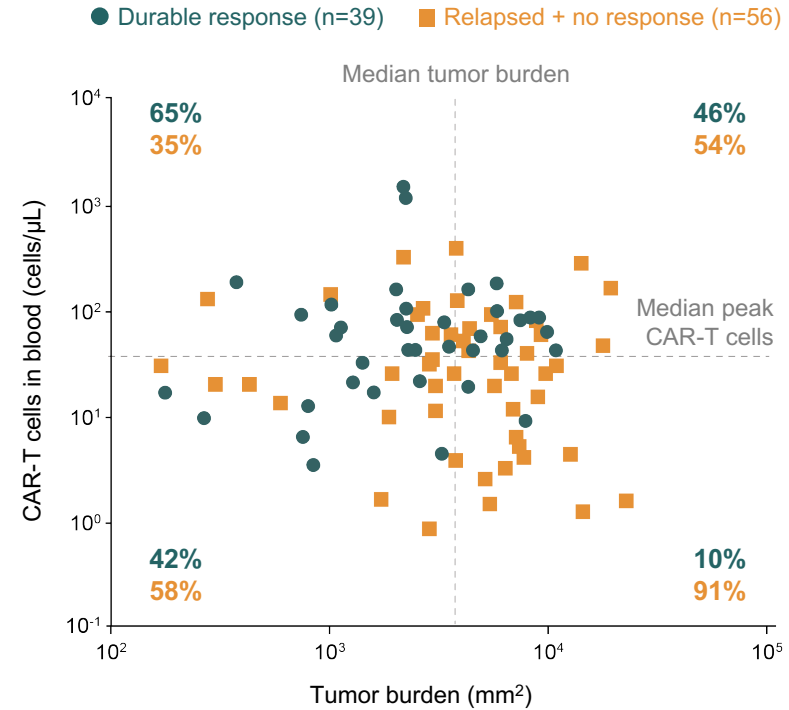
TB: tumor burden; CRP: C-reactive protein; IL: interleukin; IFN: interferon; LDH: lactate dehydrogenase

Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma

Logistic regression analysis

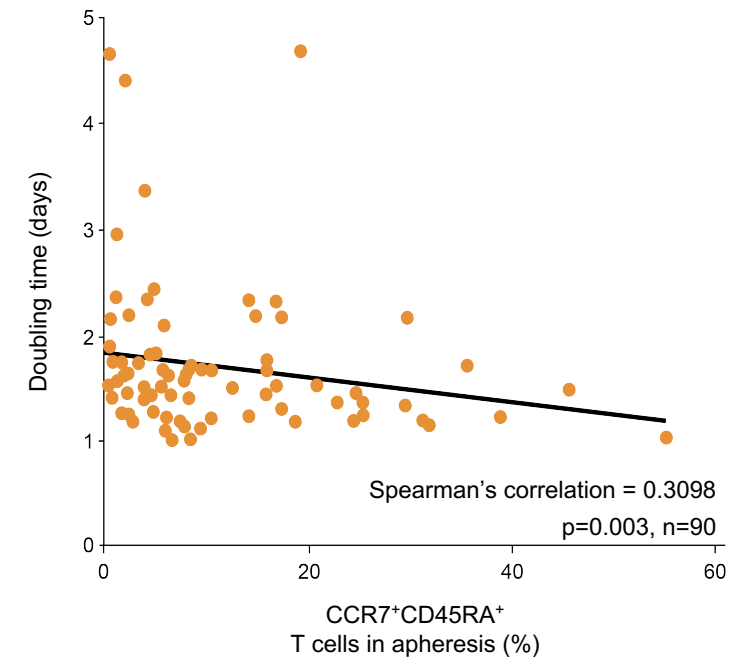
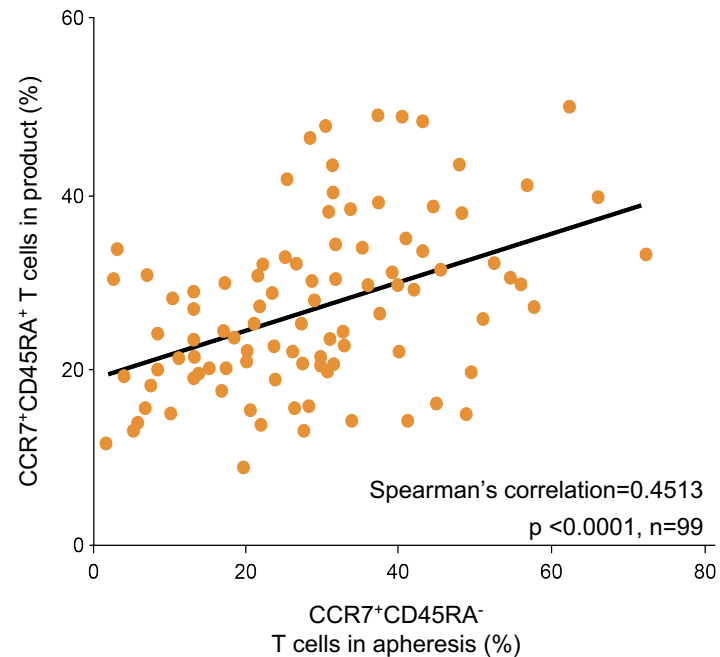
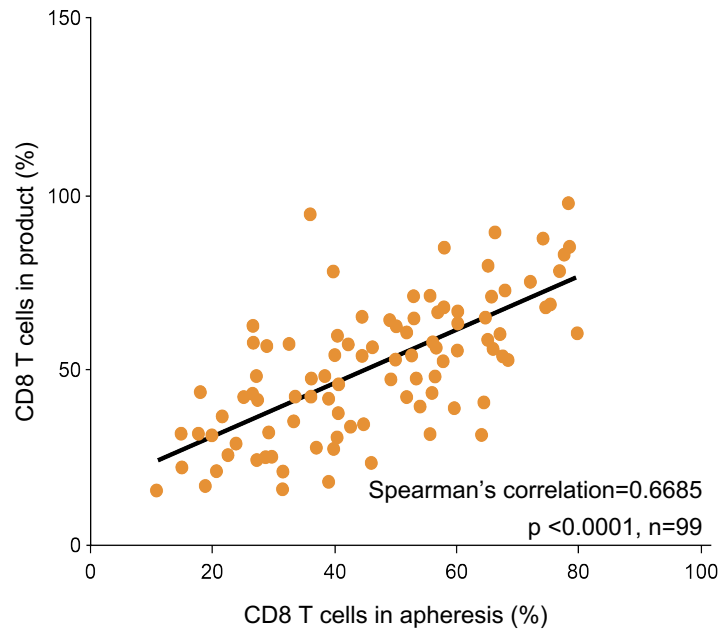


Peak expansion by tumor burden

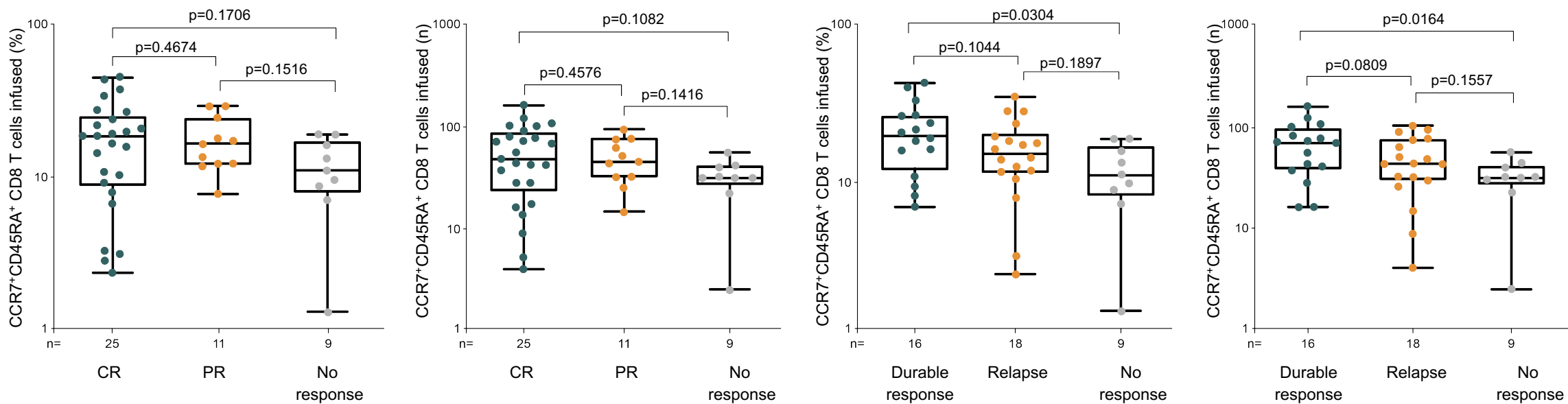


CAR-T cell expansion commensurate with baseline TB is associated with durable responses after axi-cel

T-cell phenotypes in apheresis material is associated with product phenotype and product doubling time

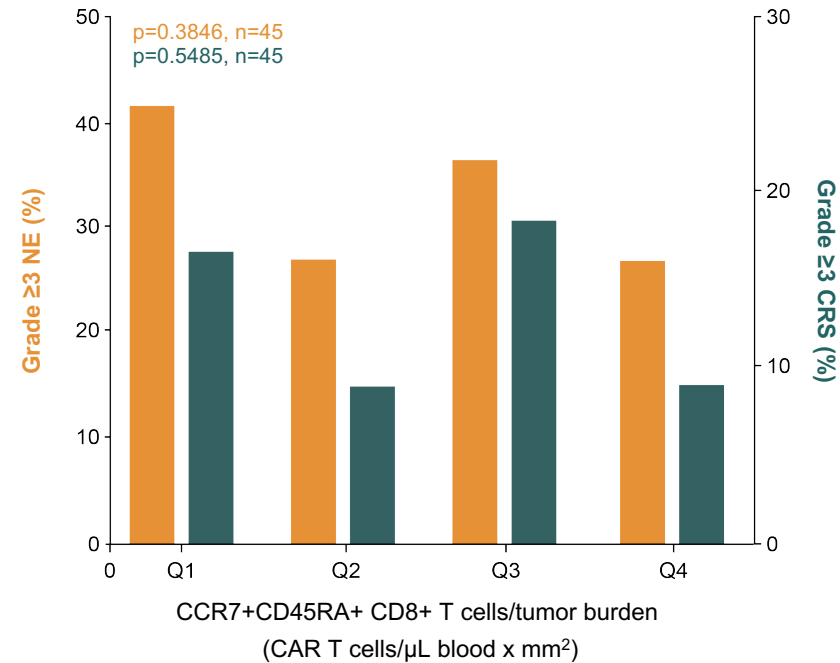
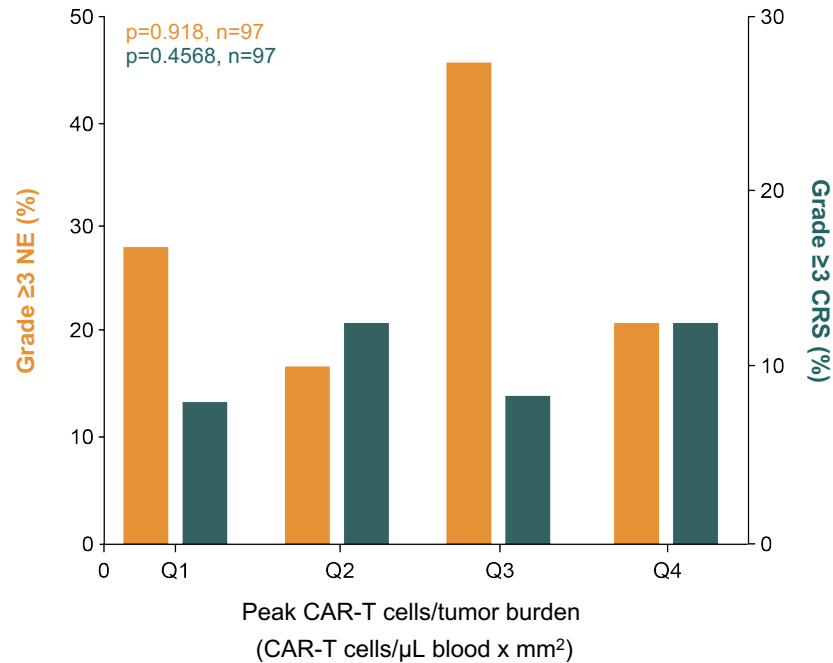


- The proportion of T cells with a more juvenile phenotype in the apheresis material directly associates with a lower product doubling time (DT). DT (before infusion) is associated with CAR-T expansion (after infusion)
- Frequency of CCR7⁺CD45RA⁺ T cells was negatively associated with DT



The number of CD8 and CCR7 CD45RA T cells commensurate with TB is critical to achieving durable response after axi-cel

CCR7⁺CD45RA⁺ T cells from peripheral blood differentiate in vitro into stem-like memory cells, provides a biological link for our observations that CCR7⁺CD45RA⁺ T cells in both the apheresis material and the product associate with DT and outcomes



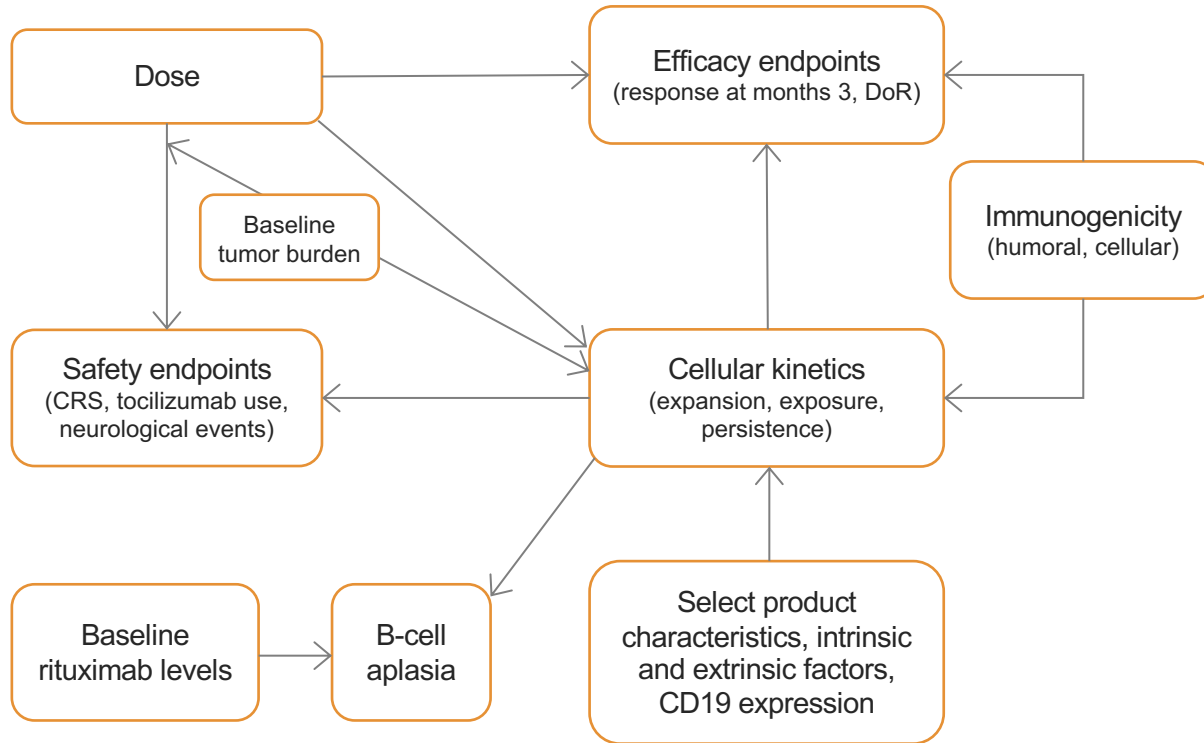
CAR-T cell levels normalized to either pretreatment TB or body weight, DT, and CD8 T cells or CCR7+CD45RA+ CD8 T cells normalized to TB were associated with efficacy but not with severe toxicities

NE: neurologic events; CRS: cytokine release syndrome

n, median (range)	Prior lines of therapy before enrollment on ZUMA-1			
	1–2 (n=31)	3 (n=29)	4 (n=29)	≥5 (n=12)
Tumor burden at baseline, SPD	31 3014 (180–12,795)	29 3355 (171–19,201)	28 4310 (268–23,297)	12 4448 (310–14,354)
Ferritin at baseline (mg/L)	27 567.2 (LLOQ–2752.2)	25 776.8 (LLOQ–5016)	27 1038 (LLOQ–10,576.1)	11 1174.8 (LLOQ–8795.1)
LDH at baseline (U/L)	31 329 (148–2105)	29 331 (153–2165)	29 320 (150–7802)	12 866 (116–3062)
Doubling time (days)	28 1.42 (1.04–3.37)	26 1.51 (1.11–2.37)	25 1.7 (1.11–4.67)	12 1.68 (1.26–4.67)
Transduction rate (%)	31 59.5 (22.4–85.1)	29 52 (25.5–72.4)	29 50.4 (34.2–76.4)	12 53.7 (21.6–67)
CCR7⁺ T cells (%)	31 48 (25.7–85)	29 42.4 (16.7–82.7)	29 42.1 (17.6–71.6)	11 37.4 (14.9–60.6)
CCR7⁺CD45RA⁺ T cells in product (%)	31 19.3 (4.9–76)	29 12.6 (3.4–52.8)	29 11.4 (1–52.2)	11 9.1 (1.6–38.9)
CCR7⁺CD45RA⁺ cells in product bag (x 10⁶ cells)	31 54.8 (10.6–215.0)	29 36.0 (11.3–158.0)	29 31.5 (2.1–200.6)	11 22.1 (5.5–110.1)

TB, markers of baseline inflammation, and DT increased with increasing number of lines of therapy, but the proportion and absolute numbers of CCR7⁺CD45RA⁺ cells decreased

SPD: sum of product diameters; LLOQ: lower limit of quantification



No relationship between dose and peak expansion or exposure

Summary of peripheral blood cellular kinetic parameters by flow cytometry for tisagenlecleucel by response at month 3

Parameter	CR/PR	SD/PD/unknown
AUC_{0-28d}, % CD3⁺CAR⁺ cells* x d	n=35**	n=44**
Geometric mean (% CV)	36.9 (214.7)	42.0 (299.3)
C_{max}, % CD3⁺CAR⁺ cells*	n=34	n=50
Geometric mean (% CV)	4.81 (169.7)	4.18 (232.9)
Range	(0.600–40.9)	(0.300–61.5)
t_{max}, d	n=34	n=50
Median (range)	6.35 (2.91–271)	7.64 (2.82–25.9)
C_{last}, % CD3⁺CAR⁺ cells*	n=35	n=50
Geometric mean (% CV)	0.289 (165.2)	0.539 (313.2)
t_{last}, d	n=35	n=50
Median (range)	280 (21–554)	28.1 (9.01–400)

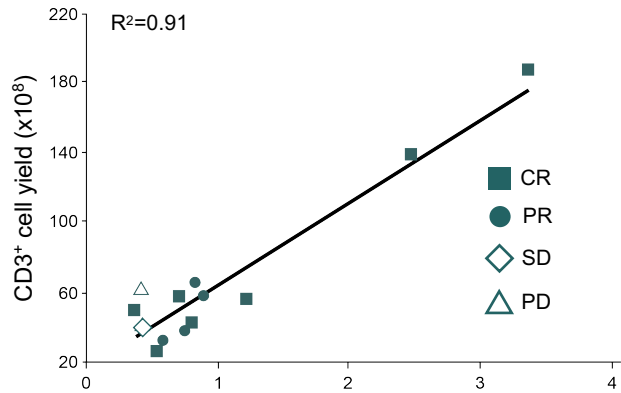
* Percentage of CAR⁺ cells among CD3⁺ T cells

**Patients who had ≥1 sample with evaluable cellular kinetics data were included

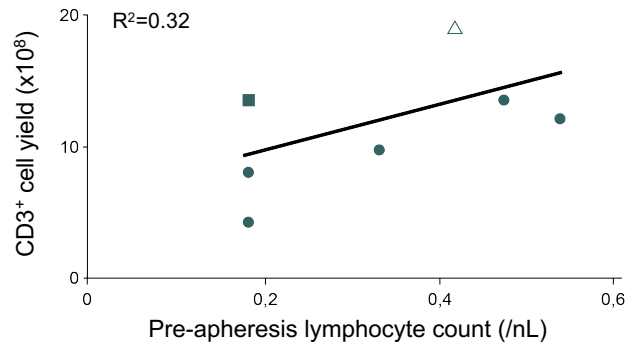
CV: coefficient of variation; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; DoR: duration of response

Overview pre-leukapheresis lymphocyte count and CD3+ cell yield by response (axi-cell)

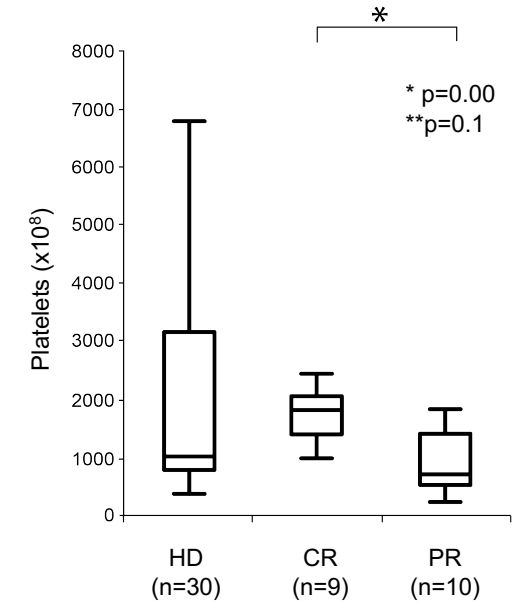
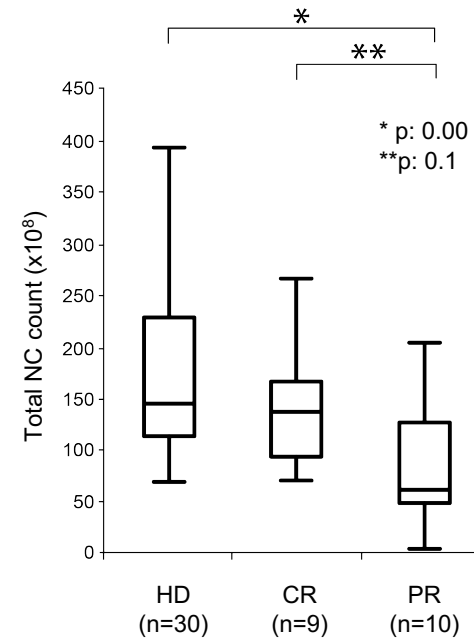
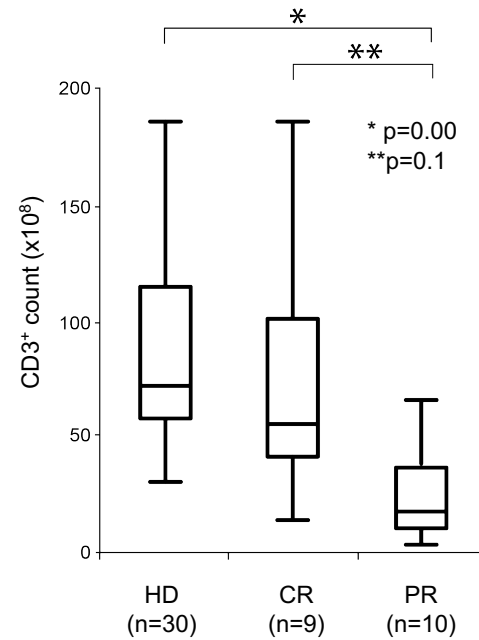
CD3+ cell yield of >20 (x10⁸)

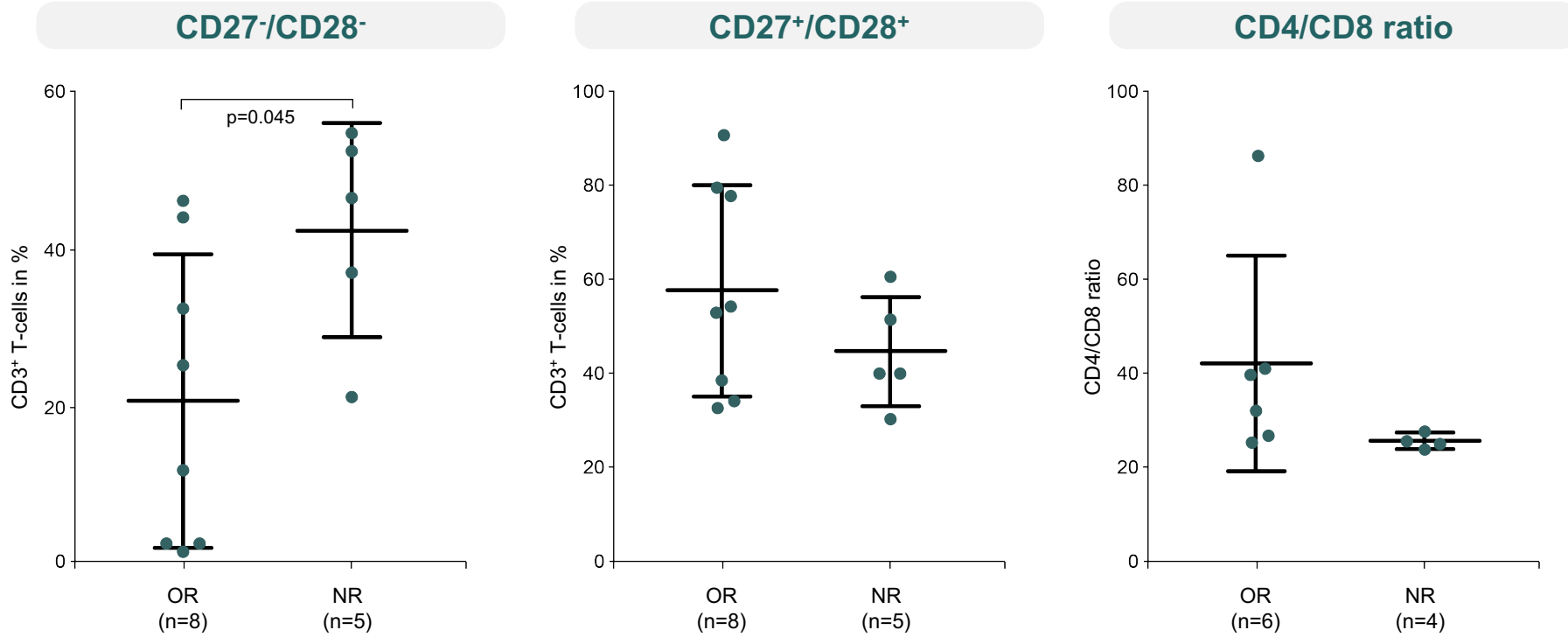


CD3+ cell yield of ≤20 (x10⁸)



Comparison of leukapheresis products from healthy donors and patients by remission status



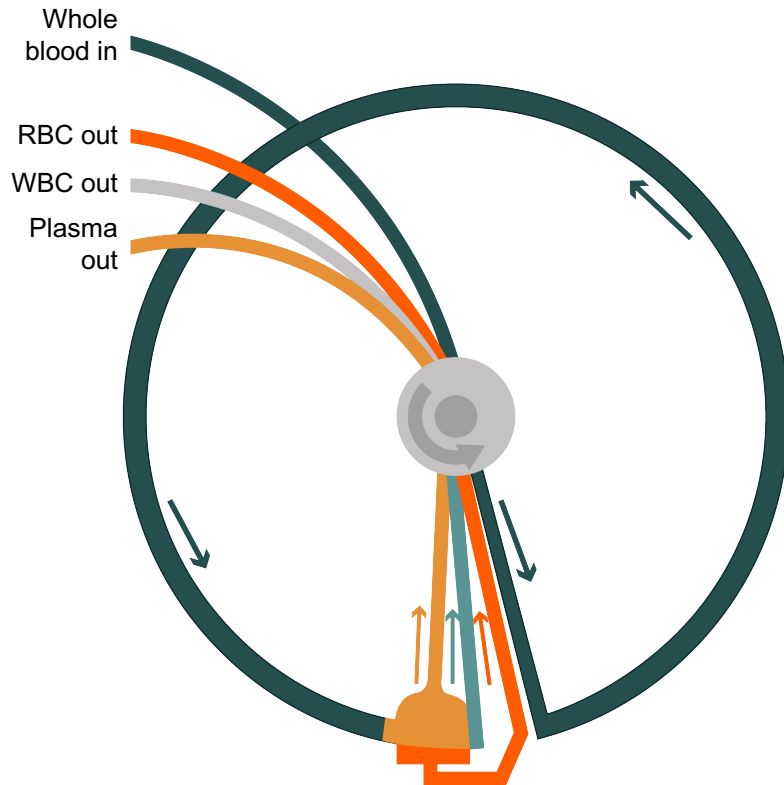


Individuals with a lower percentage of more differentiated, senescent or exhausted T cells are more likely to respond to CAR-T

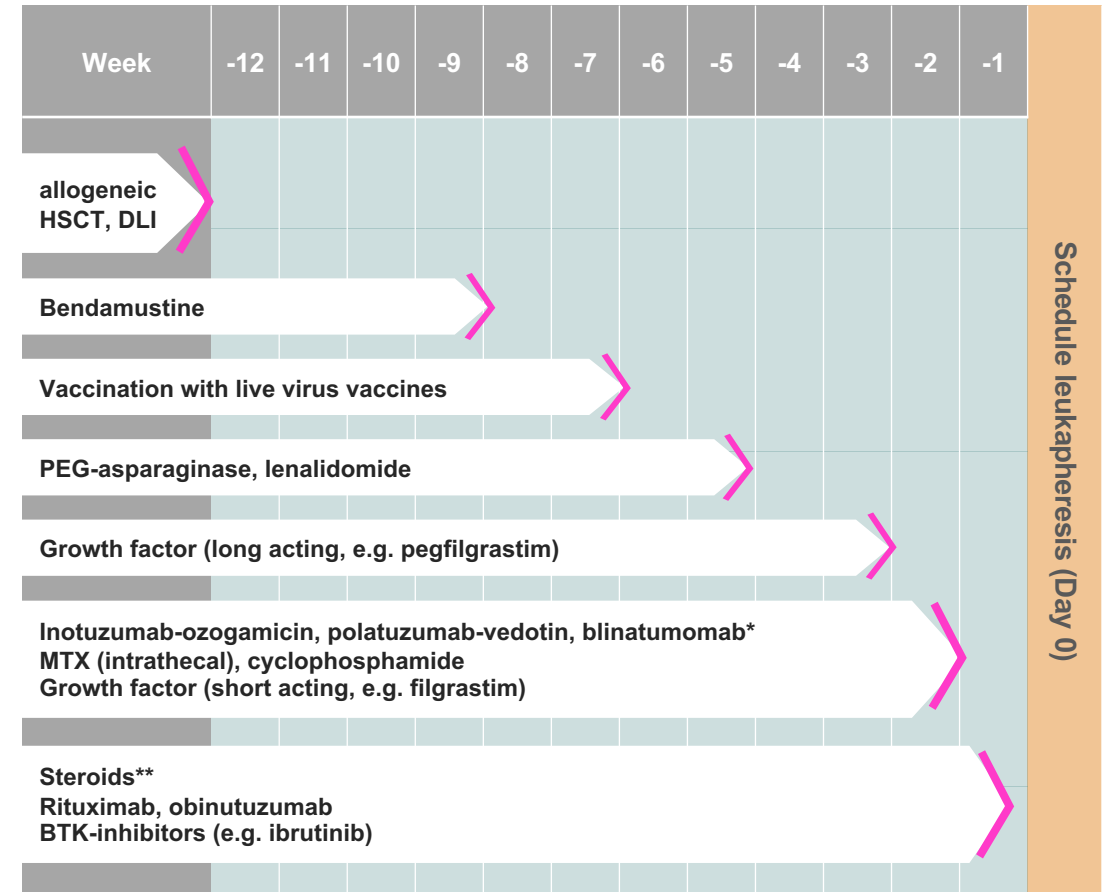
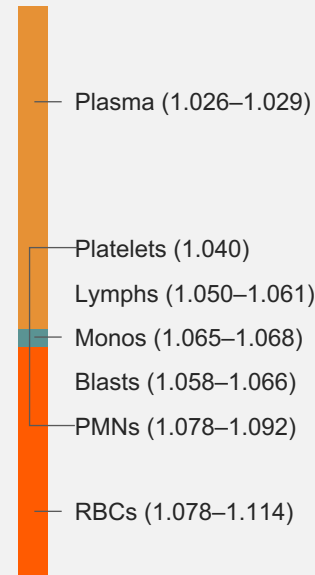
A significantly lower frequency of CD27-/CD28- T cells was found in responders (n=8) compared to non-responders (n=5)

OR: responder; NR: non-responder

Separation by centrifugation



Specific gravity



NHL: non-Hodgkin lymphoma; ALL: acute lymphoblastic leukemia; WBC: white blood cell; RBC: red blood cell; PMS: polymorphonuclear leukocytes; HSCT: hematopoietic stem cell transplantation; DLI: donor lymphocyte infusion; MTX: methotrexate