AMERICAN SOCIETY of GENE & CELL THERAPY

CAR T and Related Immune Effector Cell Therapies Workshop

Co-chairs: Cameron Turtle and Marcela Maus

Session 1: Clinical long-term follow-up

Session 2: Novel engineering and gene editing

Session 3: Beyond autologous CAR-T cells for cancer

Factors impacting duration of response after CD19 CAR-T cells for adult B-cell ALL and NHL

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Disclosures

- Research funding
  - Juno Therapeutics
  - Nektar Therapeutics
- Intellectual property
  - Patents – pending and/or licensed to Juno Therapeutics/Celgene and Nektar Therapeutics
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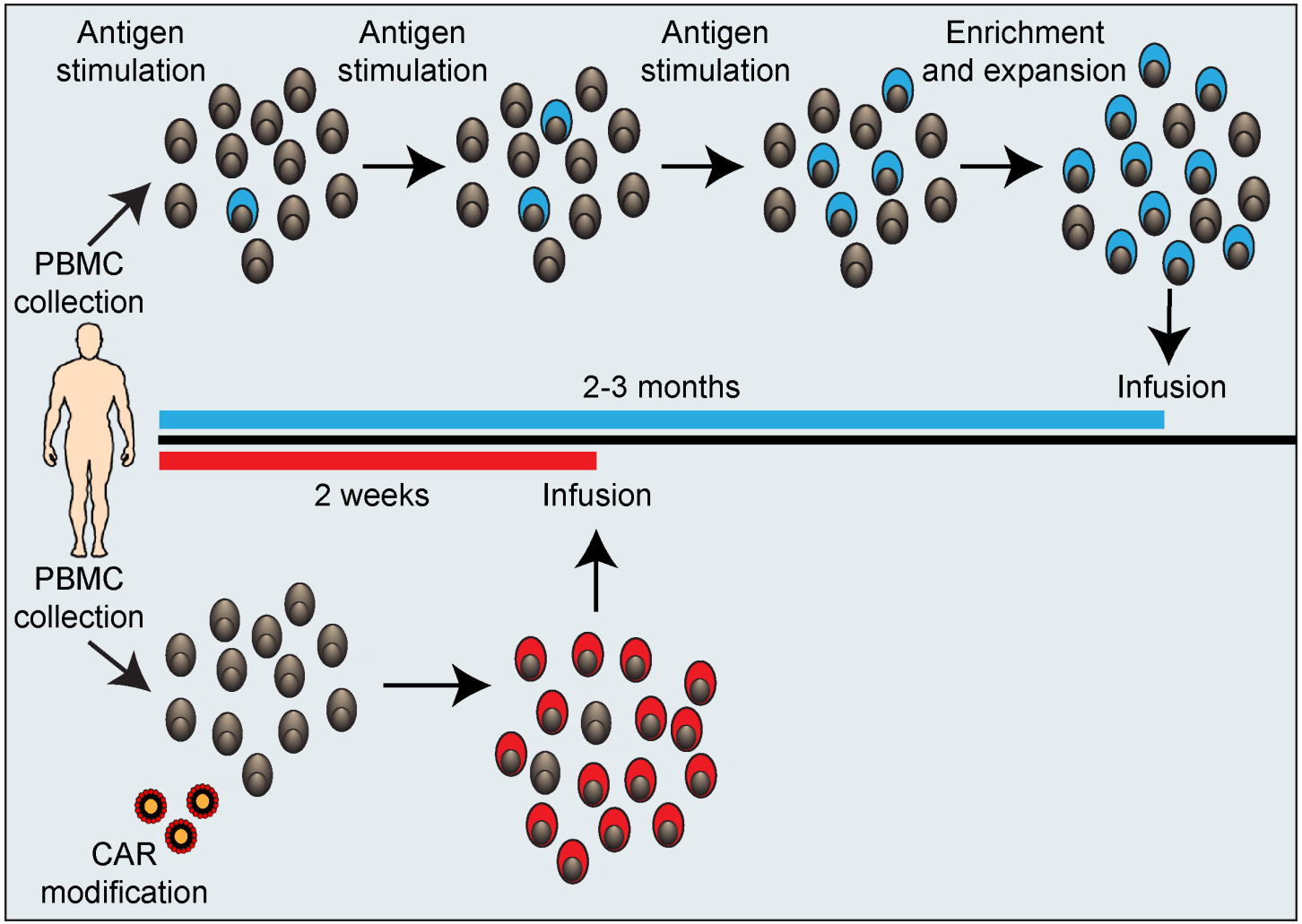
Redirection of T cell specificity by genetic modification

Repetitive antigen stimulation

Genetic redirection of the specificity of competent T cells to tumors



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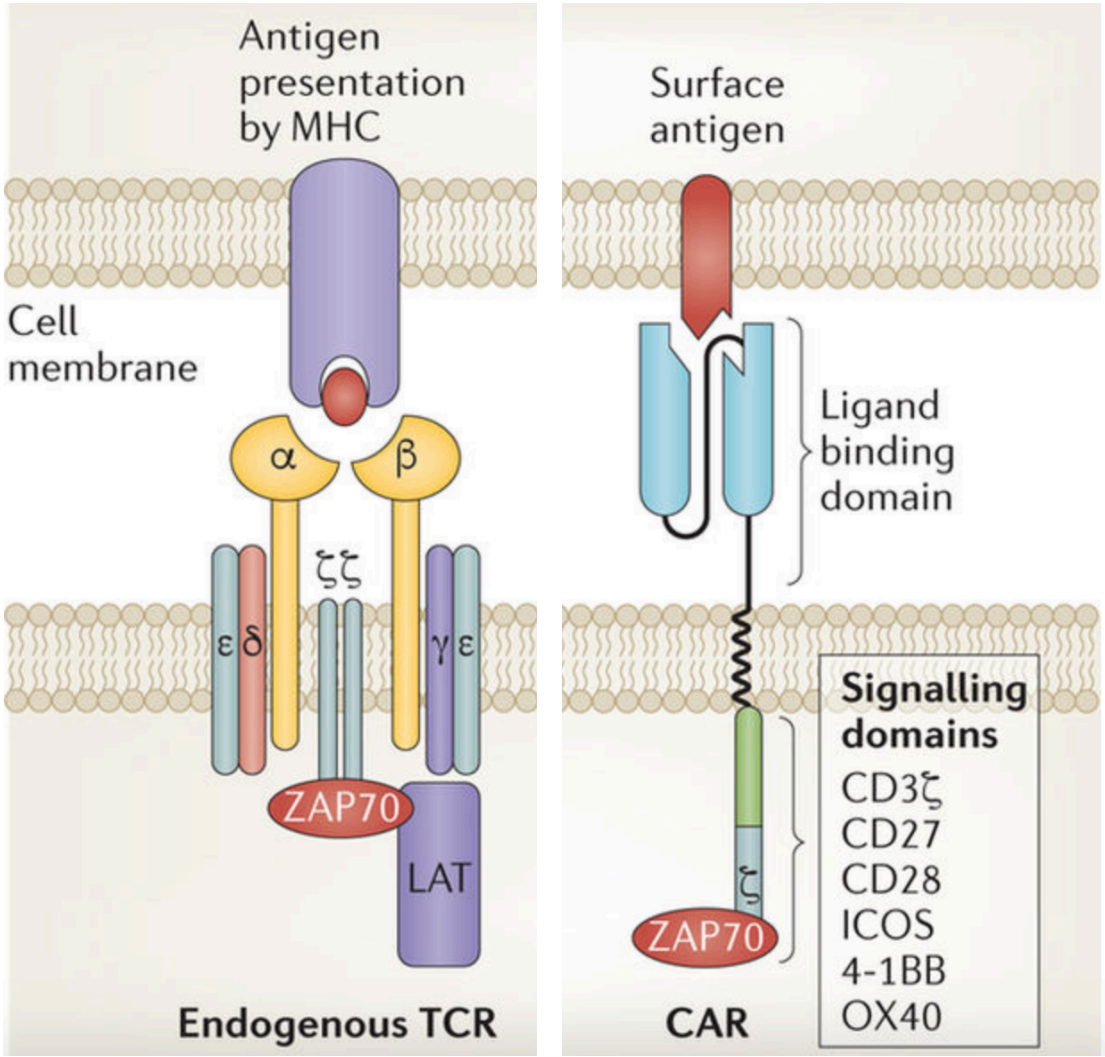
Turtle. Int J Heme, 2013

Structure of native T cell receptors and recombinant chimeric antigen receptors

Tumor cell

T cell

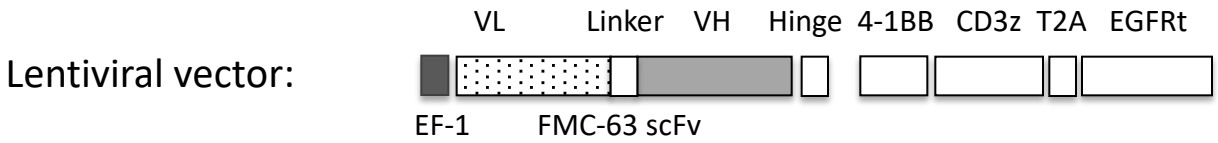


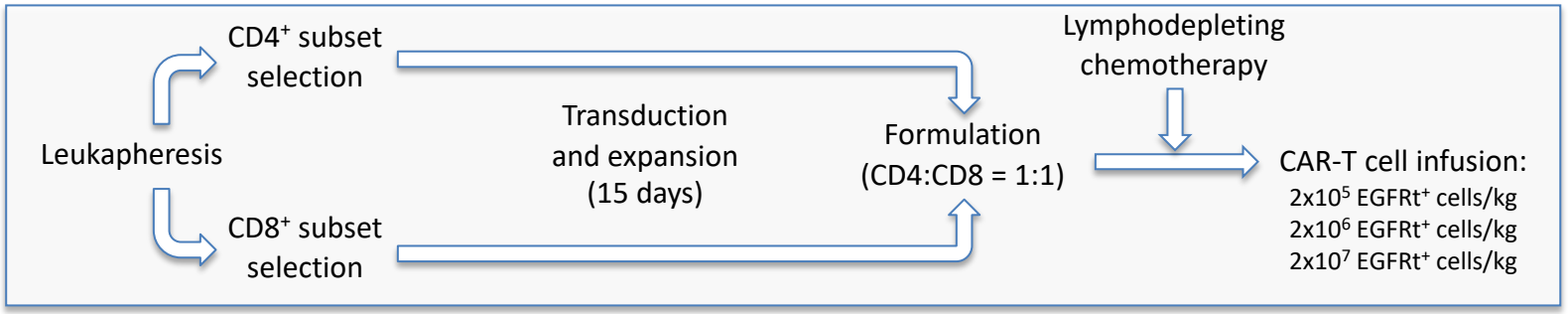
CARs

- Target surface molecules
- No HLA restriction
- 'In-line' costimulation
- Engineered cell subsets can be redirected to an appropriate target antigen

Fesnak, Nat Rev Cancer, 2016

Clinical trial of defined composition CD19 CAR-T cells for B cell malignancies





Study Objectives

- Safety
- Feasibility of manufacturing

Eligibility

- R/R CD19$^+$ B cell malignancy (B-ALL, NHL, CLL)
- ≥ 18 years
- No inclusion/exclusion based on: ALC, circulating tumor, transplant, test expansion

196 treated

ALL, n=65

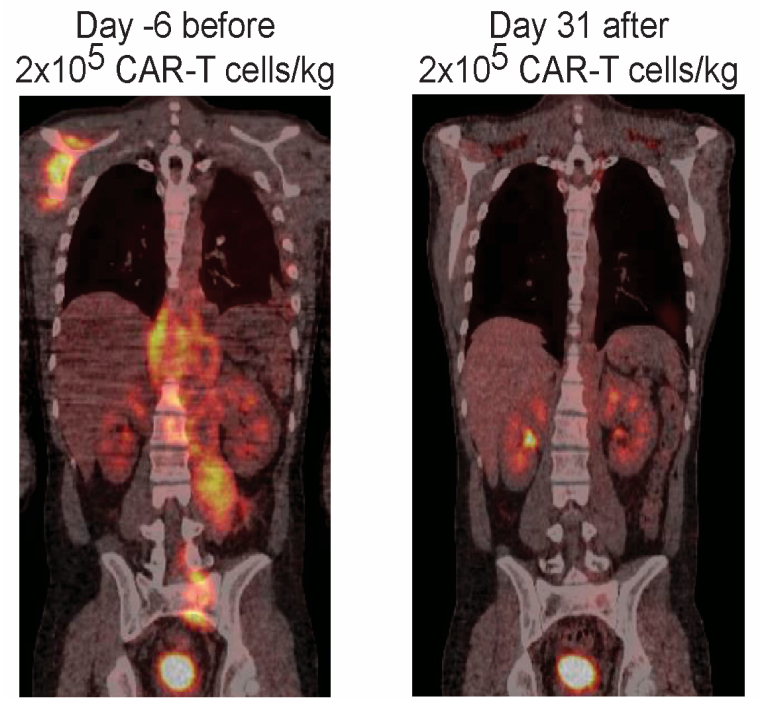
NHL, n=84

CLL, n=47

Turtle et al, J Clin Invest, 2016; Gardner et al, Blood, 2016; Turtle et al, Sci Trans Med, 2016; Turtle et al, J Clin Oncol, 2017; Gust et al, Cancer Discovery, 2017; Hay et al, Blood, 2017; Hill et al, Blood, 2018; Hay et al, Blood, 2019; Hirayama et al, Blood, 2019

Anti-tumor efficacy of CD19 CAR-T cells in B-ALL, NHL and CLL patients

- B-ALL
  - MRD-negative (flow) CR in 94%
  - IGHseq-negative in 65%
- NHL: Cy/Flu and $2x10^6$ CAR-T cells/kg
  - ORR 80%
  - CR 50%
- CLL: Ibrutinib-refractory: Cy/Flu and $\leq 2x10^6$ CAR-T cells/kg
  - BM flow-negative: 86%
  - ORR by IWCLL imaging (CT): 69%
  - CR by IWCLL imaging (CT): 25%
  - CR by Lugano (PET-CT): 67%



<u>Phase 1 lessons</u>: Infused CAR-T cell dose, disease burden, the immune response, and the lymphodepletion regimen impact CAR-T cell counts, anti-tumor response, and toxicity

Turtle et al, J Clin Invest, 2016; Gardner et al, Blood, 2016; Turtle et al, Sci Trans Med, 2016; Turtle et al, J Clin Oncol, 2017; Gust et al, Cancer Discovery, 2017; Hay et al, Blood, 2017; Hill et al, Blood, 2018; Hay et al, Blood, 2019; Hirayama et al, Blood, 2019

Acute lymphoblastic leukemia

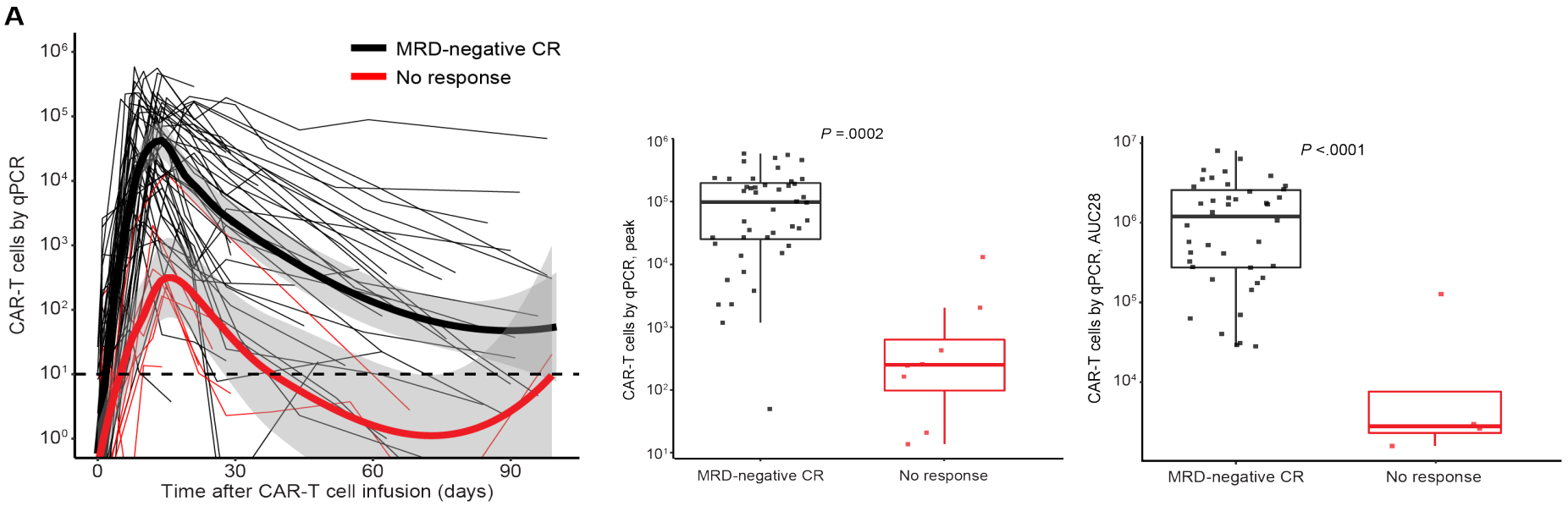
Responses after CD19 CAR-T cells for adult ALL

	Fred Hutch[1]	ZUMA-3 (axi-cel)[2]	MSKCC (19-28z)[3]
BM MRD-negative CR by flow (%)	85%*	75%	67%

Data from patients (n=53) who received ≤ 2 x 10^6 CAR-T cells/kg (MTD) in the phase 1 study and the subsequent expanded cohort

[1]Hay K et al, Blood. 2019;
[2]Wierda et al. ASH abstract. 2018; [3]Park et al. NEJM. 2018

Better CAR-T cell expansion *in vivo* in patients achieving MRD-negative CR

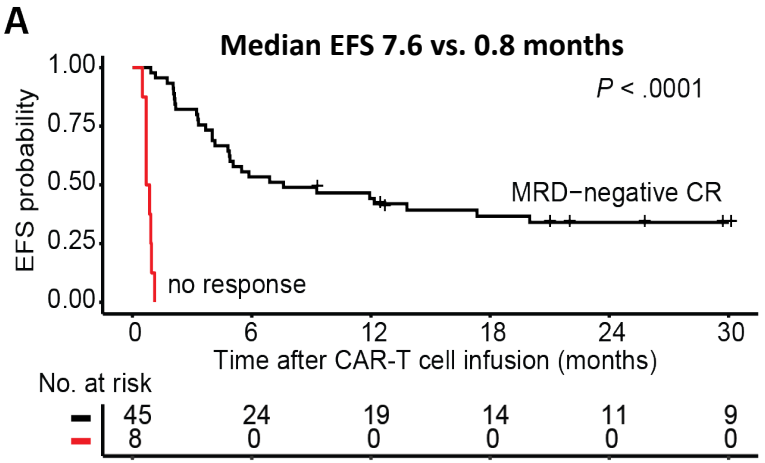


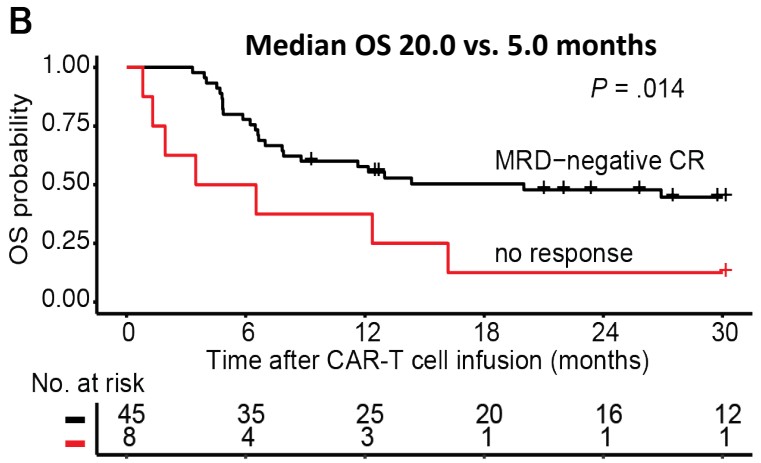
*All had flow-based marrow and/or extramedullary disease before therapy

Hay et al, Blood, 2019

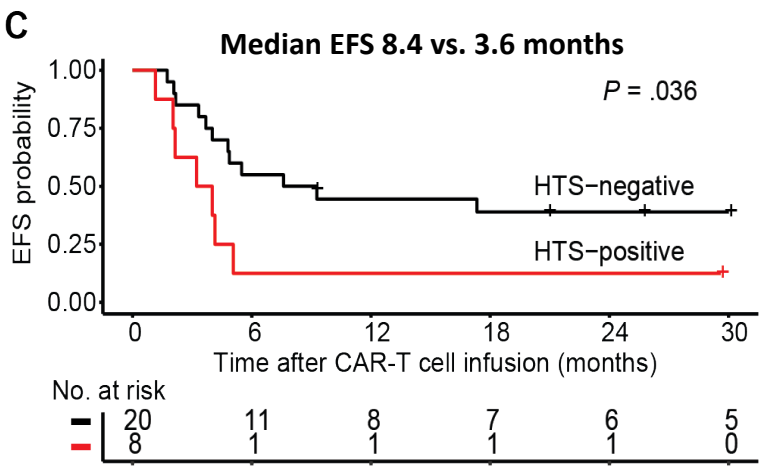
Depth of remission after CD19 CAR-T cells is associated with event-free survival in B-ALL patients

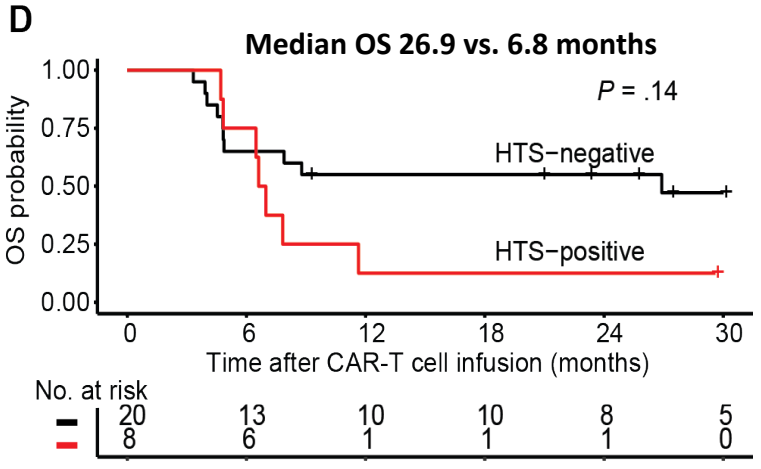
All pts (median F/U 30 mos)





Pts in MRD-neg CR by flow

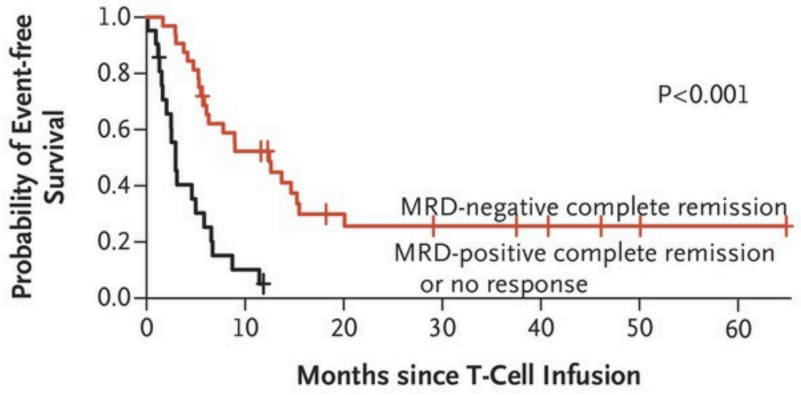




Hay et al, Blood, 2019

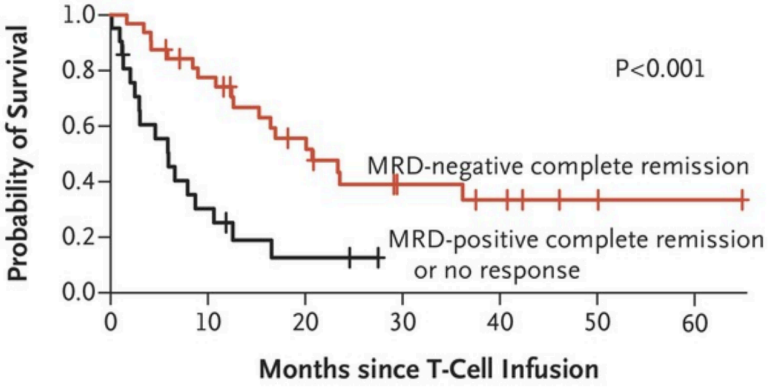
Survival after 28z CD19 CAR-T cells for adult ALL

Event-free Survival, According to MRD Status and Response



No. at Risk							
MRD-negative complete response	32	16	7	5	4	2	1
MRD-positive complete response or no response	21	2	0	0	0	0	0

Overall Survival, According to MRD Status and Response



No. at Risk							
MRD-negative complete response	32	23	14	7	5	2	1
MRD-positive complete response or no response	21	6	2	0	0	0	0

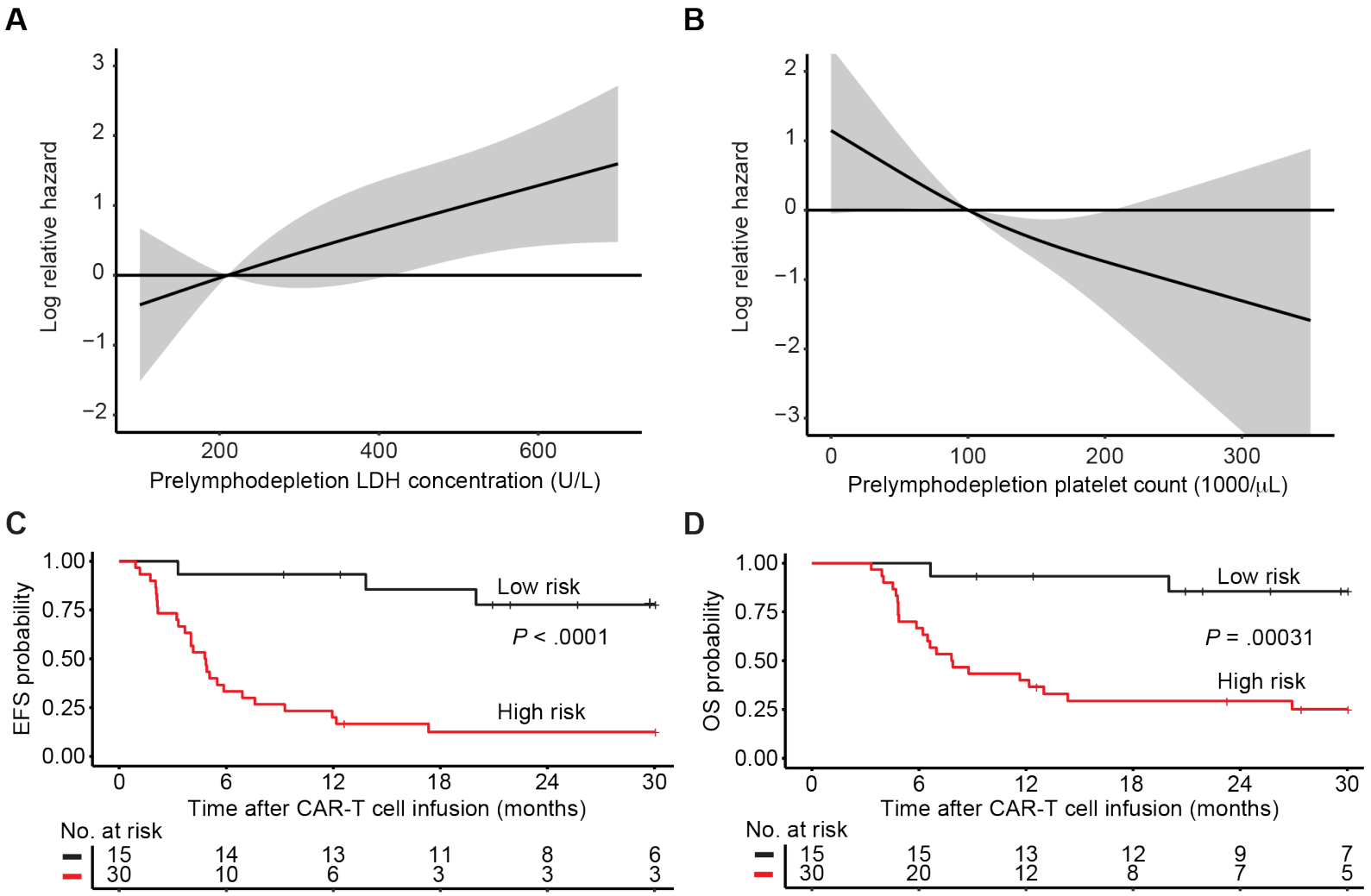
Park et al, NEJM, 2018

Stepwise multivariable analysis of factors impacting EFS after achieving MRD-negative CR

Variable	Univariate HR (95% CI)	*P* value	Multivariable HR (95% CI)	*P* value
LDH (per 100 U/L, pre-lymphodepletion)	1.49 (1.22-1.80)	<.0001	1.39 (1.12-1.74)	.003
Bridging systemic therapy[a]	5.66 (2.56-12.5)	<.0001	-[b]	-
Platelet count (per 50,000/μL, pre-lymphodepletion)	0.57 (0.42-0.76)	.0002	0.65 (0.47-0.88)	.006
Extramedullary disease (Y)	3.57 (1.66-7.65)	.001	-	-
Fludarabine added to lymphodepletion (Y)	0.30 (0.13-0.66)	.003	0.34 (0.15-0.78)	.011
IL-6 (pg/mL, pre-lymphodepletion)	1.02 (1.01-1.03)	.005	-	-
Marrow blasts by flow cytometry (%)	1.01 (1.00-1.03)	.006	-	-
Neutrophil count (1000/μL, pre-lymphodepletion)	0.73 (0.55-0.97)	.03	-	-
Soluble TNFRp55 (pg/mL, Day 0)	4.84 (1.07-21.8)	.04	-[c]	-
IL-2 (pg/mL, Day 0)	3.24 (1.05-10.0)	.04	-	-
IL-8 (pg/mL, pre-lymphodepletion)	1.78 (1.00-3.15)	.05	-	-
Soluble TIM-3 (ng/mL, pre-lymphodepletion)	1.05 (1.00-1.11)	.06	-	-
MLL rearrangement (Y)	2.19 (0.95-5.06)	.07	-	-
Dose level ($2x10^5$ vs $2x10^6$ CAR-T cells/kg)	0.51 (0.24-1.11)	.09	-	-
Prior regimens (n)	1.13 (0.97-1.32)	.1	-	-
Prior allogeneic hematopoietic cell transplantation (Y)	1.65 (0.79-3.44)	.2	-	-
Philadelphia chromosome-positive	0.68 (0.26-1.78)	.4		
Prior blinatumomab therapy	1.27 (0.52-3.12)	.6		
ECOG performance status (n)	1.18 (0.62-2.26)	.6	-	-
Age (years)	1.00 (0.98-1.01)	.7	-	-
CAR-T cell counts (transgene log_{10} copies/μg DNA, AUC28)	0.98 (0.56-1.71)	.9	-	-

Hay et al, Blood, 2019

Patients with normal LDH and platelets ≥100 who receive Cy/Flu (Low risk) have better EFS and OS

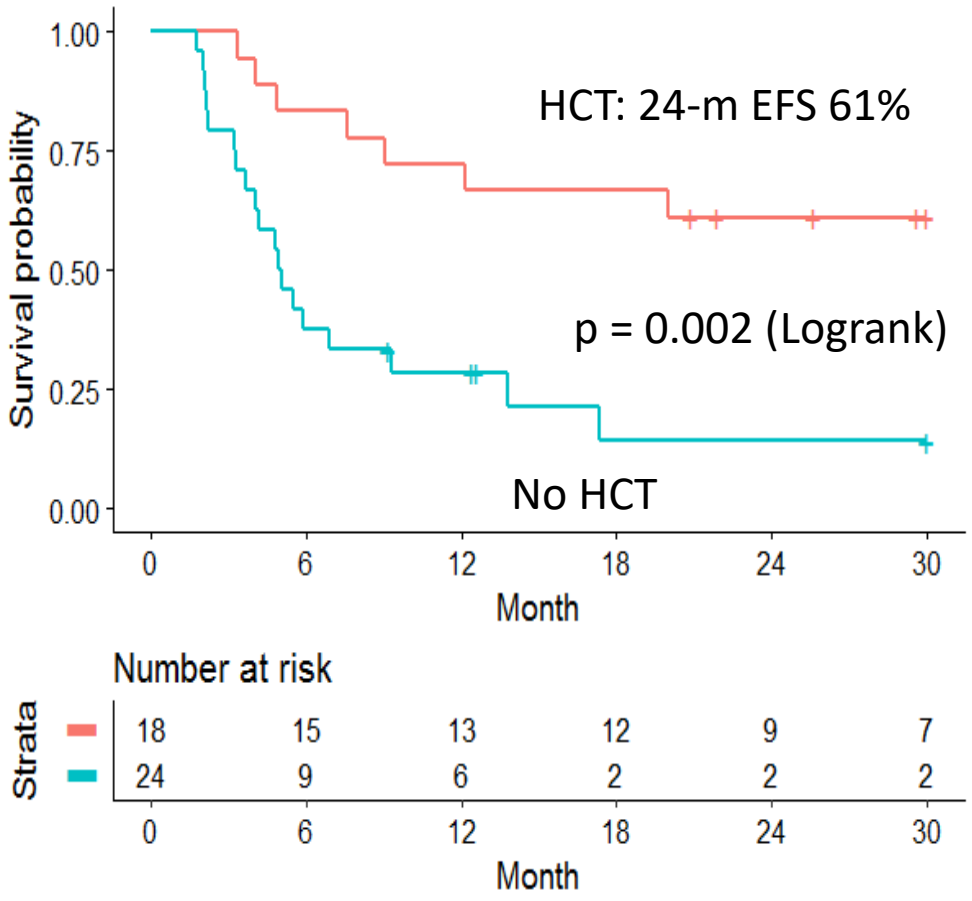


40% of pts in MRD-negative CR underwent allo-HCT; not censored at allo-HSCT

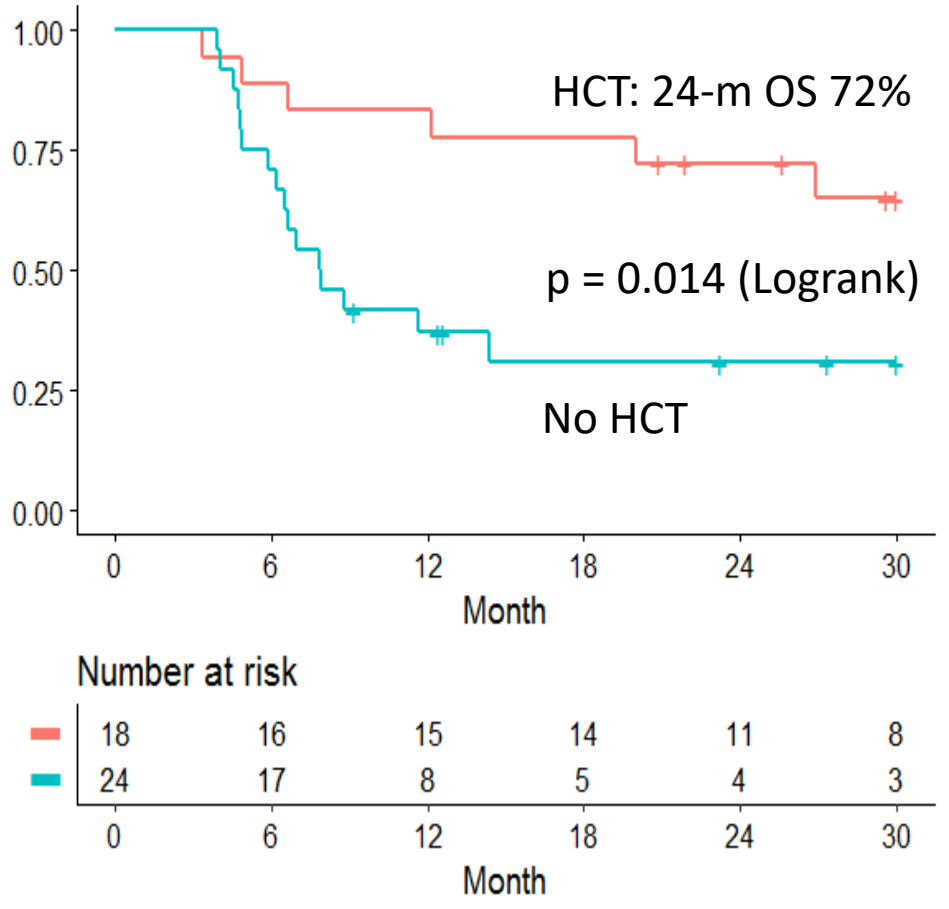
Hay et al, Blood, 2019

Allogeneic HSCT while in MRD-negative CR after CD19 CAR-T cells may improve EFS and OS

EFS



OS

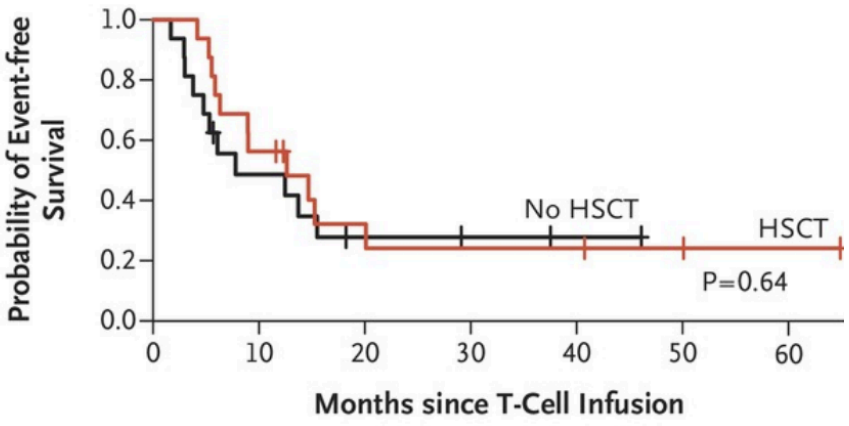


Similar findings were noted on analysis of patients with no prior HCT history

Hay et al, Blood, 2019

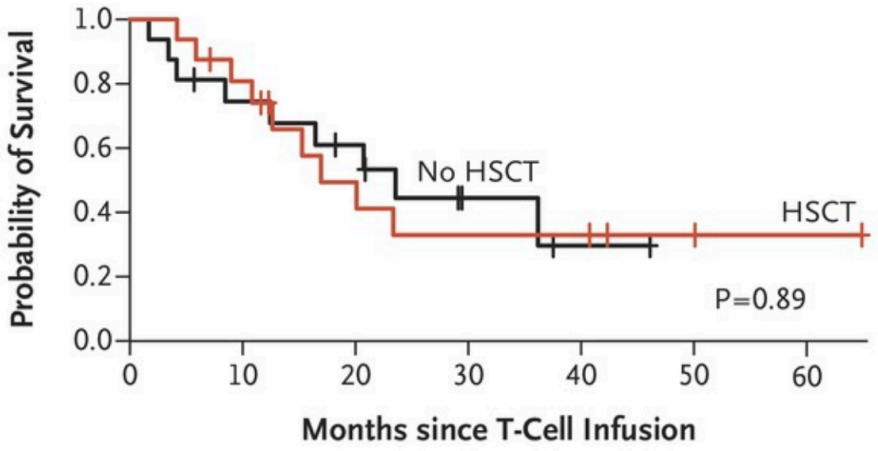
Survival after 28z CAR-T cells for adult ALL

EFS



No. at Risk							
No HSCT	16	7	3	2	1	0	0
HSCT	16	9	4	3	3	2	1

OS



No. at Risk							
No HSCT	16	11	8	3	1	0	0
HSCT	16	12	6	4	4	2	1

Park et al, NEJM, 2018

Allogeneic HCT while in MRD-negative CR after CD19 CAR-T cells may improve EFS

Multivariable analysis for factors impacting event-free survival in patients who achieved MRD-negative CR, adjusted for HCT after CAR-T cell therapy as a time-dependent covariate

Variable	HR (95% CI)	p value
LDH pre-lymphodepletion (per 100 U/L increment)	1.38 (1.11-1.73)	0.004
Platelets pre-lymphodepletion (per 50,000/mL increment)	0.74 (0.53-1.03)	0.069
Fludarabine added to lymphodepletion (Y)	0.25 (0.15-0.78)	0.003
Allogeneic HCT after CAR-T cell infusion	0.39 (0.13-1.15)	0.088

An interaction test demonstrated no significant interaction between risk group and allogeneic HCT after CAR-T cells (p=0.53), suggesting benefit in both low and high risk groups

Hay et al, Blood, 2019

Summary – B-ALL

- High MRD-negative CR rates in relapsed/refractory B-ALL
- Tumor burden, cell dose, and lymphodepletion regimen drive CAR-T cell expansion and impact response and toxicity
- Good risk patients can be defined among those in MRD-negative CR
  - Normal LDH and platelets before lymphodepletion; received Cy/Flu
- Allogeneic HSCT after CD19 CAR-T cells is feasible and may provide a survival benefit in good and poor risk groups

Non-Hodgkin lymphoma

NHL expansion: Cy/Flu and $2x10^6$/kg CAR-T cells

Characteristic	Aggressive histology	Indolent histology	All patients
Number (no.) of patients	48	9	57
Disease type – no. (%)			
Diffuse large B-cell lymphoma*	28 (58)	0	28 (58)
NOS	18 (37)	0	18 (32)
Transformed from indolent	10 (21)	0	10 (17)
HGBL-DH/TH	8 (17)	0	8 (14)
Other aggressive†	5 (11)	0	5 (9)
Mantle cell lymphoma‡	6 (12)	0	6 (10)
Follicular lymphoma	1 (2)§	8 (89)	9 (16)
Marginal zone lymphoma	0	1 (11)	1 (2)
Age			
Median (range) – years	56.5 (27-71)	56 (33-69)	56.5 (27-71)
≥ 65 years – no. (%)	8 (17)	1 (11)	9 (16)
Male sex – n (%)	35 (73)	5 (56)	40 (70)
ECOG performance-status score ≥ 1 – no. (%)	20 (42)	4 (44)	24 (42)
LDH, pre-lymphodepletion > ULN – no. (%)	32 (67)	1 (11)	33 (58)
Disease stage – no. (%)			
I or II	1 (2)	2 (22)	3 (5)
III or IV	47 (98)	8 (78)	54 (95)
Extranodal disease – no. (%)			
Yes	43 (90)	6 (67)	49 (86)
No	5 (10)	3 (33)	8 (14)
International Prognostic Index (IPI) score – no. (%)‖			
0 or 1	7 (15)	4 (44)	11 (19)
2	16 (33)	4 (44)	20 (35)
3 or 4	25 (52)	1 (11)	26 (46)
Bulky disease (≥ 10 cm)¶			
Yes	8 (17)	0	8 (14)
No	40 (83)	9 (100)	49 (86)
Tumor cross-sectional area#			
Median – mm^2	3249	3511	3343
Range – mm^2	124-16765	406-8452	124-16765
≥ Median – no. (%)	26 (54)	3 (33)	29 (51)
Prior therapies			
Median (range)	4 (1-11)	4 (2-7)	4 (1-11)
≥ Four prior lines of therapy – no. (%)	34 (71)	8 (89)	36 (63)
Prior autologous hematopoietic stem cell transplantation – no. (%)			
Yes	19 (40)	3 (33)	22 (39)
No	29 (60)	6 (67)	35 (61)
Prior allogeneic hematopoietic stem cell transplantation – no. (%)			
Yes	7 (15)	1 (11)	8 (14)
No	41 (85)	8 (89)	49 (86)
Bridging therapy between leukapheresis and lymphodepletion			
Intensive chemotherapy – no. (%)**	7 (15)	0	7 (12)
High dose corticosteroid – no. (%)††	9 (19)	1 (11)	10 (18)
Other – no. (%)‡‡	2 (4)	0	2 (4)
Any therapy between leukapheresis and lymphodepletion – no. (%)	12 (25)	1 (11)	13 (23)

Hirayama, Blood, 2019

High response rates in NHL patients after CD19 CAR-T cell immunotherapy

	All patients (n=56)	Indolent (n=9)	Aggressive (n=47)
ORR*	57%	89%	51%
CR*	48%	89%	40%

Data from patients (n=56) who received 2 x 10^6 CAR-T cells/kg (MTD) and Cy/Flu lymphodepletion in the phase 1 study and the subsequent expansion cohort

*Best responses

Hirayama, Blood, 2019

Responses after CD19 CAR-T cell immunotherapy for aggressive NHL

	Fred Hutch (JCAR014)[1]			ZUMA-1 (axi-cel)[2]			JULIET (tisagenlecleucel)[3]		
Best response	ORR	CR	PR	ORR	CR	PR	ORR	CR	PR
	51%	40%	11%	75%	55%	20%	52%	40%	12%
Median F/U	27 months			27 months			28 months		

Historical data in refractory DLBCL (SCHOLAR-1)

ORR 26% (CR rate 7%)

Median OS 6 months (15 months in CR patients)

*Full dataset

[1]Hirayama A, Gauthier J al, Blood. 2019; [2]Locke FL et al, Lancet Oncology, 2019; [3]Schuster SJ et al, NEJM, 2019; [4]Abramson JS et al, ASCO abstract. 2018; Crump et al, Blood. 2017

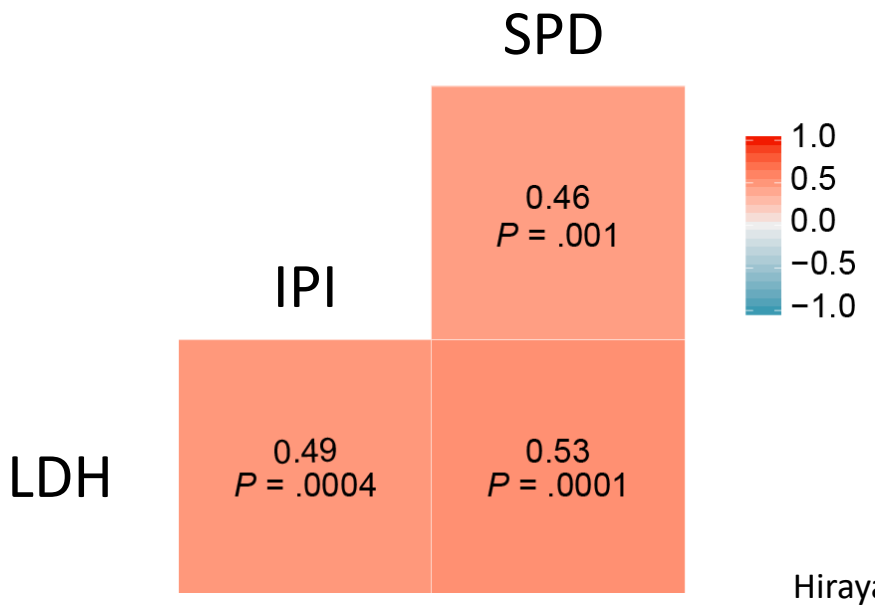
Multivariable analysis of factors impacting CR in aggressive NHL after Cy/Flu and CD19 CAR-T cells

Multivariable analysis using elastic net selection of clinical, manufacturing, treatment, and biomarker variables

Variable	HR (95% CI)	P value
LDH, pre-lymphodepletion*	0.24 (0.08 – 0.53)	0.003
MCP-1Δ, pre-LD to day 0#	1.36 (1.12– 1.79)	0.007

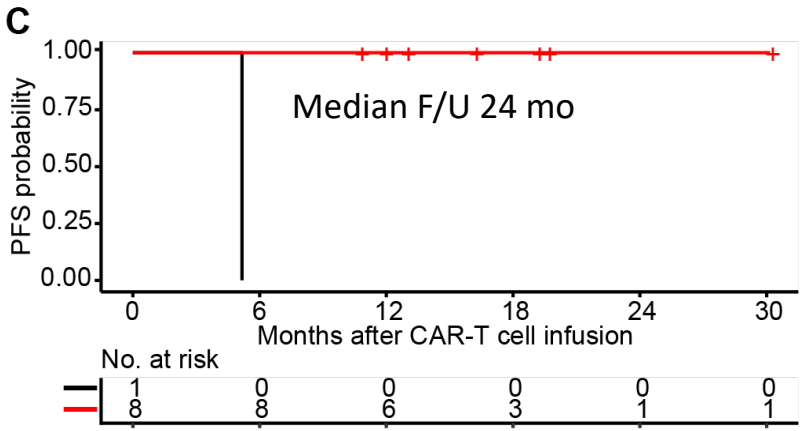
*Per 100 U/L increment
Per 50 ng/mL increment

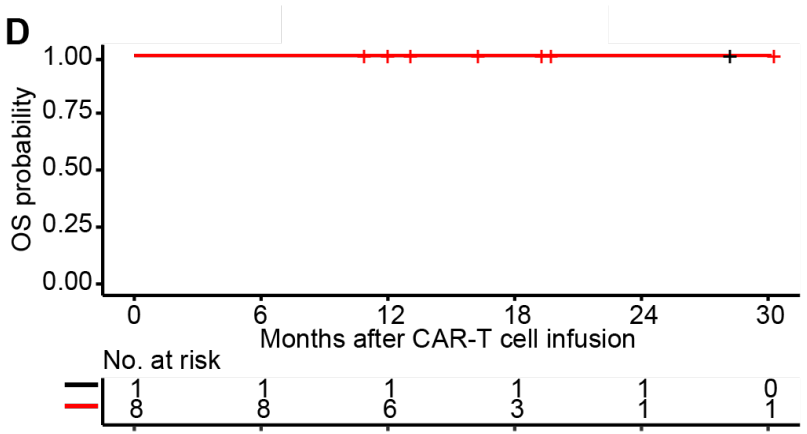
Pre-lymphodepletion LDH correlates with IPI and SPD



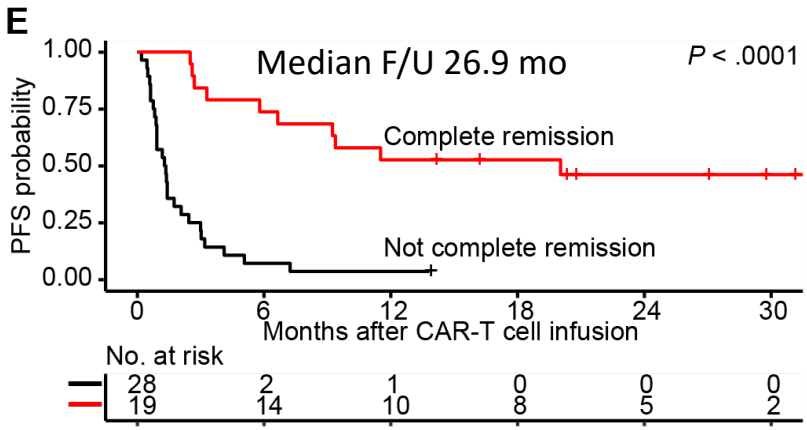
Hirayama, Blood, 2019

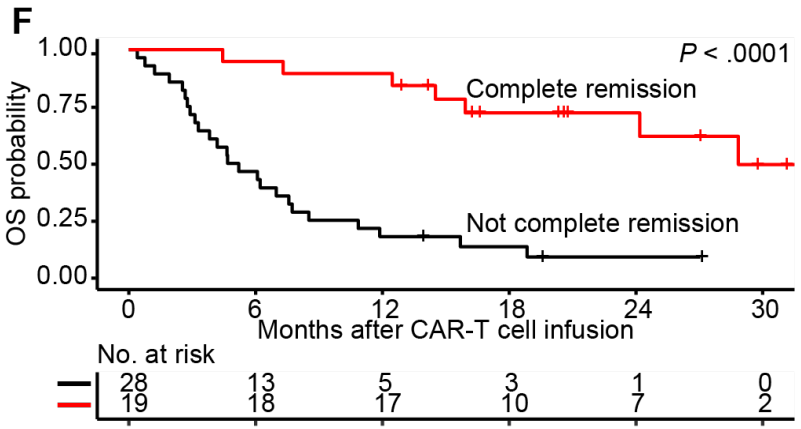
PFS and OS in NHL patients after Cy/Flu and CD19 CAR-T cell immunotherapy





Indolent



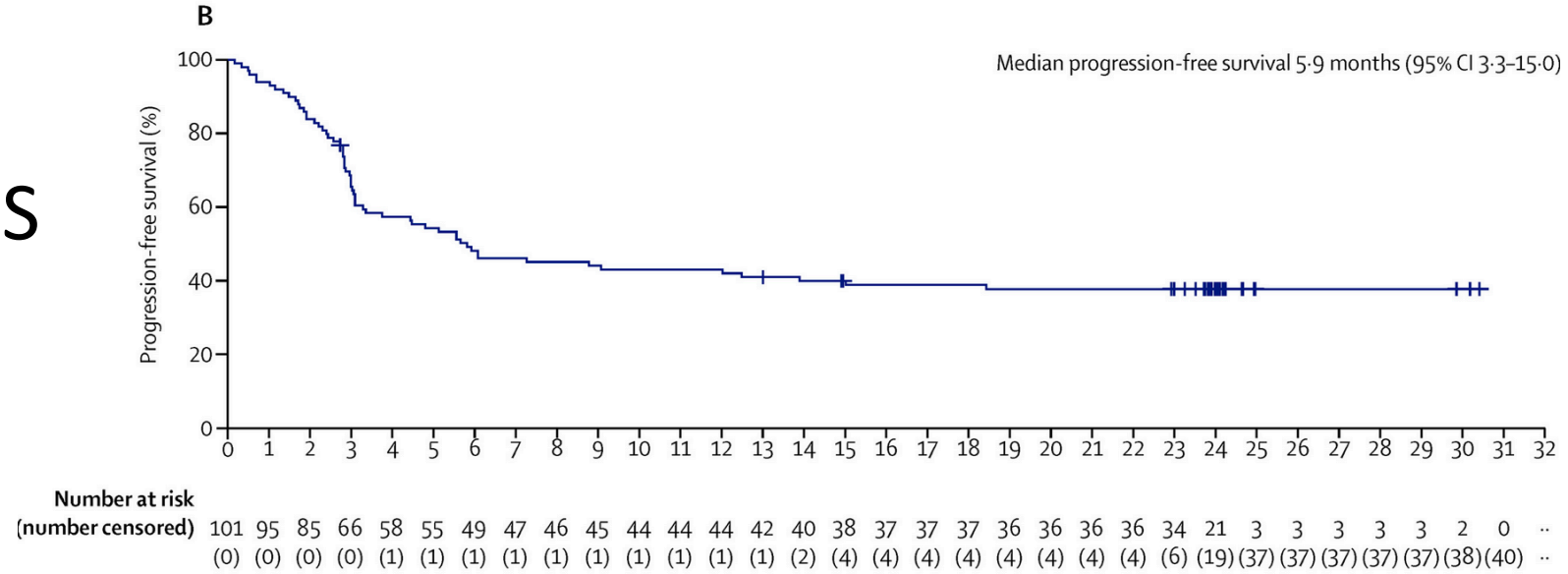


Aggressive

Hirayama, Blood, 2019

Durable responses to axi-cel for DLBCL (ZUMA-1)

PFS



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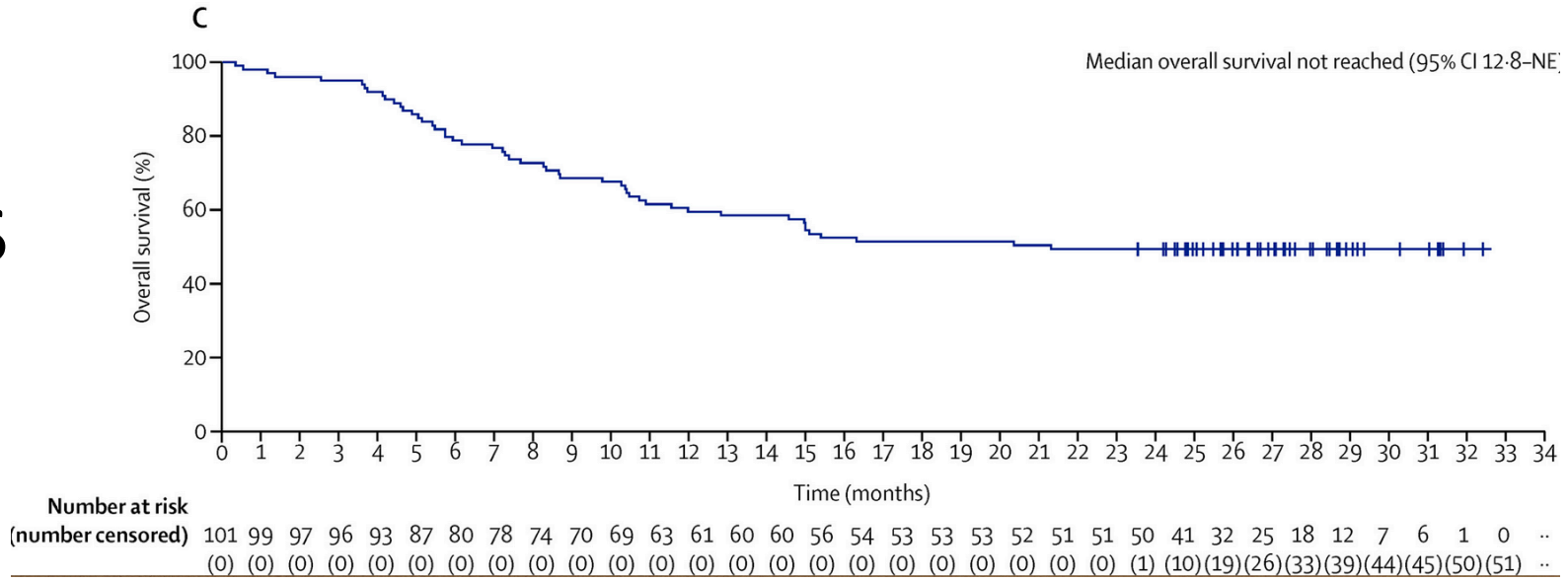


Locke FL et al, Lancet Oncology, 2019

Factors impacting PFS in aggressive NHL patients treated with Cy/Flu and CD19 CAR-T cells

Variable	Univariate*		Multivariate†	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
LDH, pre-lymphodepletion‡	1.24 (1.04-1.47)	.02	1.37 (1.14-1.63)	.0006
MCP-1, day 0 (pre-CAR-T cell infusion)§	0.25 (0.10-0.60)	.002	0.29 (0.09-0.90)	.03
IL-7, peak‖	0.84 (0.74-0.95)	.01	0.89 (0.77-1.04)	.14

PFS, progression-free survival; HR, Hazard Ratio; 95% CI, 95% confidence interval

* Univariate Cox regression model; variables chosen for the final multivariate model are presented (complete univariate results available in the supplemental Table 2).

† Cox regression model using elastic net was performed to select variables associated with PFS, where $\log_{10}$ values were used to transform data as appropriate, with 0.001 substituting for values of 0.

‡ Per 100 U/L increment.

§ Per $\log_{10}$ pg/mL serum concentration increment.

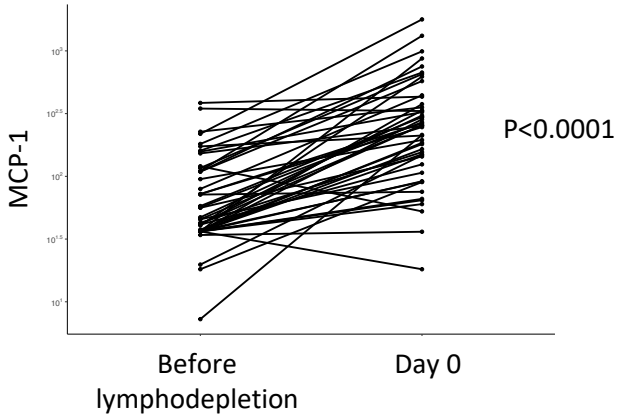
‖ Per 5 pg/mL serum concentration increment.

LDH likely represents disease kinetic and/or bulk

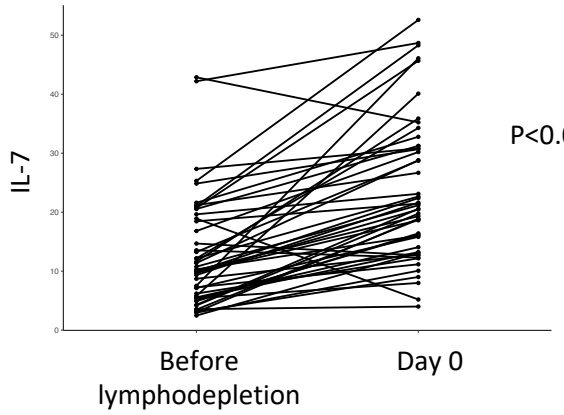
Are MCP-1 and IL-7 associated with lymphodepletion chemotherapy?

Hirayama, Blood, 2019

MCP-1 and IL-7 are increased by lymphodepletion chemotherapy



P<0.0001



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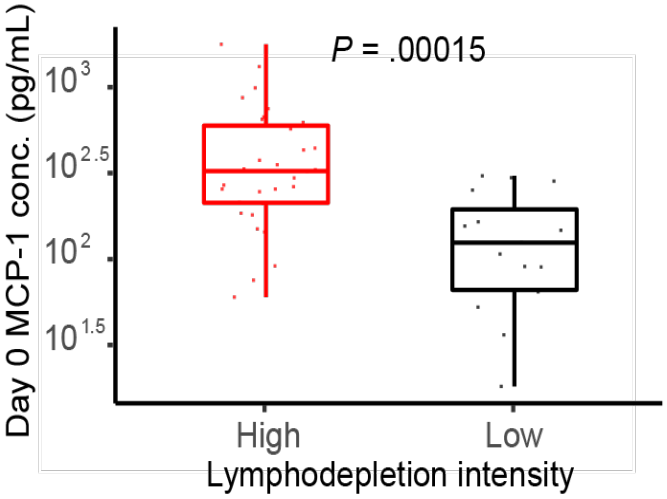
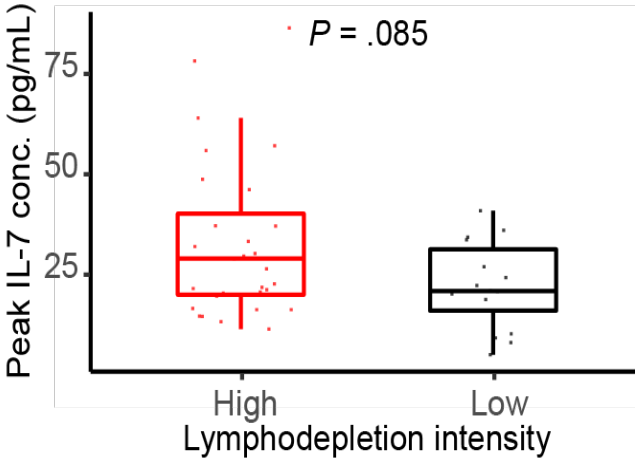
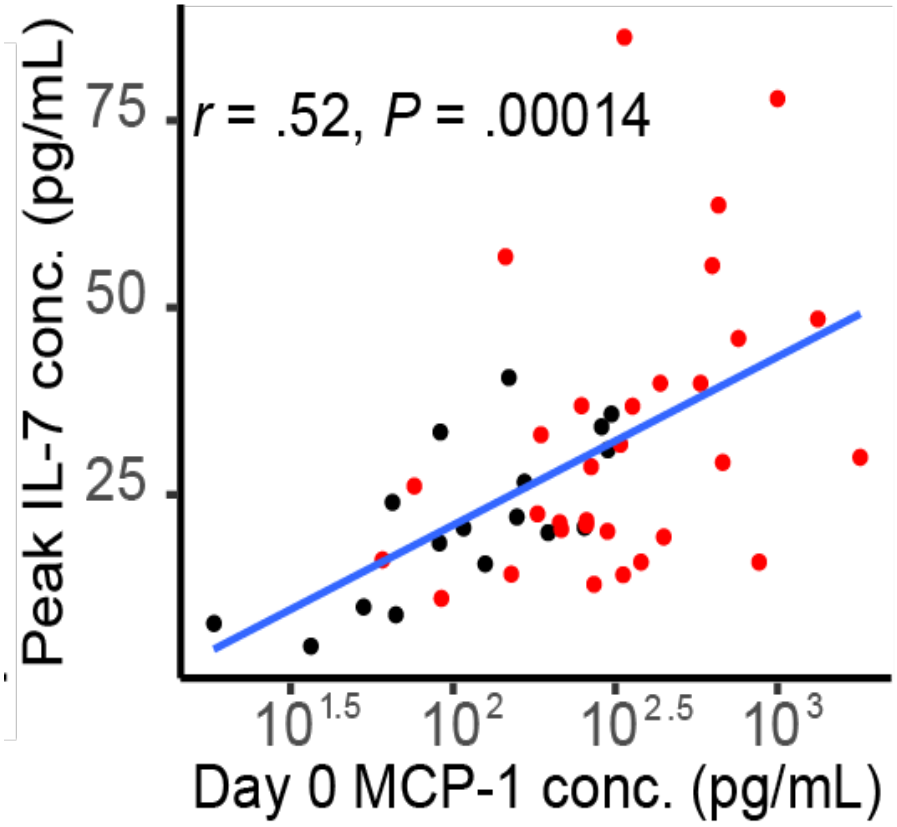




Wilcoxon rank-sum

Hirayama, Blood, 2019

Failure to achieve favorable cytokines in a subset of patients after high intensity lymphodepletion



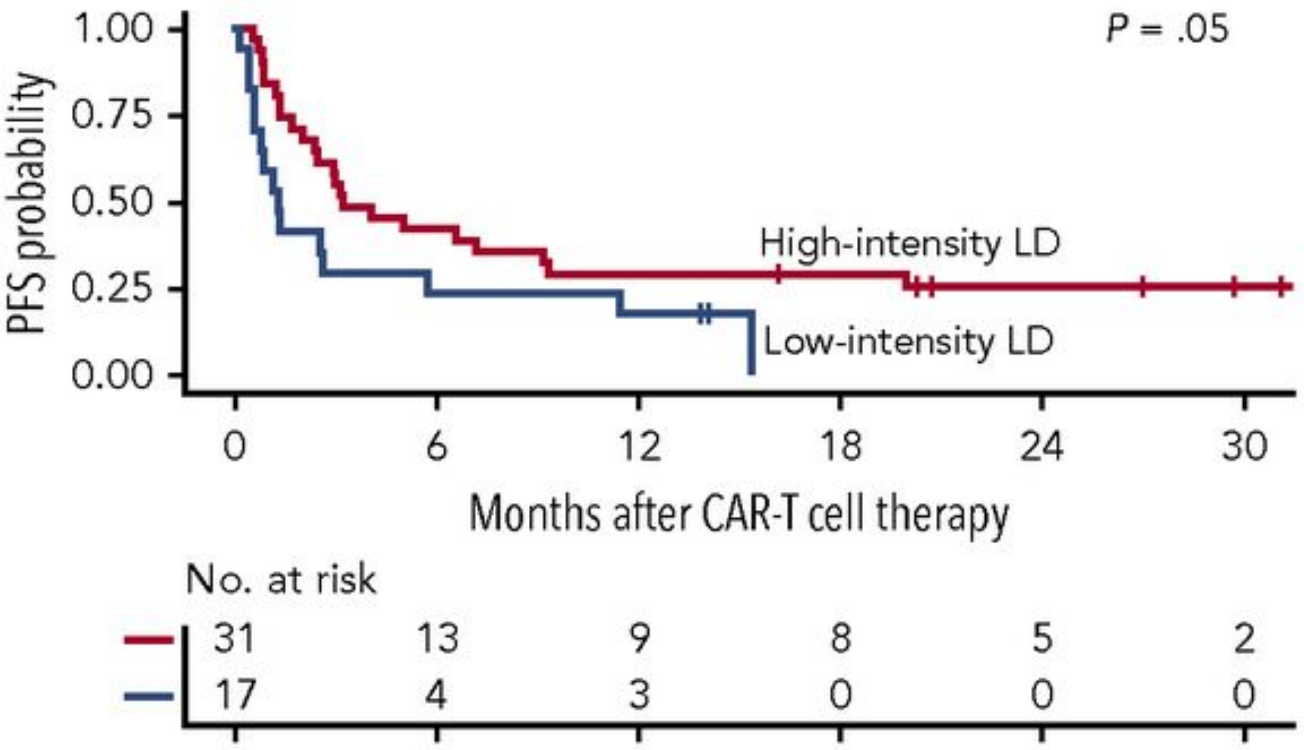
High intensity lymphodepletion

Low intensity lymphodepletion

Spearman

Hirayama, Blood, 2019

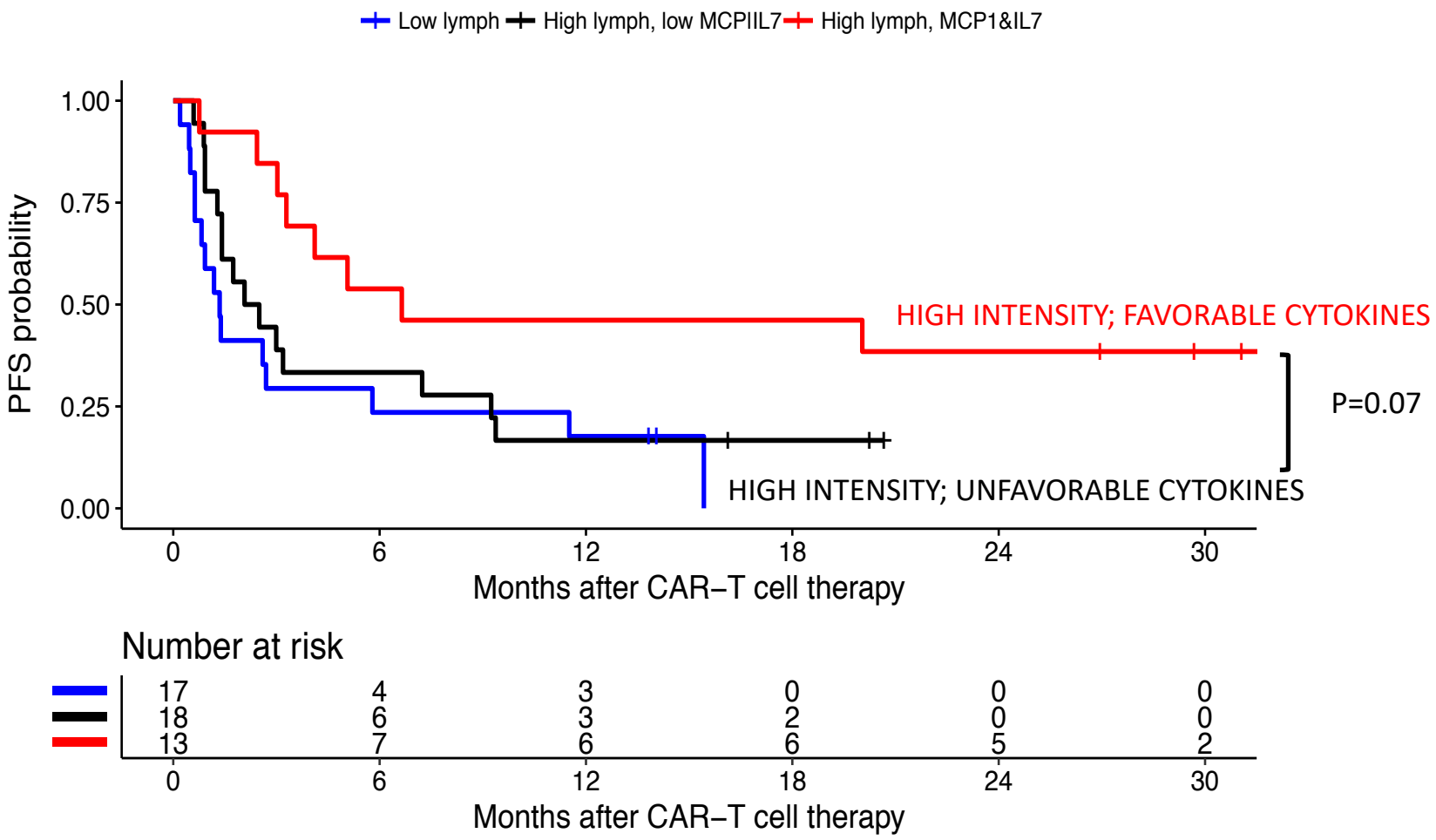
Better PFS after high-intensity lymphodepletion chemotherapy



Is it a direct anti-tumor effect or is it related to the cytokine profile?

Hirayama, Blood, 2019

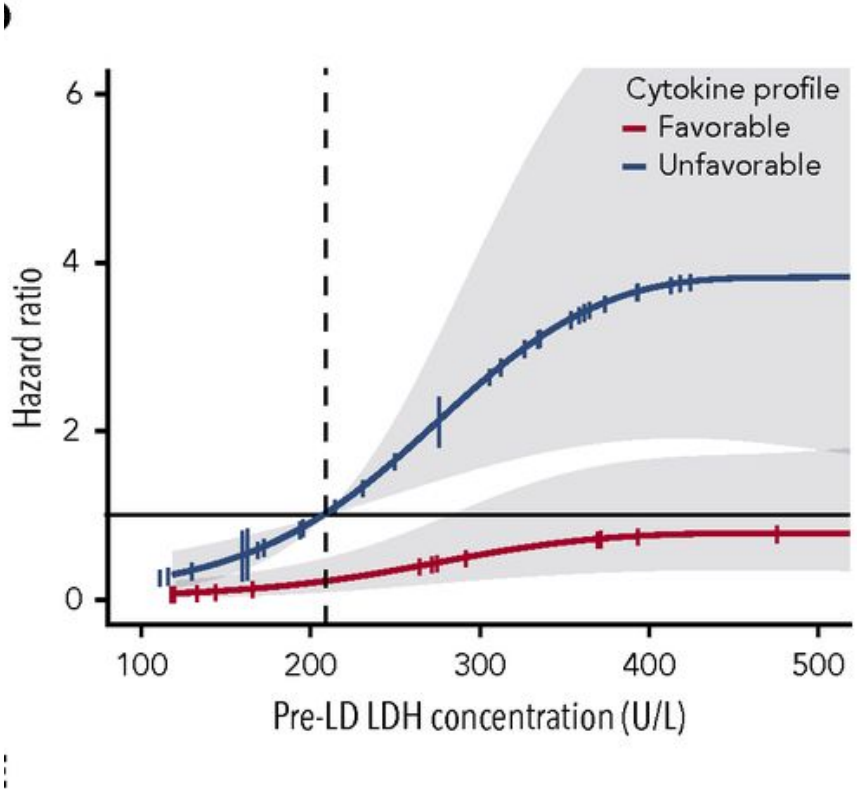
A favorable cytokine profile is associated with better PFS after CD19 CAR-T cells for aggressive NHL

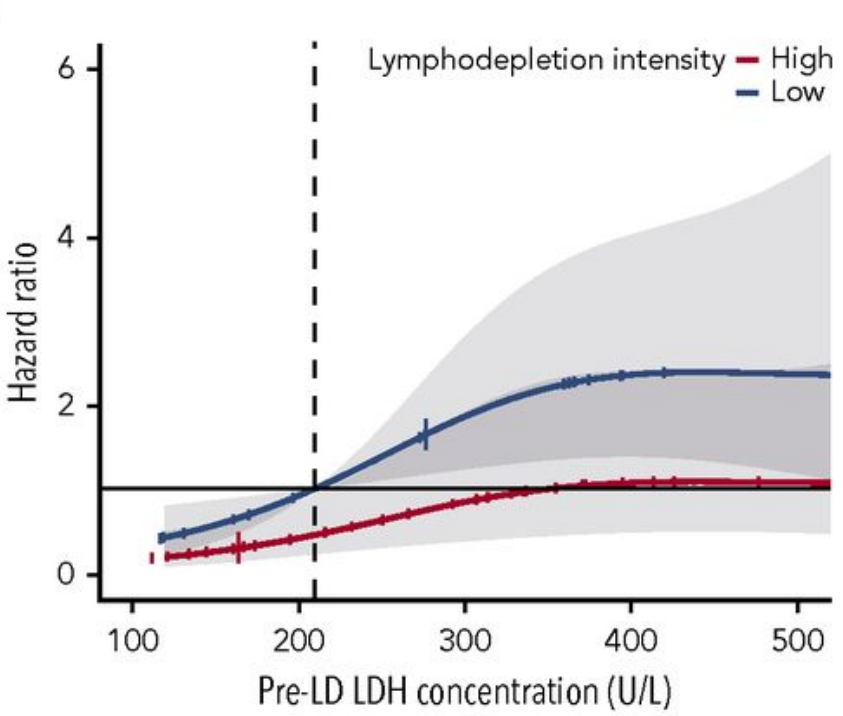


Log-rank

Hirayama, Blood, 2019

Associations of cytokine profiles and LD intensity with hazard of a PFS event





Cytokines were modeled as a cubic spline with three knots.
The horizontal line shows the hazard ratio of a PFS event in the whole cohort.

Hirayama, Blood, 2019

Summary – NHL

- Very high CR rate in indolent NHL and a low risk of relapse
- High OR and CR rate in aggressive NHL, but relapse remains a problem
- High LDH and low MCP-1/IL-7 are associated with increased risk of relapse in aggressive NHL
- A favorable cytokine profile is associated with a low relapse risk, even in high-risk patients

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