UNIVERSITÀ DEGLI STUDI
DI MILANO

FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI

Terapie cellulari a confronto dal punto di vista di attivazione/espansione/formulazione

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Divisione di Ematologia

Fondazione IRCCS Istituto Nazionale dei Tumori - Milano

Novità nella terapia cellulare nel mondo dei pazienti R/R DLBCL
Milano, 12 luglio 2023

CAR-T:
c'è posta per te!

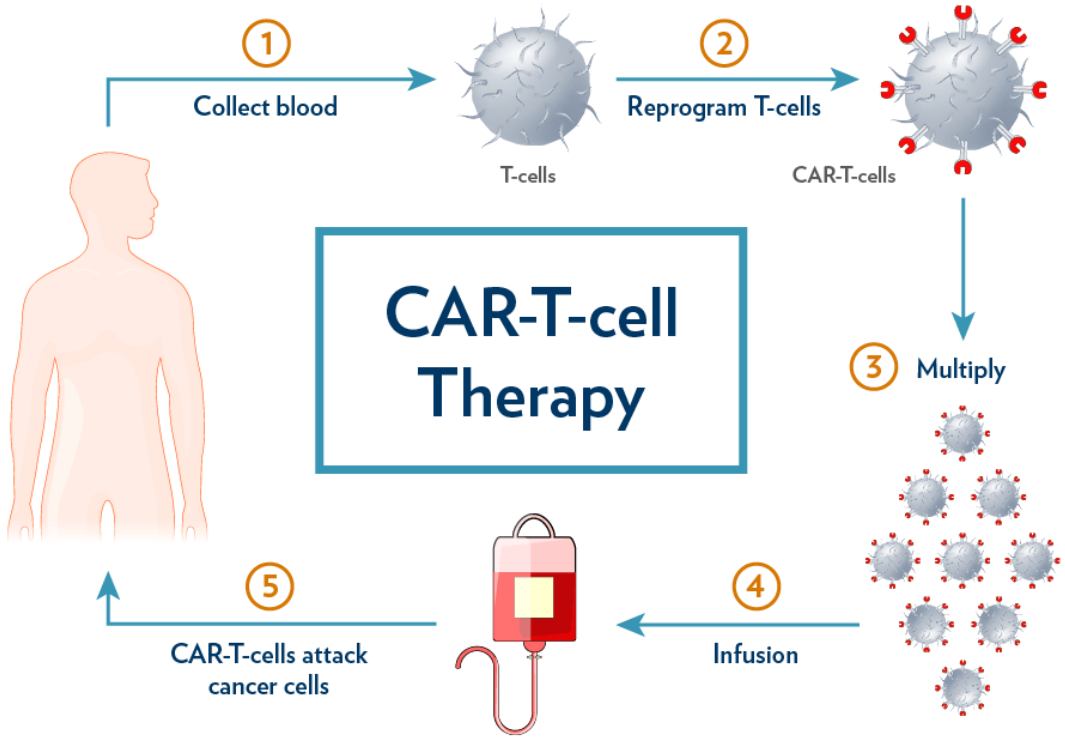
Disclosure Statement

Travel and accommodations paid by profit health care companies during the past 2 years: Novartis

CAR T cell therapy

CAR: Chimeric Antigen Receptor

- Synthetic receptors that reprogram immune cells for therapeutic purposes
- They comprise three canonical domains for antigen recognition, T cell activation, and co-stimulation
- Autologous CAR T cells are generated from the patient's peripheral blood T cells and expand in the recipient to eliminate the targeted tumor
- CAR T cells specifically recognize and eliminate malignant cells expressing a target antigen (CD19 for NHL , CLL and ALL, BCMA for MM)



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FDA-Approved CAR T therapies for B-cell Lymphomas

Product	Lymphoma Indications (FDA Approval Date)
Axicabtagene ciloleucel *(Yescarta)* ▪ Anti–CD19-CD28-CD3z construct ▪ Uses retroviral transduction	▪ **Adults with LBCL** either refractory to first-line chemoimmunotherapy or relapsed within 12 mo of first-line chemoimmunotherapy (April 1, 2022) ▪ **Adults with R/R LBCL** after ≥2 lines of systemic therapy, including DLBCL NOS, DLBCL arising from FL, primary mediastinal LBCL, high-grade B-cell lymphoma (October 18, 2017) ▪ **Adults with R/R FL** after ≥2 lines of systemic therapy (March 5, 2021)
Brexucabtagene autoleucel *(Tecartus)* ▪ Anti–CD19-CD28-CD3z construct ▪ Uses retroviral transduction	▪ **Adults with R/R MCL** (July 24, 2020)
Lisocabtagene maraleucel *(Breyanzi)* ▪ Anti–CD19-41BB-CD3z construct ▪ Uses lentiviral transduction	▪ **Adults with LBCL**, including DLBCL NOS, DLBCL arising from indolent lymphoma, high-grade B-cell lymphoma, primary mediastinal LBCL, and FL grade 3B, who have disease that is: • Either refractory to first-line chemoimmunotherapy or relapsed within 12 mo of first-line chemoimmunotherapy (June 24, 2022), *or* • Refractory to first-line chemoimmunotherapy or relapsed after first-line chemoimmunotherapy and ineligible for HSCT due to comorbidities or age (June 24, 2022), *or* • R/R after ≥2 lines of systemic therapy (February 5, 2021)
Tisagenlecleucel *(Kymriah)* ▪ Anti–CD19-41BB-CD3z construct ▪ Uses lentiviral transduction	▪ **Adults with R/R LBCL** after ≥2 lines of systemic therapy, including DLBCL NOS, high-grade B-cell lymphoma, and DLBCL arising from FL (May 1, 2018) ▪ **Adults with R/R FL** after ≥2 lines of systemic therapy (May 27, 2022)

Axicabtagene ciloleucel PI. Brexucabtagene autoleucel PI. Lisocabtagene maraleucel PI. Tisagenlecleucel PI.

Slide credit: clinicaloptions.com

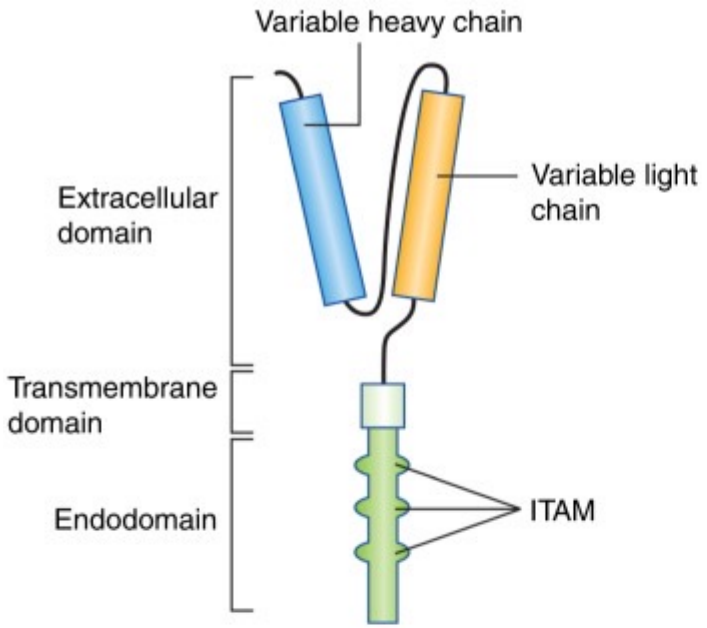
Pivotal Trials Leading to FDA Approval: Lymphomas

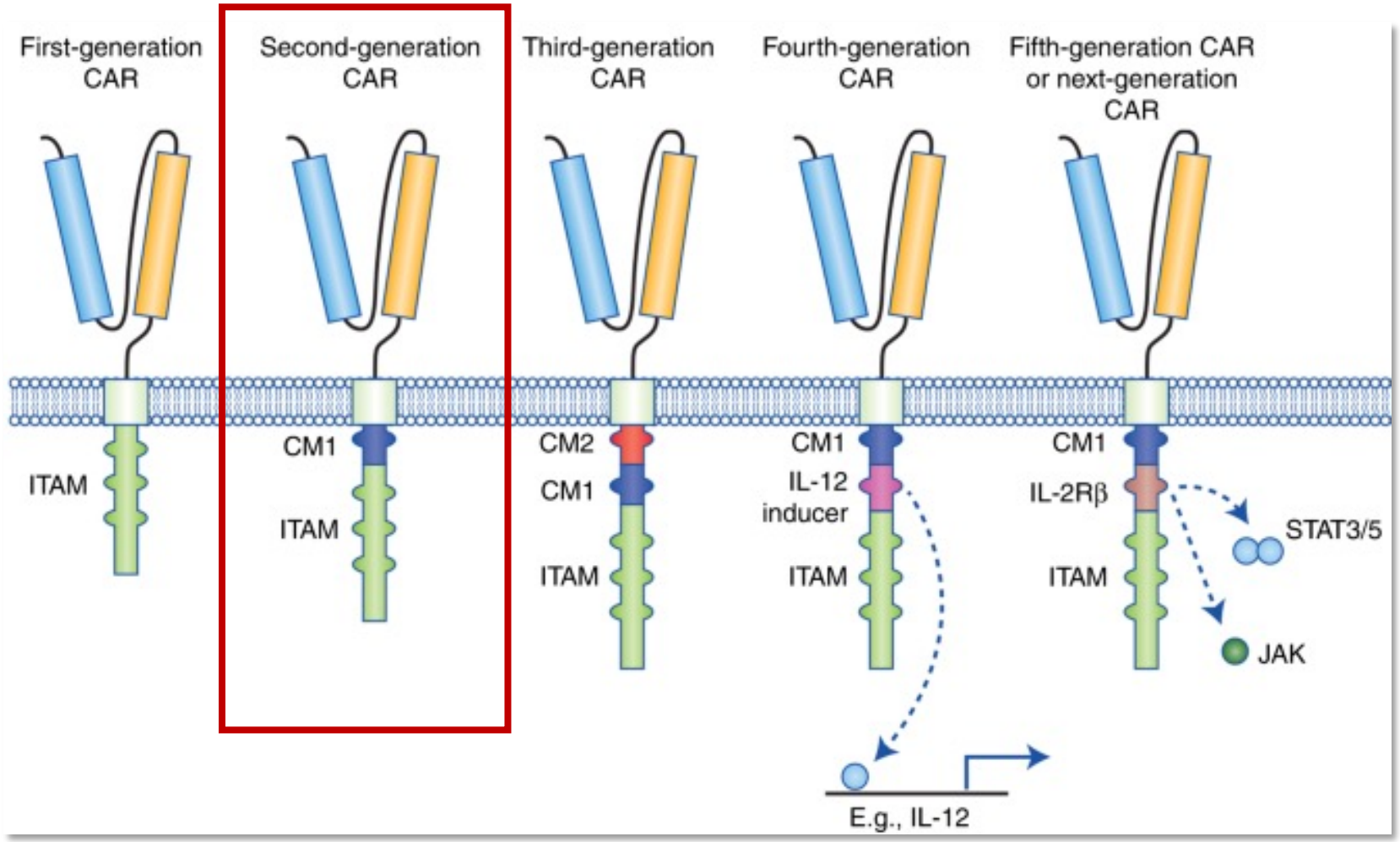
Outcome	Phase II ZUMA-1[1-3]	Phase II ZUMA-5[1,4-5]	Phase II JULIET[6-8]	Phase II ELARA[6,9-11]	Phase I TRANSCEND NHL 001[12-15]	Phase II ZUMA-2[16-18]
CAR T-cell product	Axi-cel (*Yescarta*)	Axi-cel (*Yescarta*)	Tisa-cel (*Kymriah*)	Tisa-cel (*Kymriah*)	Liso-cel (*Breyanzi*)	Brexu-cel (*Tecartus*)
Patient population	Adults with R/R LBCL	Adults with R/R FL	Adults with R/R LBCL post/ineligible for autoHSCT	Adults with R/R FL	Adults with R/R LBCL	Adults with R/R MCL
Pheresed/ treated, n	111/101	127/124	165/111	98/97	344/269	71/68
Bridging tx, %	Not permitted	4	92	44	59	37
ORR/CR, %	82/52	94/79	52/40	86.2/69.1	73/53	85/59
OS/PFS rate, %	1 yr: 59/44 5 yr: 42.6/--	2 yr: 81.2/63.4	1 yr: 49/-- 2 yr: 41.1/33.5	1 yr: --/67.0	1 yr: 58/44 2 yr: 50.5/40.6	1 yr: 83/61 2 yr: --/52.9

1. Axicabtagene ciloleucel PI. 2. Neelapu. NEJM. 2017;377:2531. 3. Jacobson. TCT 2022. Abstr 10. 4. Jacobson. Lancet Oncol. 2022;23:91. 5. Neelapu. EBMT 2022. Abstr OS08-01. 6. Tisagenlecleucel PI. 7. Schuster. NEJM. 2019;380:45. 8. Schuster. Leuk Lymphoma. 2022;63:845. 9. Fowler. Nat Med. 2022;28:325. 10. Thieblemont. TCT 2022. Abstr 74. 11. Schuster. ASCO 2021. Abstr 7508. 12. Lisocabtagene maraleucel PI. 13. Abramson. ASH 2019. Abstr 241. 14. Abramson. Lancet. 2020;396:839. 15. Abramson. EBMT 2022. Abstr OS08-07. 16. Brexucabtagene autoleucel PI. 17. Wang. NEJM. 2020;382:1331. 18. Wang. ASCO 2022. Abstr 7518.

Slide credit: clinicaloptions.com

Structure of different CAR generations

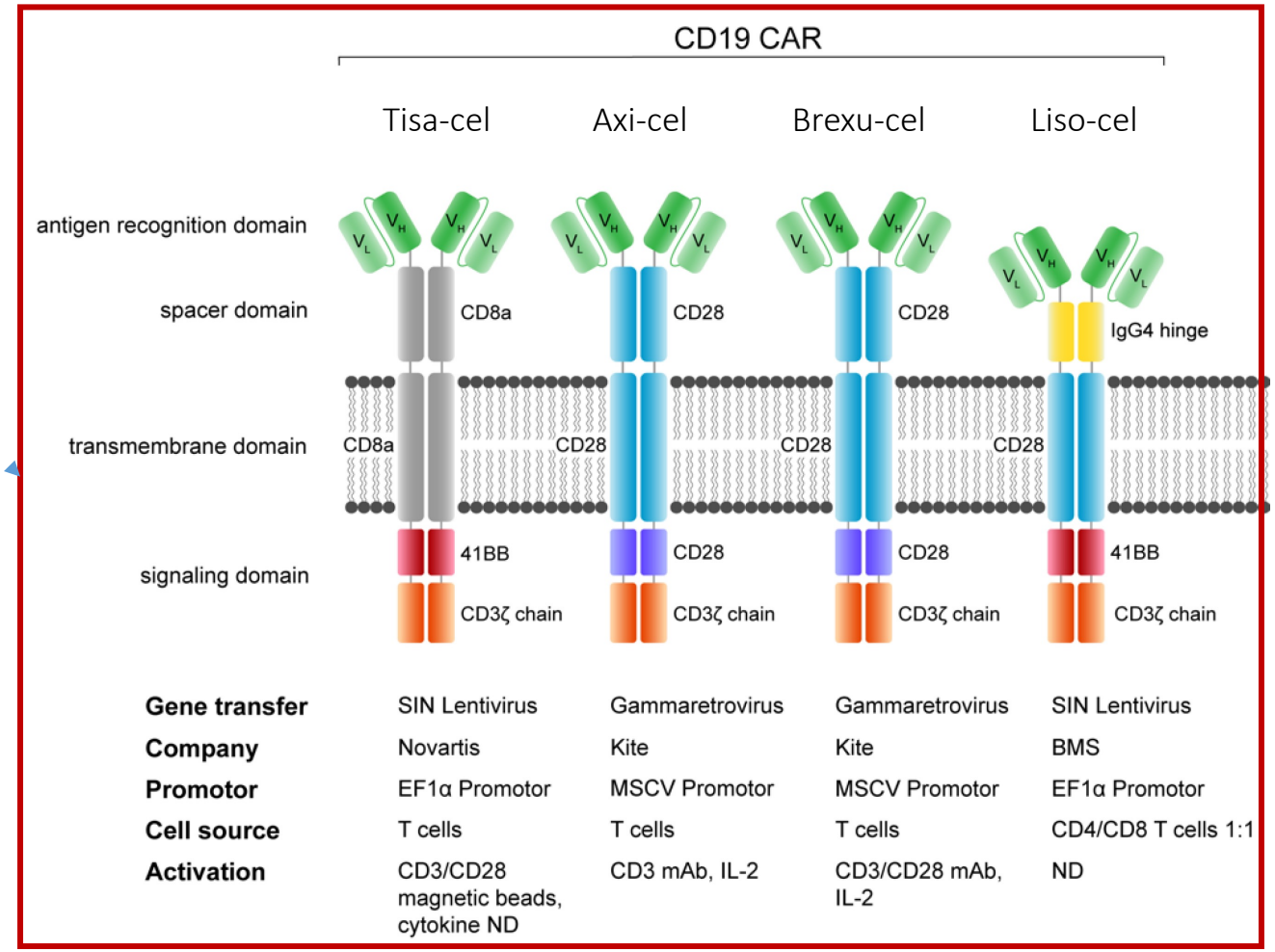




adapted from Tokarew et al, 2019 BJC

CM: co-stimulatory molecule; ITAM: immunoreceptor tyrosine-based activation motif

Key Differences in approved products for B-cell Lymphomas

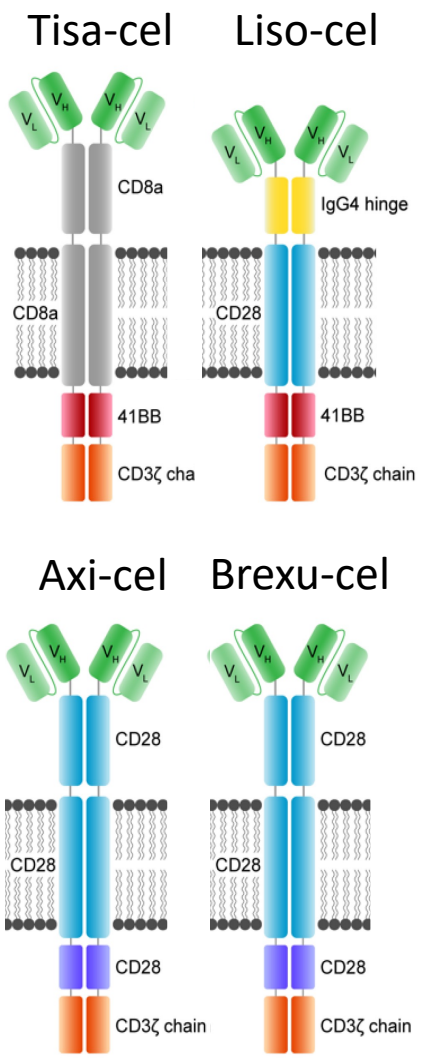


	Tisa-cel	Axi-cel	Brexu-cel	Liso-cel
Gene transfer	SIN Lentivirus	Gammaretrovirus	Gammaretrovirus	SIN Lentivirus
Company	Novartis	Kite	Kite	BMS
Promotor	EF1α Promotor	MSCV Promotor	MSCV Promotor	EF1α Promotor
Cell source	T cells	T cells	T cells	CD4/CD8 T cells 1:1
Activation	CD3/CD28 magnetic beads, cytokine ND	CD3 mAb, IL-2	CD3/CD28 mAb, IL-2	ND

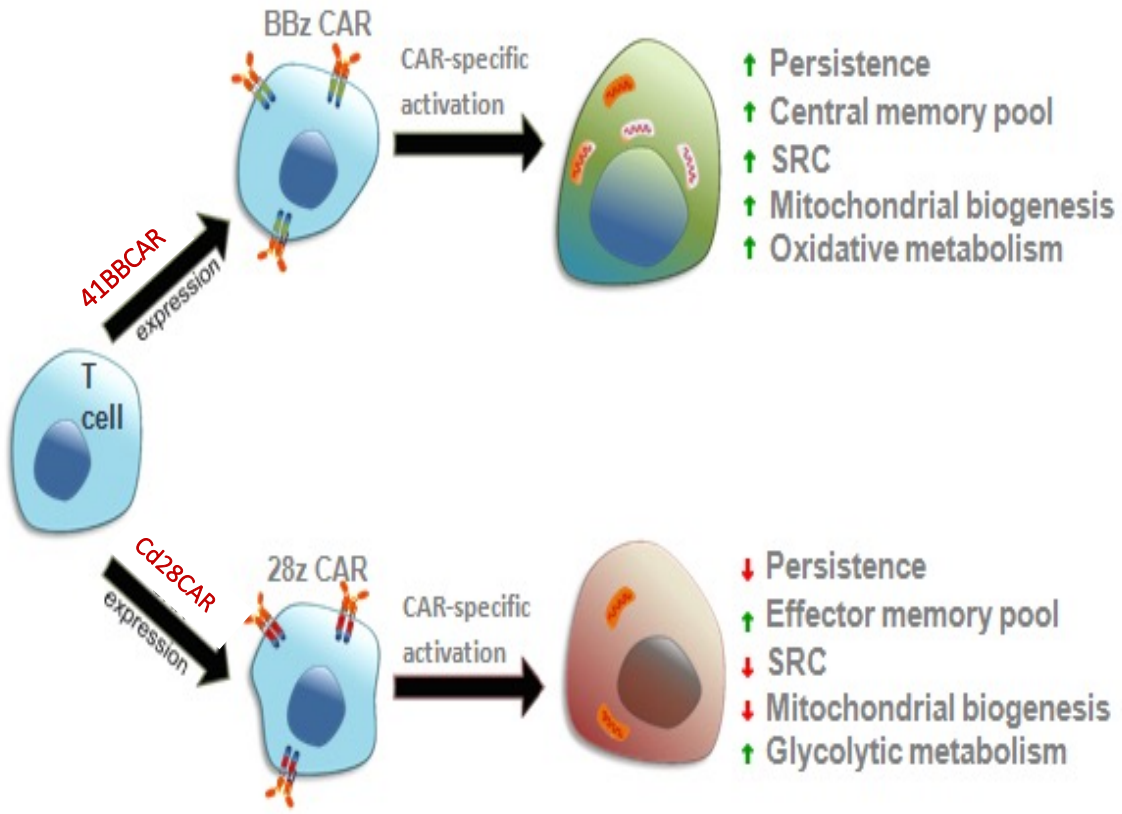
adapted from Boettcher et al. 2022

Despite these differences, all CD19-CAR-T cell constructs use the same single-chain variable fragments (scFvs) derived from a murine FMC63 monoclonal antibody and have demonstrated outstanding clinical performance in various B-lineage malignancies

CAR Signaling Domains Program Cells for Metabolic Fitness



adapted from Boettcher et al. 2022



Adapted from Cerrano et al, Front Immunol 2020

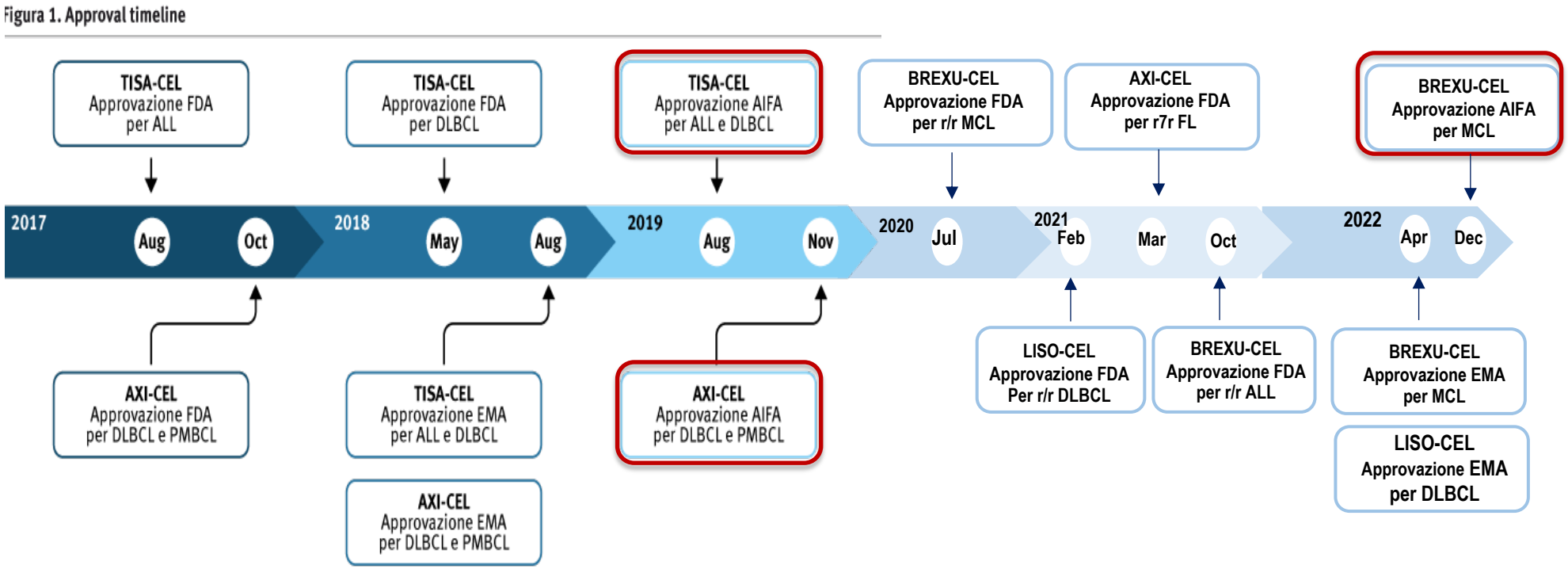
- **Tisa-cel and Liso-cel (4-1BB domains)**

enhance mitochondrial biogenesis associated with elevated persistence

- **Axi-cel and Brexu-cel (CD28 domains)**

enhance glycolysis via "Warburg" effect, causing higher effector function and decreased persistence

AIFA-approved CAR T therapies for B-cell Lymphomas



adapted from https://news.accmed.org/ga-ematologia

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Assignement to either Tisa-cel or Axi-cel

Tisa-cel target dose: 0,6 to $6x10^8$ CAR-positive viable cells

Axi-cel target dose: 2 $x10^6$ CAR-positive viable cells per Kg body weight

Maximum dose: $2x10^8$ CAR-positive viable cells

- Efficacy depends on cellular and molecular features of infused CAR-T cell product as a memory CD8 phenotype in CAR-T was associated with superior efficacy among CLL patients receiving CTL019 therapy
- Axi-cel durable responses were associated with high product T_{CM} among patients of the the ZUMA-1 trial

→ Still patients are assigned to either Tisa-cel or Axi-cel based on slot production availability and histology

Within the prospective observational study enrolling all consecutive LBCL pts receiving CAR T at INT, we wanted to:

- ✓ encover differences between Tisa-cel and Axi-cel
- ✓ identify properties of CAR-T cells that enable their *in vivo* proliferation and their efficacy

Jacobson et al. JCO 2020, Vercellino et al. Blood Adv 2020; Awasthi et al. Blood Adv 2020; Ayuk et al. Blood Adv 2021, Fraietta et al. Nat Med 2018, Deng et al Nat Med 2020

Are IP characteristics associated with *in vivo* CAR T expansion and response

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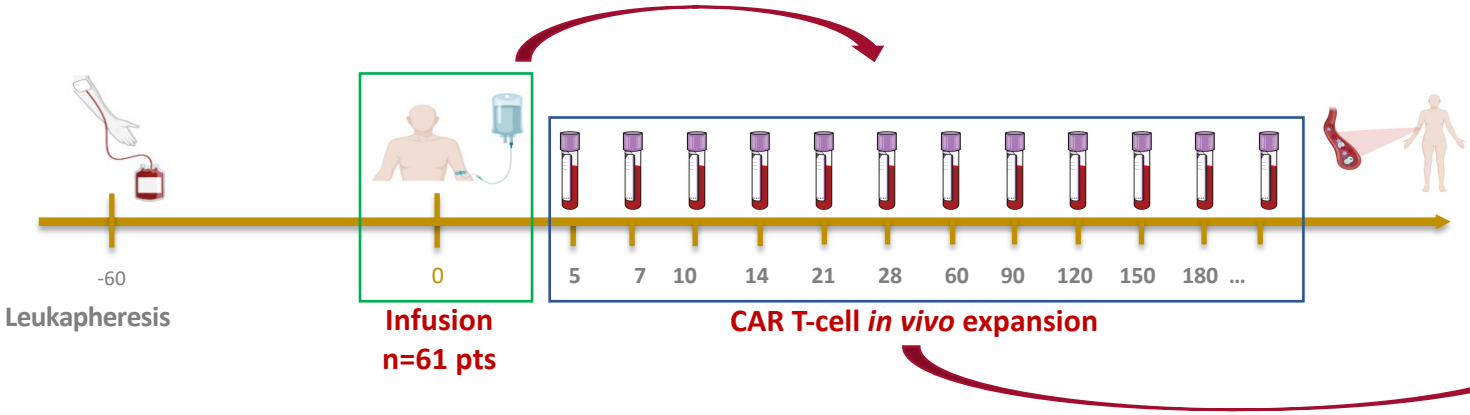

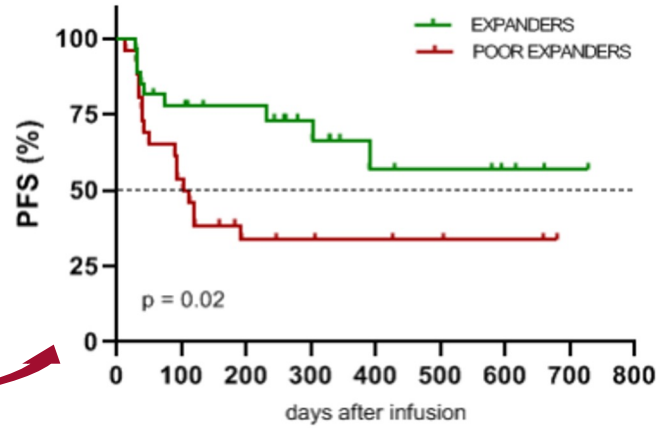

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RESEARCH ARTICLE | MAY 18 2022

Phenotypic composition of commercial anti-CD19 CAR-T cells affects *in vivo* expansion and disease response in large B-cell lymphoma patients

Chiara Monfrini; Federico Stella; Vanessa Aragona; Martina Magni; Silva Ljevar; Cristina Vella; Eugenio Fardella; Annalisa Chiappella; Francesca Nanetti; Martina Pennisi; Anna Dodero; Anna Guidetti; Paolo Corradini; Cristiana Carniti

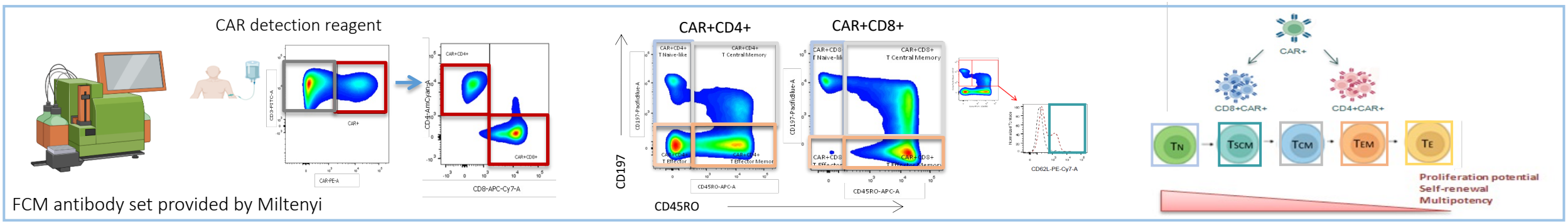
CAR: chimeric antigen receptor; IP: infusion products; PFS: progression free survival; pts: patients; T_{CM} : T central memory

PATIENTS

Characteristics	Overall (N = 61)	Axi-cel (N = 32)	Tisa-cel (N = 29)	p-value
Age – median years (range)	56 (21-70)	55 (21-70)	56 (26-70)	0.99
Hystotypes -DLBCL -tFL -PMBCL -HGBL (including DHL/THL)	 31 (51%) 8 (13%) 13 (21%) 9 (15%)	 12 (37%) 3 (9%) 13 (41%) 4 (12%)	 19 (65%) 5 (17%) 0 (0) 5 (17%)	 0.47 0.46 <0.001* 0.72
ECOG 0 / 1	46 (75%) / 13 (21%)	27 (84%) / 4 (12%)	19 (65%) / 9 (31%)	0.13/0.11
Previous lines ≥ 3	24 (39%)	11 (34%)	13 (45%)	0.44
Primary refractory (< 6 mo) (%)	46 (75%)	24 (75%)	22 (76%)	0.13
Stage III-IV (%)	45 (74%)	22 (69%)	23 (79%)	0.39
IPI 3-5 (%)	13 (21%)	3 (9%)	10 (34%)	0.03*
Bulky > 5 cm (%)	16 (26%)	10 (31%)	6 (21%)	0.6
Bridging therapy (%)	48 (79%)	24 (75%)	25 (86%)	0.34
TMTV median (range)	27.6 (0.7-389)	29.4 (1.35-256)	21.1 (0.7-389)	0.6

DLBCL: Diffuse Large B-cell Lymphoma; tFL: transformed Follicular Lymphoma; PMBCL: Primary Medistinal B-cell Lymphoma; HGBL: High Grade B-cell Lymphoma; ECOG: Eastern Cooperative Oncology Group; IPI: International Prognostic Index; TMTV:Total Metabolic Tumor Volume;

Tisa-cel and Axi-cel infusion product phenotypes by FCM



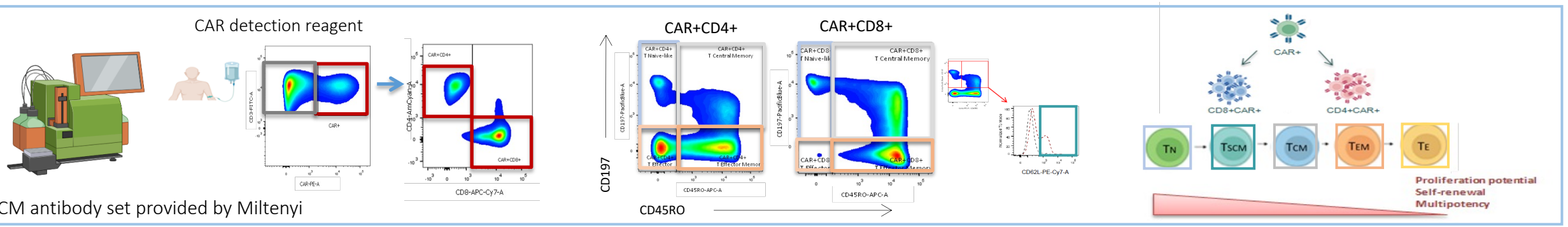
FCM antibody set provided by Miltenyi

CAR: chimeric antigen receptor; T_N: T naive; T_{SCM}: T stem cell memory; T_{CM}: T central memory; T_{EM}: T effector memory; T_E: T effector

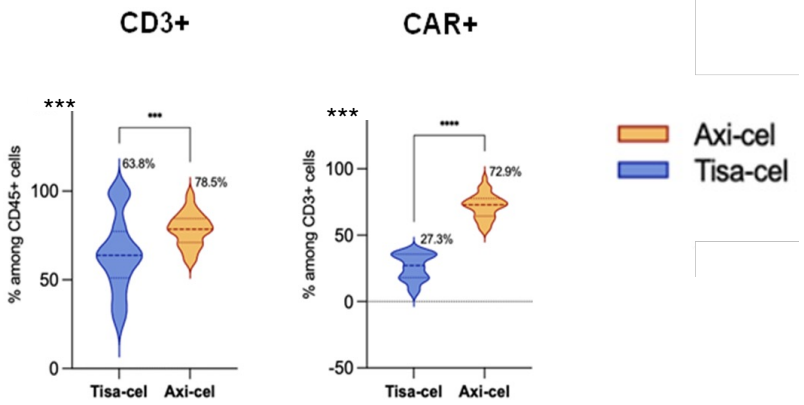

M. Magni

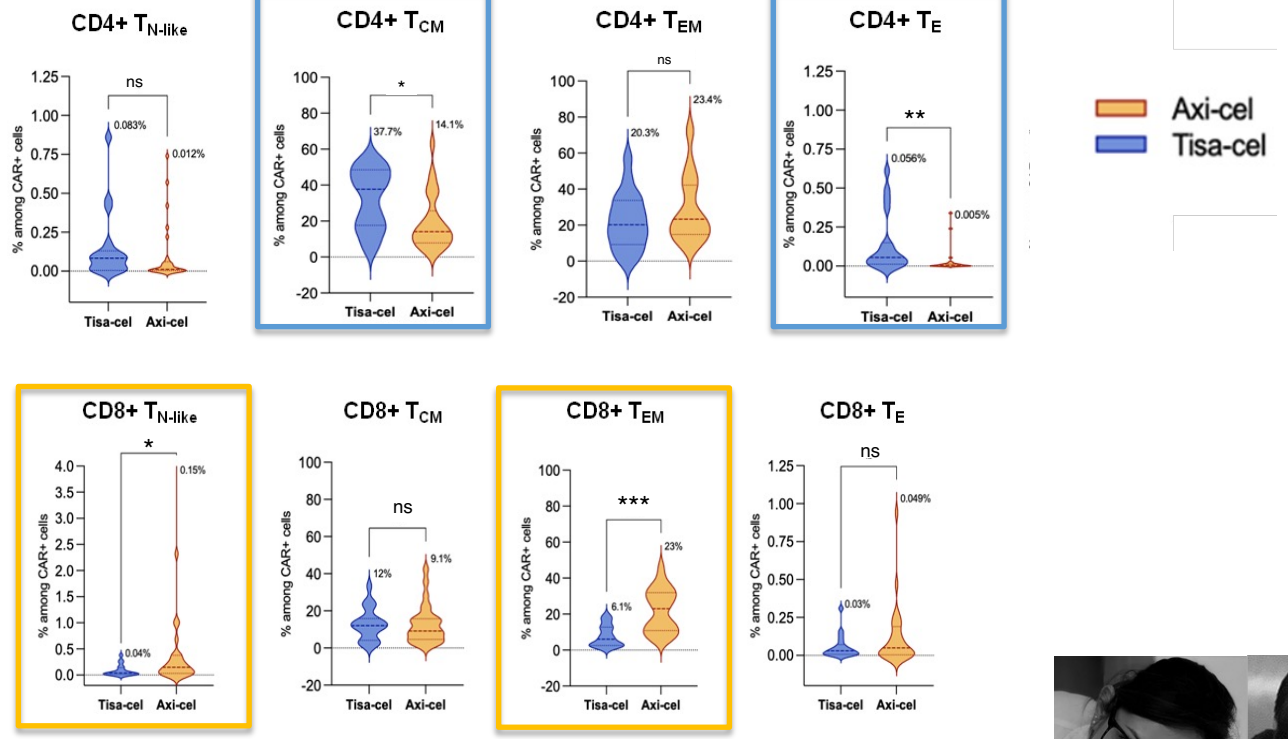

C. Monfrini

Tisa-cel and Axi-cel infusion product phenotypes by FCM



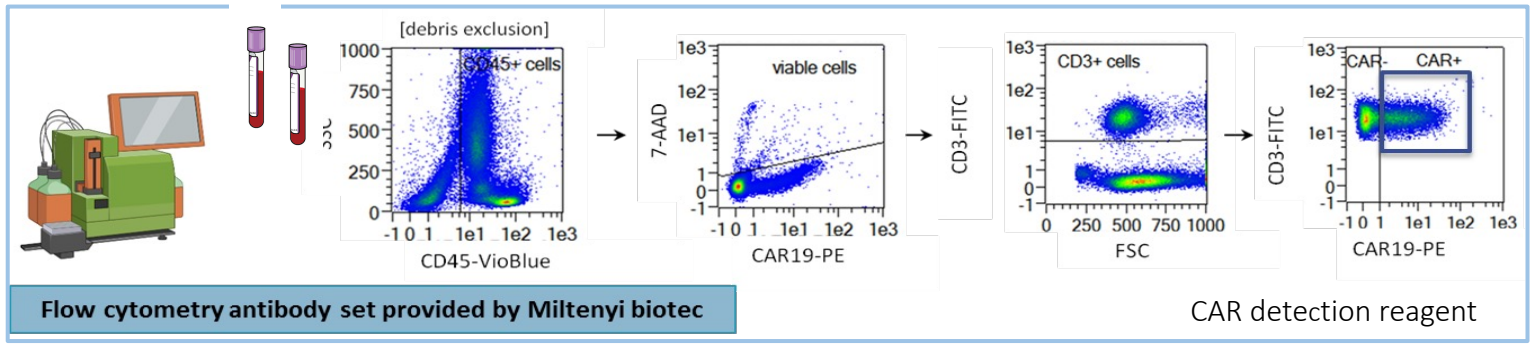
Tisa-cel and Axi-cel differ significantly in cell composition and CAR-T cell diffentiantion subsets





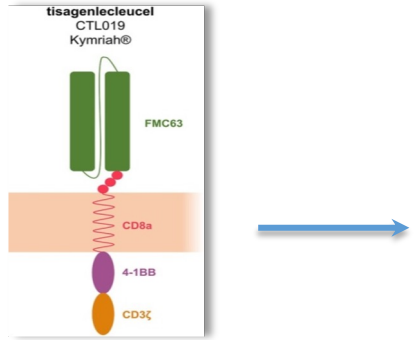
CAR: chimeric antigen receptor; T_N: T naive; T_{SCM}: T stem cell memory; T_{CM}: T central memory; T_{EM}: T effector memory; T_E: T effector

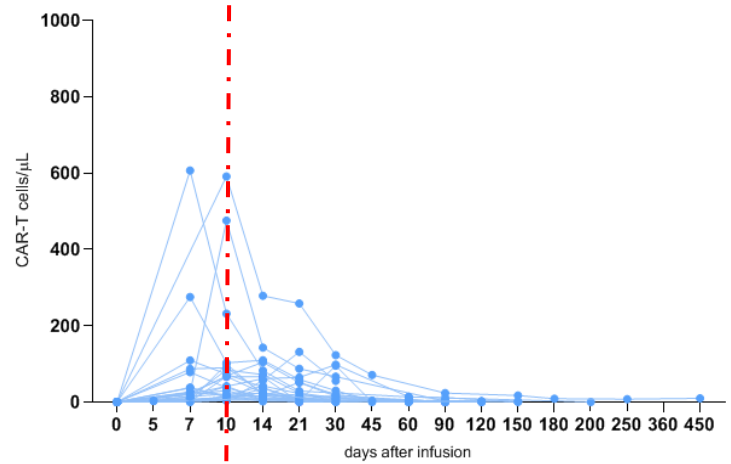
Similar expansion kinetics for Tisa-cel and Axi-cel



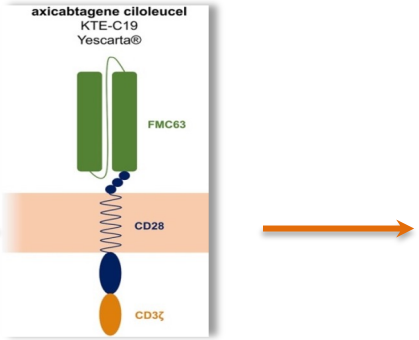
Early expansion kinetics were similar for Tisa-cel and Axi-cel; parameters strongly correlated and maximum expansion (Tmax) was achieved on day 10

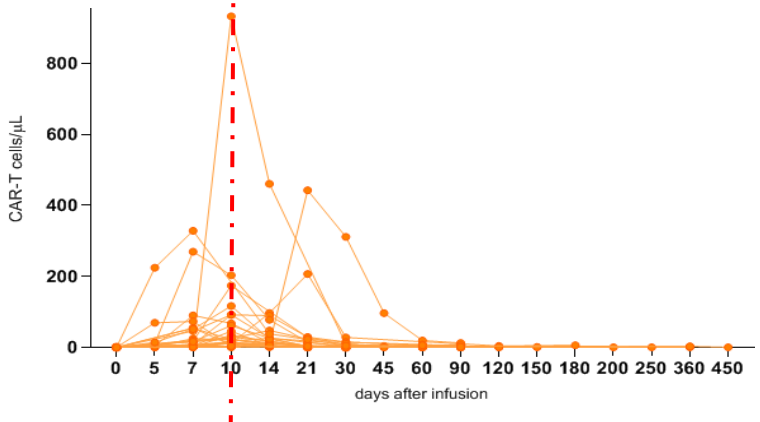
Tisa-cel

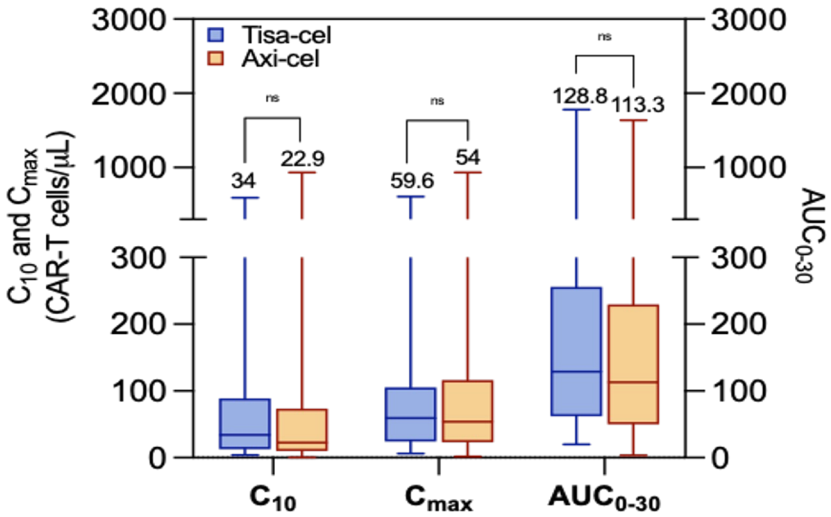




Axi-cel





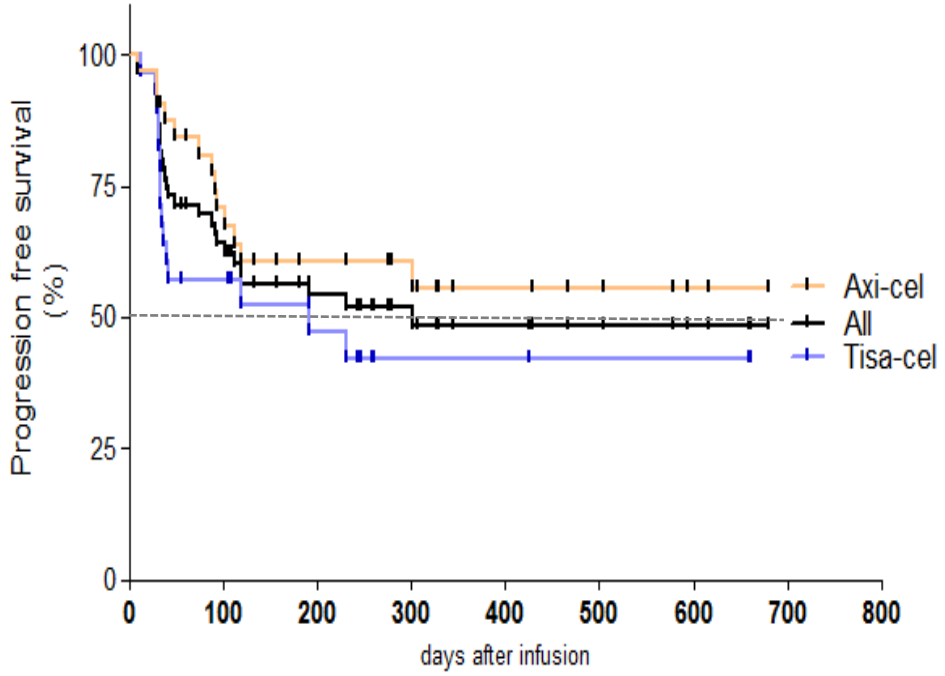


Cerrano et al, 2020 Front Immunol

No difference in PFS and OS by Tisa-cel vs Axi-cel

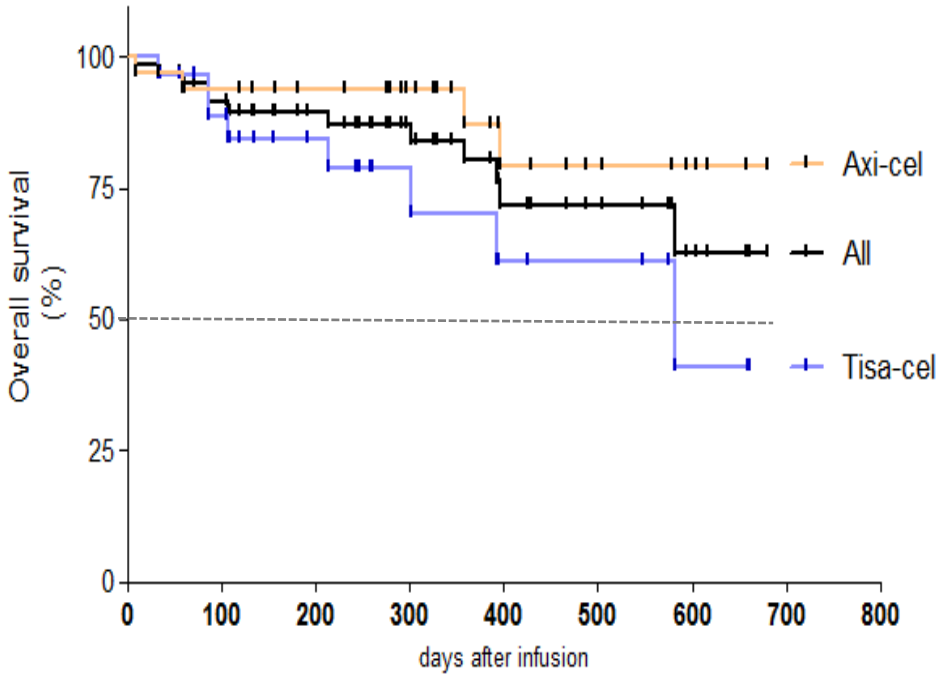
(median follow-up 10.9 months, range 1-24.2)

PFS



Median PFS
Overall = 10,1 months
Axi-cel = not reached
Tisa-cel = 6.4 months, p = ns

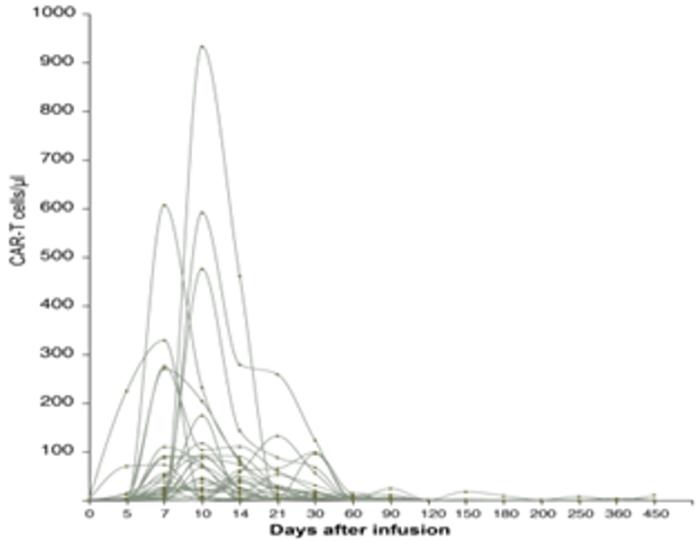
OS



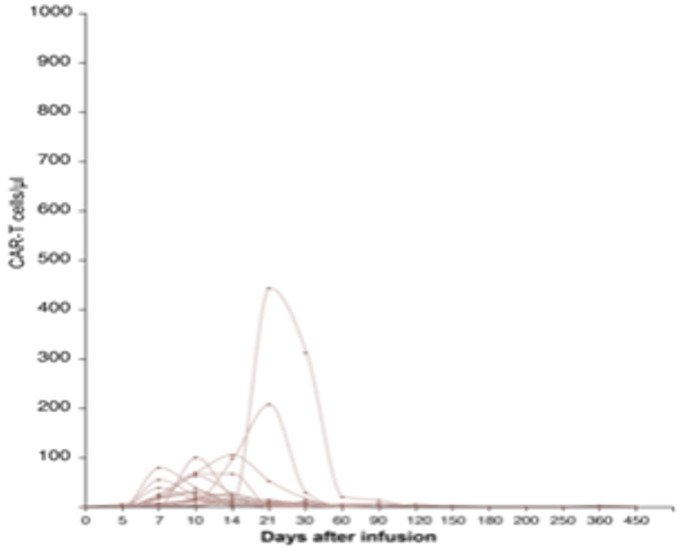
Median OS
Overall = not reached
Axi-cel = not reached
Tisa-cel = 19.4 months, p = ns

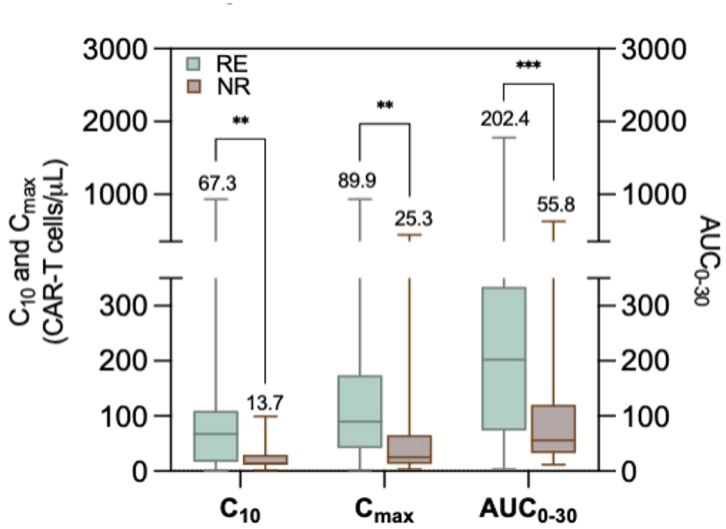
Responders have enhanced expansion

Responders
(*CR + PR by day 90*)



Non-Responders
(*PD + SD by day 90*)



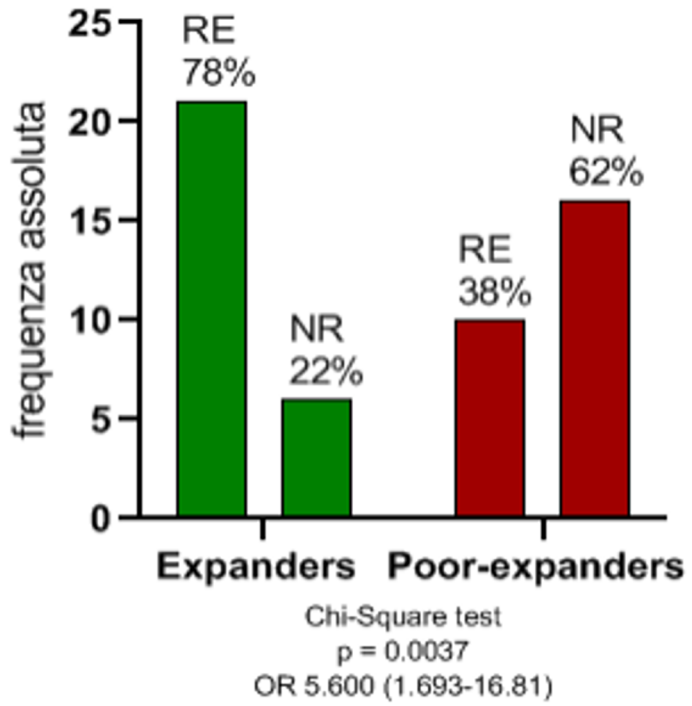


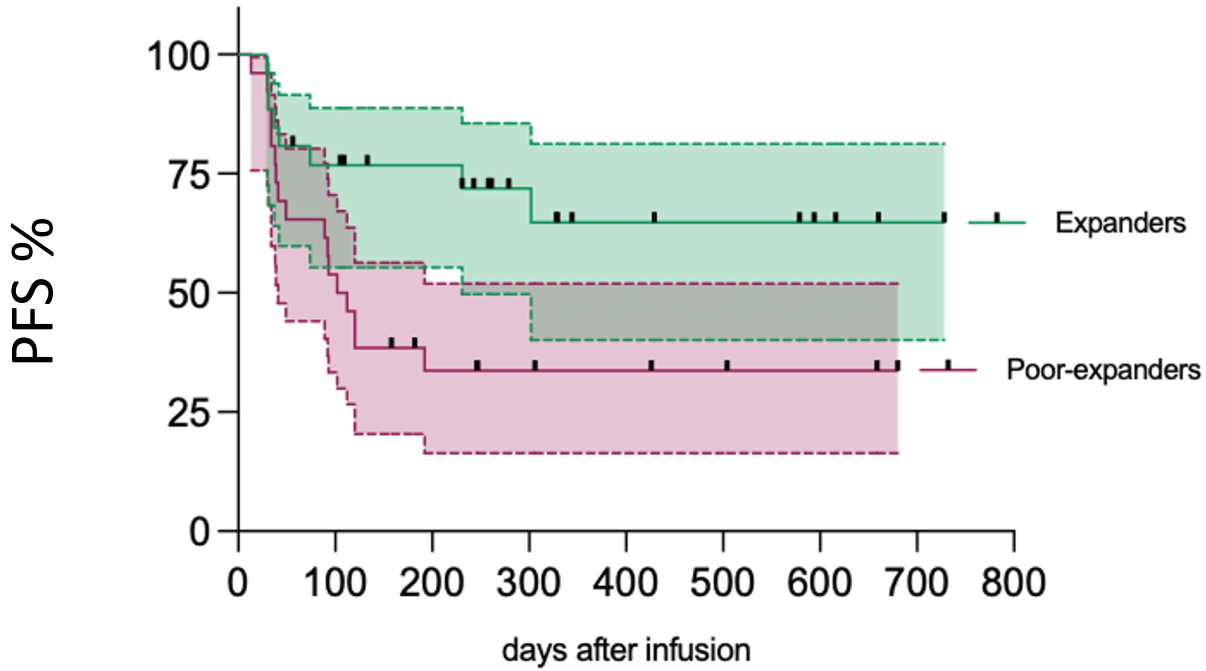
***median C_{10}** was selected to dichotomize the population into Expanders & Poor-Expanders*


M. Magni


C. Monfrini

In vivo expansion is associated with response and survival



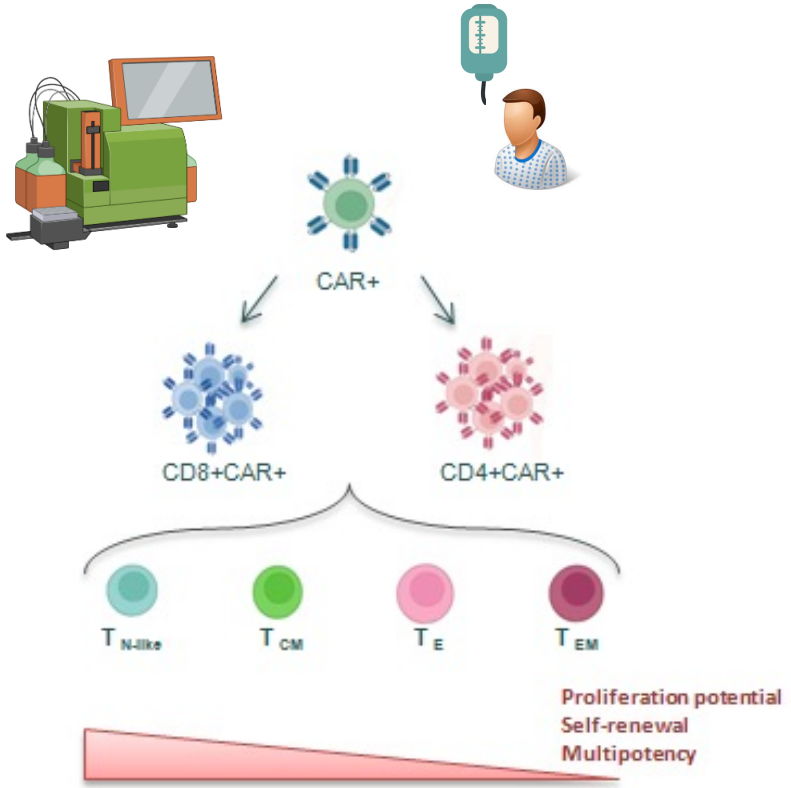


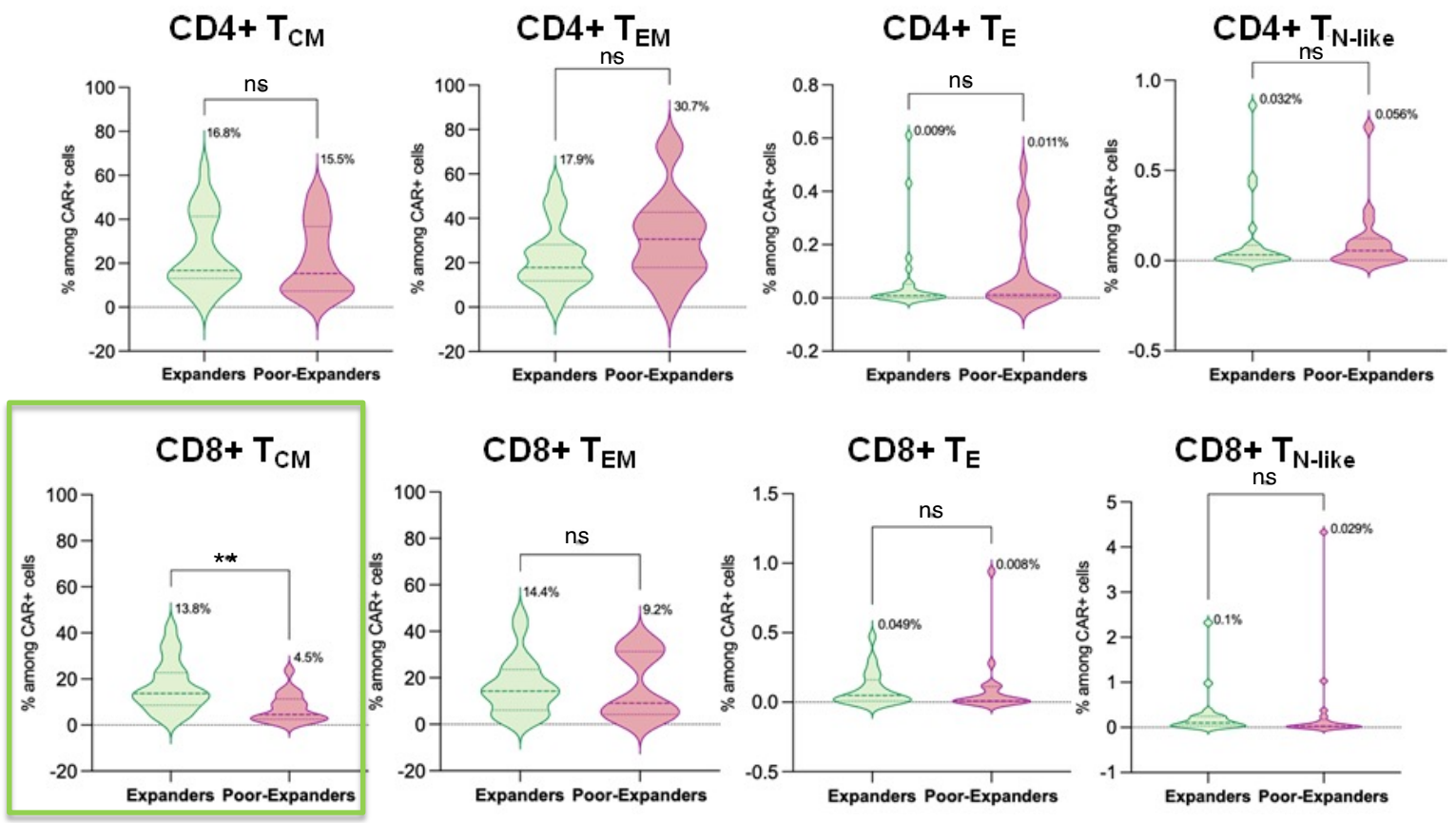
→ C_{10} could represent an early biomarker to predict response and survival in vivo on an individual patient level, regardless of the IP used


M. Magni


C. Monfrini

IP phenotypic signatures are associated with expansion





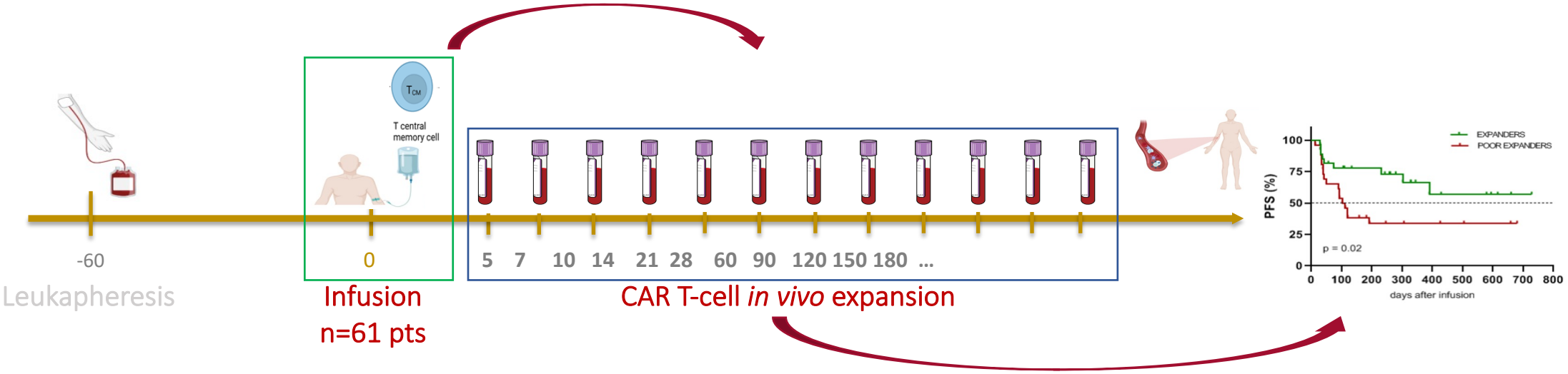
expanders received infusion products enriched in CAR+CD8+ cells with a T_{CM} phenotype (median CAR+/CD8+T_{CM}: 13.8% versus 4.5%; P<0.005)

→ irrespective of the type of IP used, CAR+CD8+ central memory could represent an early biomarker to predict in vivo CAR-T cell expansion

CAR: chimeric antigen receptor; IP: infusion product; T_N: T naive; T_{CM}: T central memory; T_{EM}: T effector memory; T_E: T effector

Summary 1:

No matter what product you use, infusion product characteristics are associated with *in vivo* CAR T expansion and response



- The presence of CD8+T_{CM} cells within the CAR+ cells favorably impact *in vivo* expansion
- Expansion is associated with better response rates and longer PFS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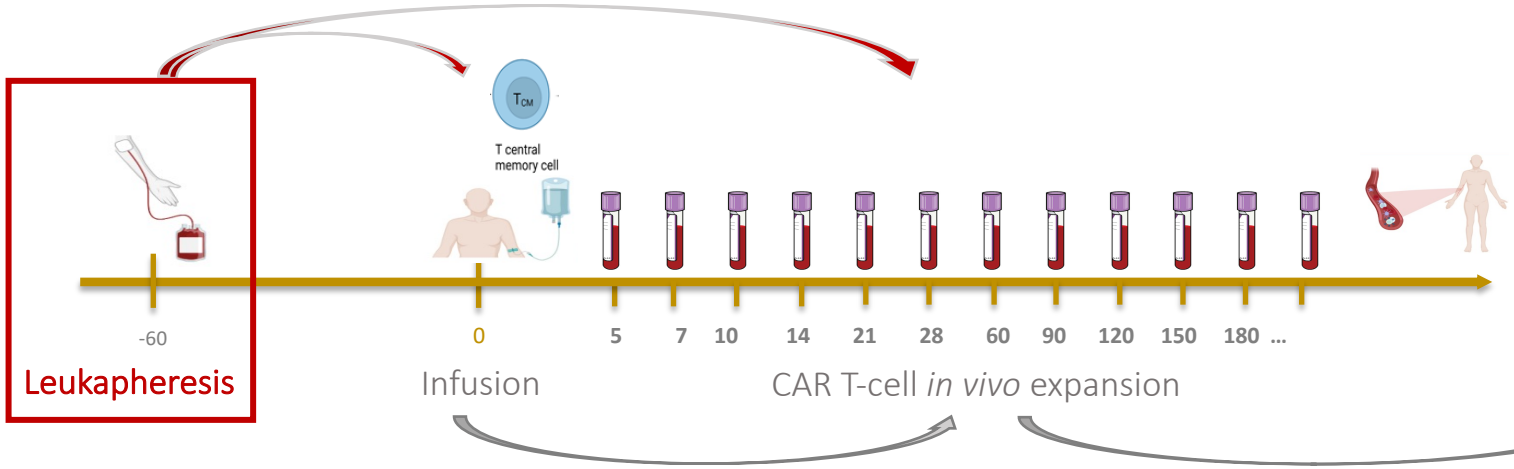
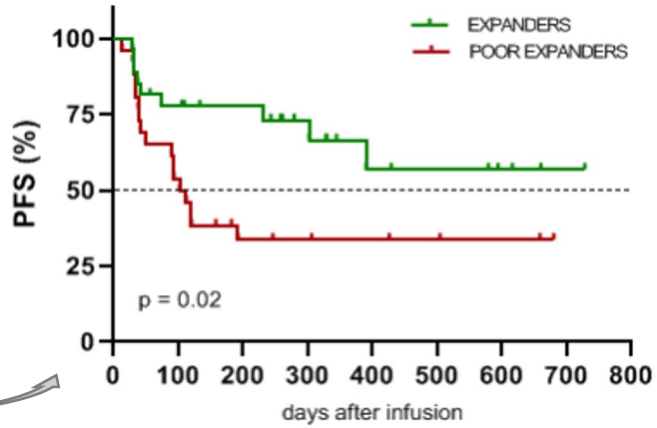
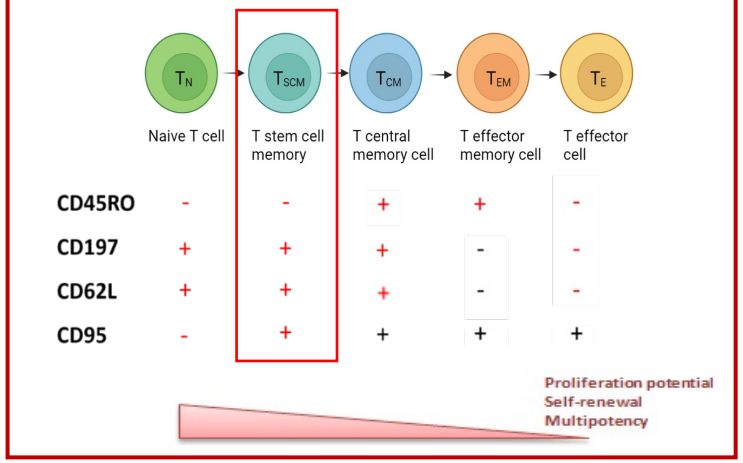
CAR: chimeric antigen receptor; IP: infusion products; PFS: progression free survival; pts: patients; T_{CM} : T central memory

Do LK cellular features matter?





LK T cell differentiation status



LK: leukapheresis; Tn: naïve T cells; Tscm: stem cell memory T cells, Tcm: central memory T cells; Tem: effector memory T cells; Te: effector T cells

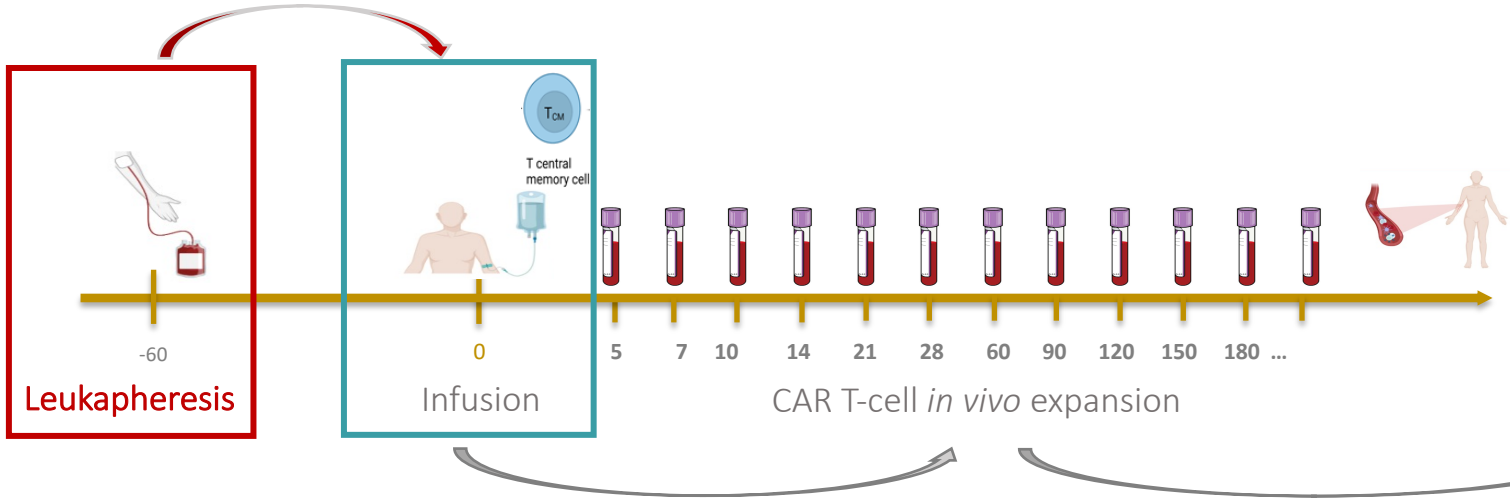
PATIENTS

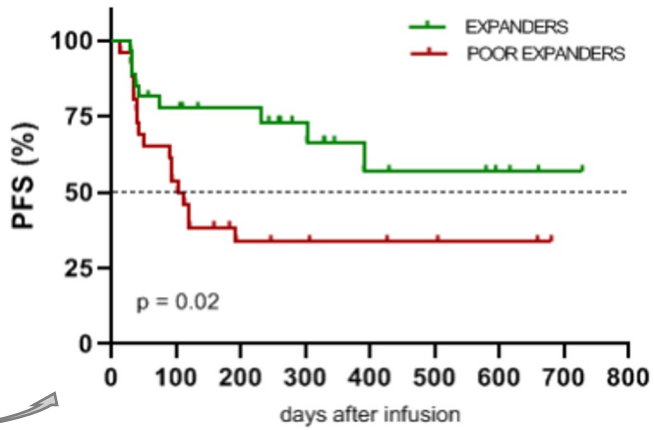
CHARACTERISTICS			N=74
Age (median, range)			56 years (24-73)
Males			46 (62%)
Histotypes	-	DLBCL	39 (53%)
	-	tFL	9 (12%)
	-	HGBL	12 (16%)
	-	PMBCL	14 (19%)
Prior lines	-	Prior lines >2 (3-7)	53 (75%)
	-	Prior ASCT	20 (27%)
	-	Prior CPI	11 (15%)
ECOG	-	0	59 (79%)
	-	1	15 (21%)
Stage	-	≤II	18 (25%)
	-	>II	56 (75%)
IPI	-	0-2	53 (72%)
	-	3-5	21 (28%)
Bulky disease (>5cm)			13 (18%)
ALC apheresis (median)			805 (230-4590)
CRP at day0 >ULN			38 (51%)
LDH at day0 >ULN			25(34%)
Ferritin at day0 >ULN			50 (67%)
Bridging therapy			63 (85%)
Status at infusion	-	CR	12 (16%)
	-	PR	10 (13%)
	-	SD	4 (5%)
	-	PD	49 (66%)
CAR T-cell product	-	Axi-cel	40 (54%)
	-	Tisa-cel	34 (46%)

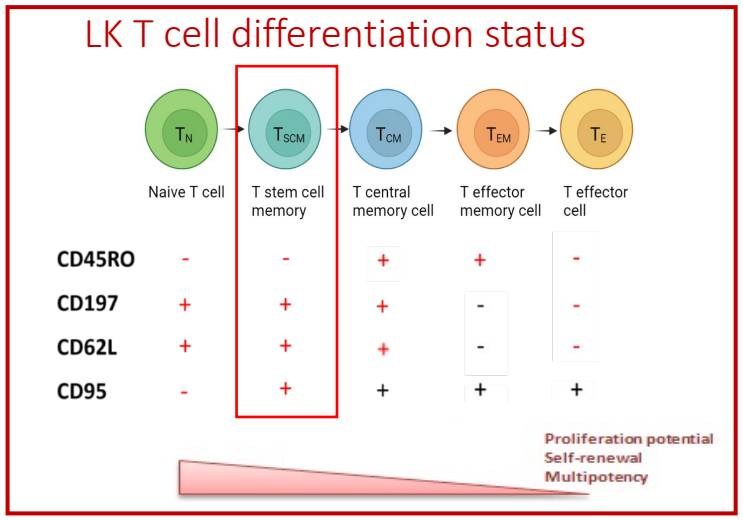
OUTCOMES	
CR @ day 30 (%)	39 (53%)
ORR @ day 30 (%)	46 (62%)
CR @ day 90 (%)	35 (47%)
ORR @ day 90 (%)	37 (50%)
CRS grade ≥ 2 (%)	16 (22%)
ICANS	12 (16%) all grade 1
Tocilizumab (%)	34 (46%)
Steroids (%)	25 (34%)

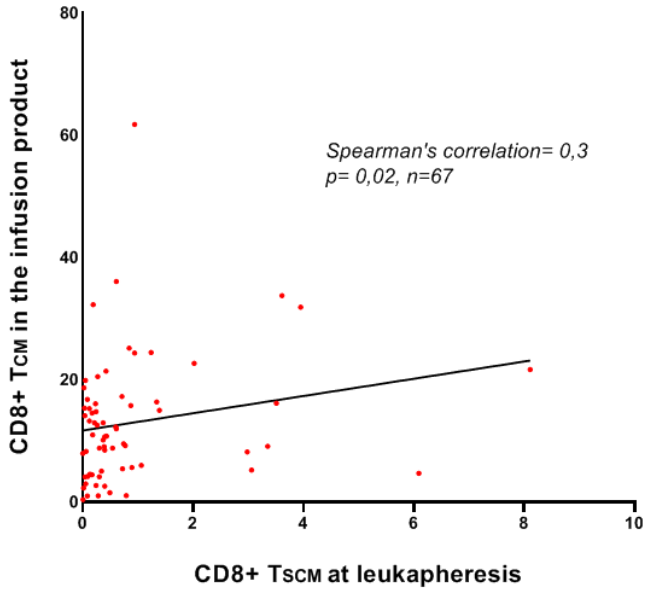
ALC: Absolute Lymphocyte Count; ASCT: Autologous Stem Cell Transplant; CPI: check-point inhibitors; CR: Complete Response; CRP: C-Reactive Protein; CRS: cytokine release syndrome; DLBCL: Diffuse Large B-cell Lymphoma; ECOG: Eastern Cooperative Oncology Group; HGBL: High Grade B-cell Lymphoma; ICANS: immune cell–associated neurologic syndrome; IPI: International Prognostic Index; LDH: Lactate Dehydrogenase; ORR: Overall response rate; PD: Progressive Disease; PMBCL: Primary Medistinal B-cell Lymphoma; PR: Partial Response; SD: Stable disease; tFL: transformed Follicular Lymphoma; TMTV:Total Metabolic Tumor Volume; ULN: Upper Level Normality.

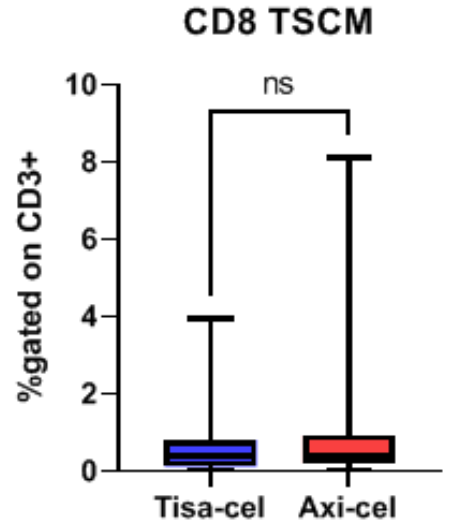
Correlation between CD8+ T_{SCM} in LK and CD8+T_{CM} in IP





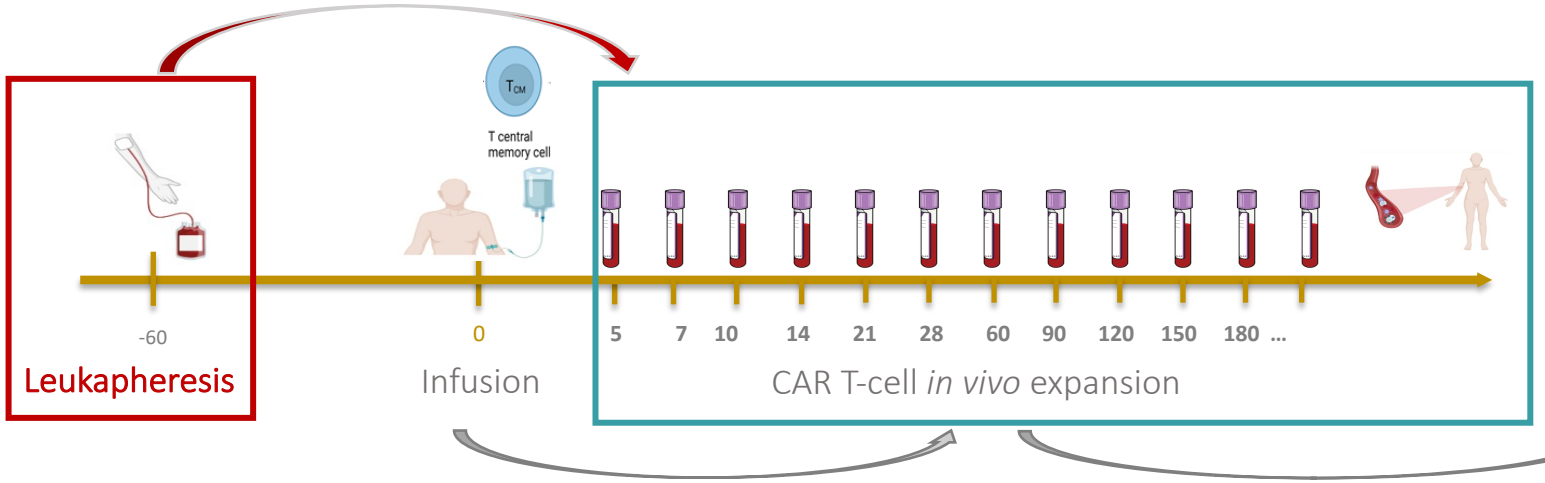


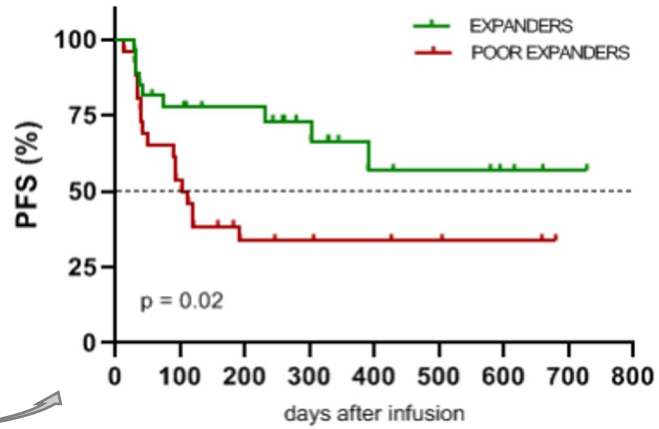


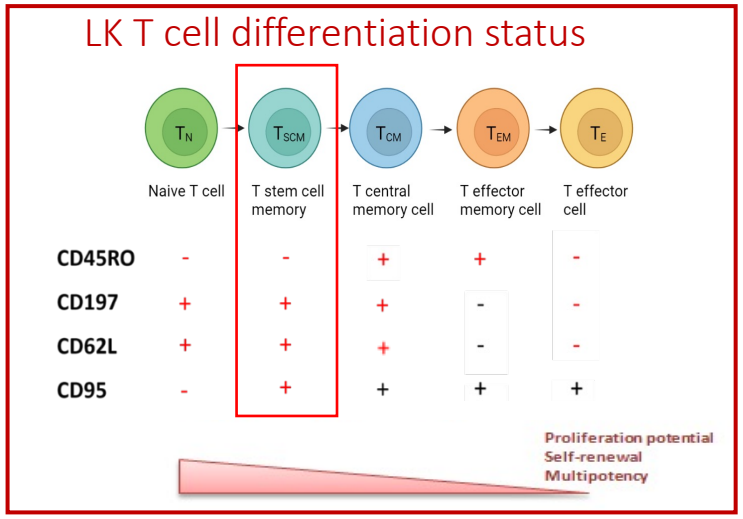


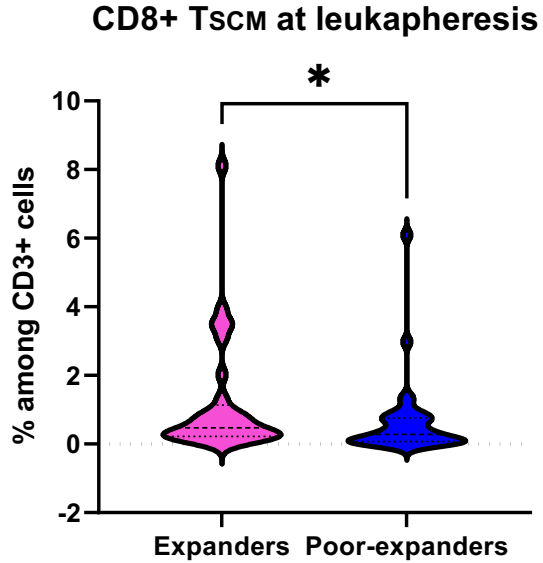
LK: leukapheresis; Tn: naïve T cells; Tscm: stem cell memory T cells, Tcm: central memory T cells; Tem: effector memory T cells; Te: effector T cells

Correlation between CD8+ T_{SCM} in LK and expansion









median C_{10}: it was selected to dichotomize the population into Expanders & Poor-Expanders

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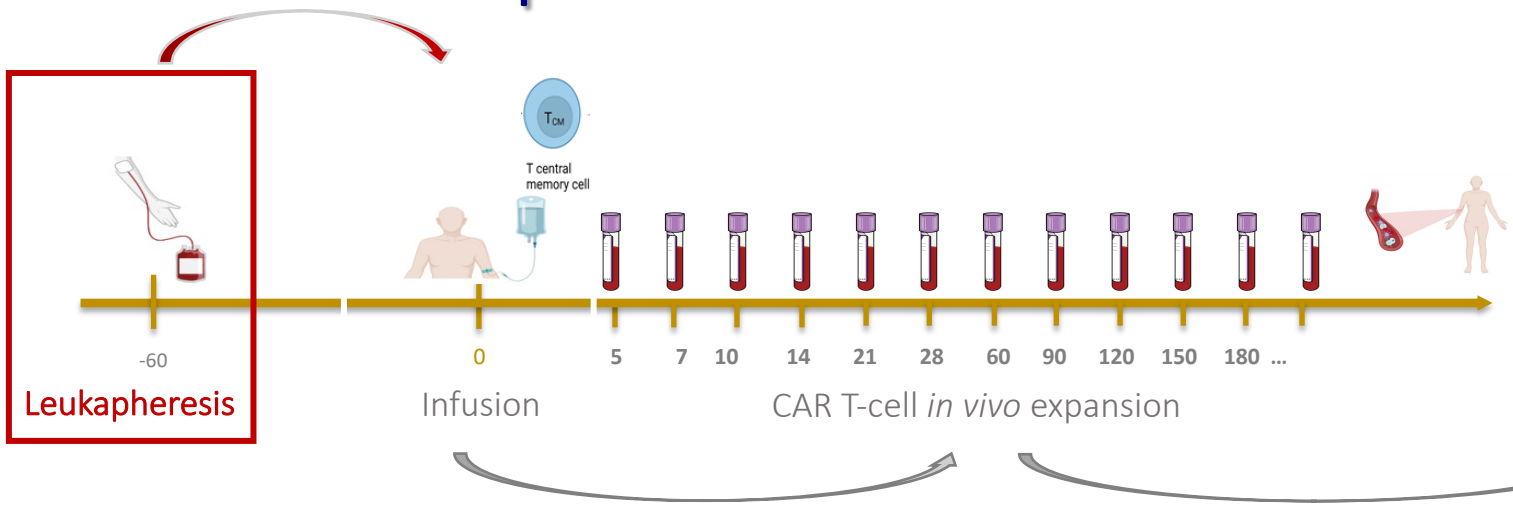
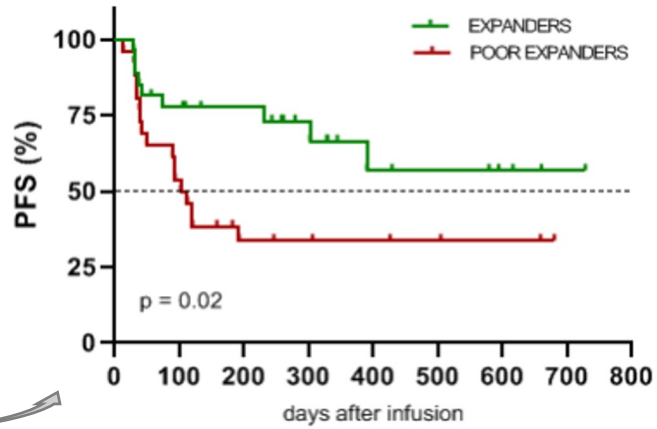
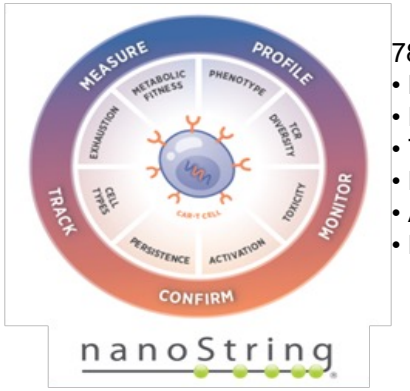
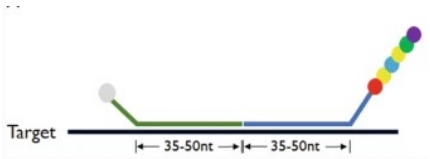
Do LK transcriptional features matter ?







780 genes covering T-cell biology and immune function
- Metabolic fitness
- Exhaustion
- T cell types
- Persistance
- Activation
- Diversity



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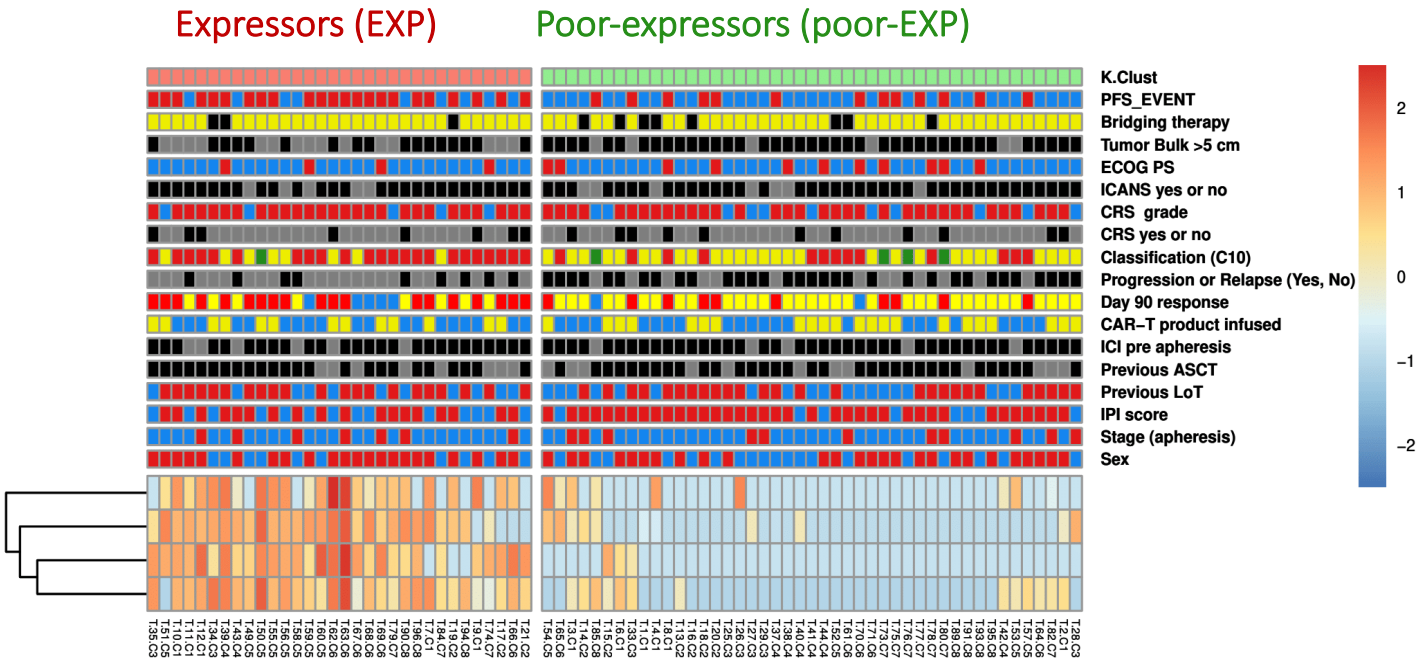
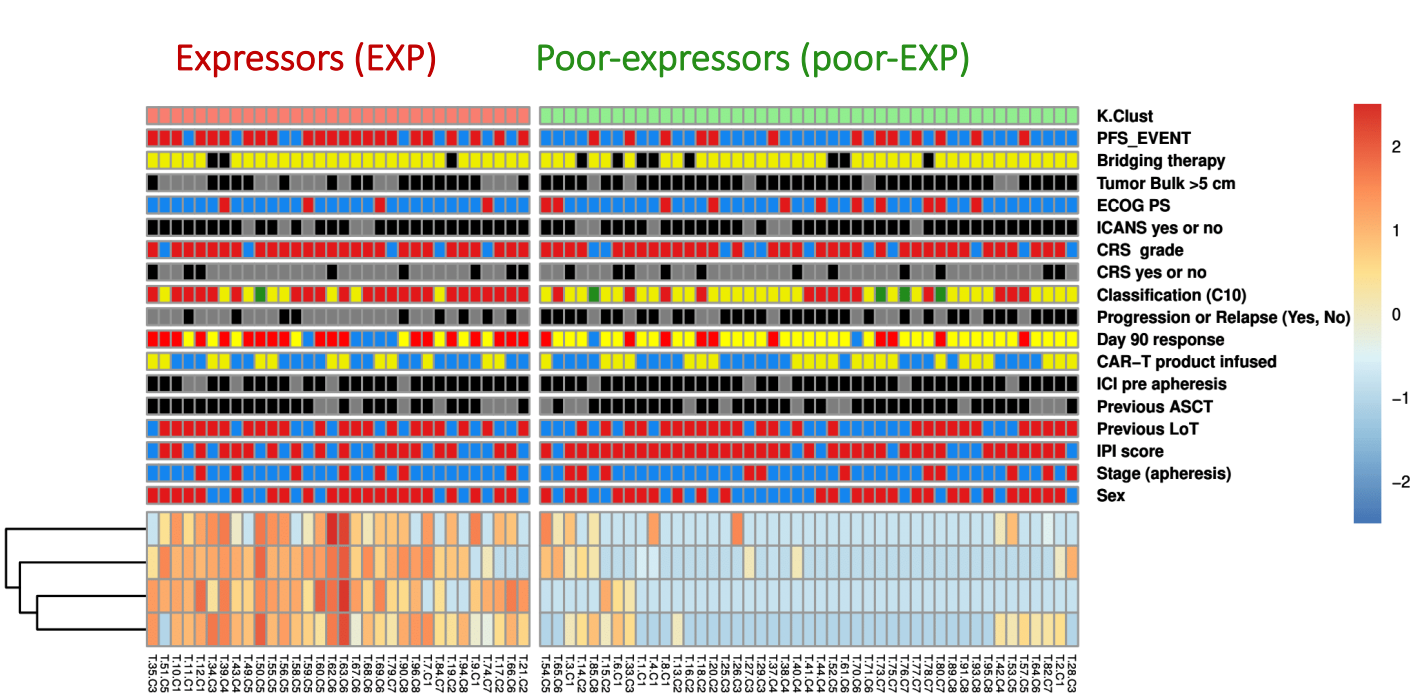
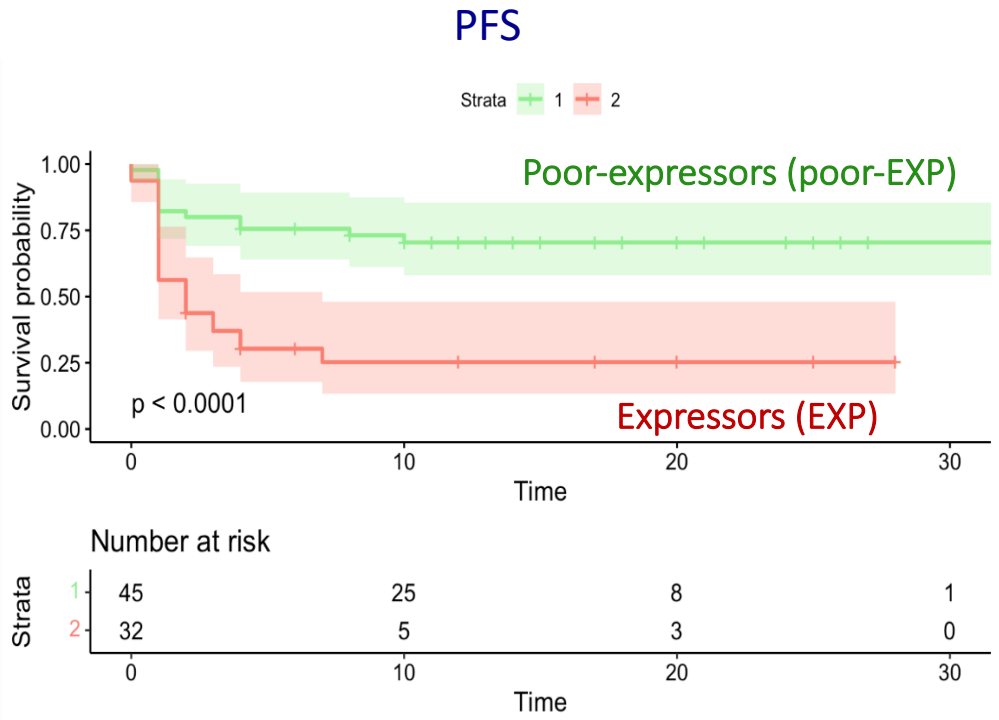
A 4-gene signature in LK segregates pts with different PFS



ASCT: Autologous Stem Cell Transplant; C_{10}: CAR T-cell concentration at day 10; CRS: cytokine release syndrome; ICP: immune check-point inhibitors; ICANS: immune cell–associated neurologic syndrome; LK: leukapheresis

A 4-gene signature in LK segregates pts with different PFS





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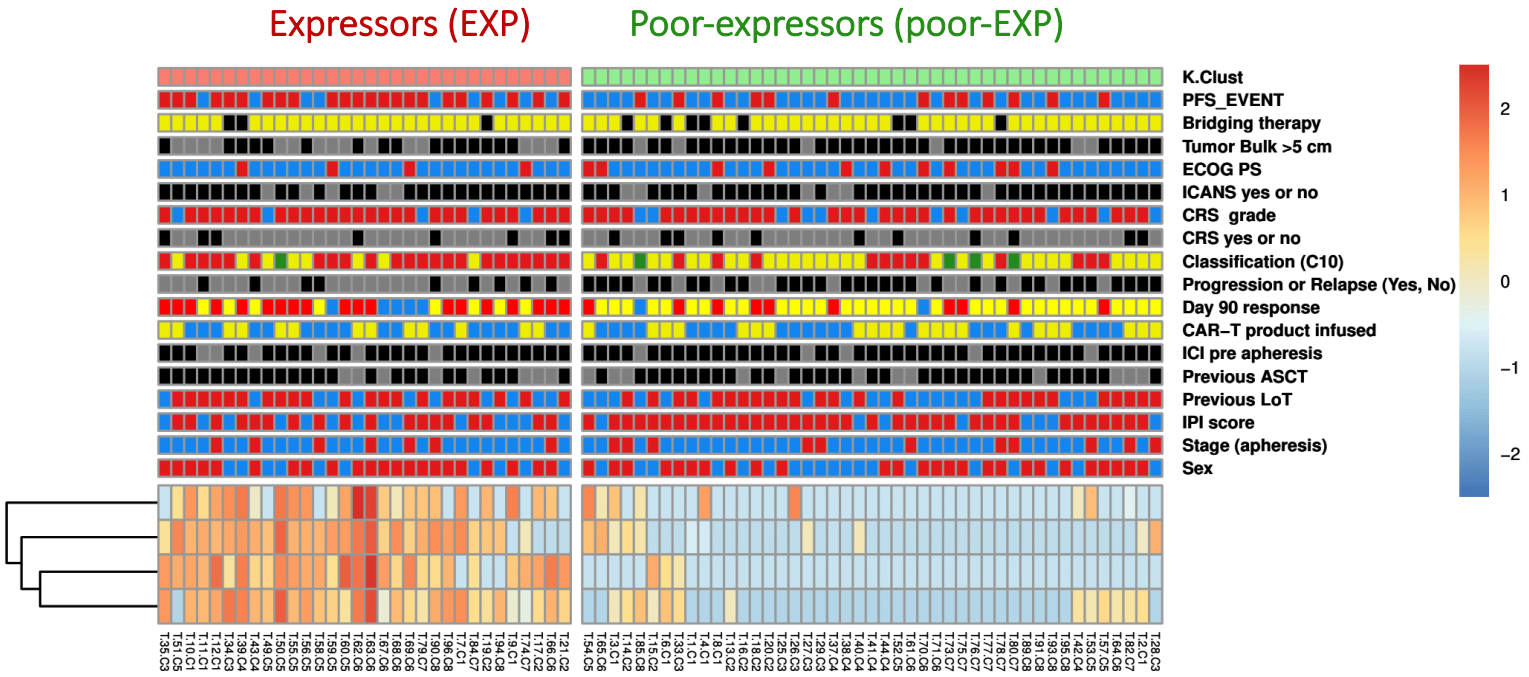
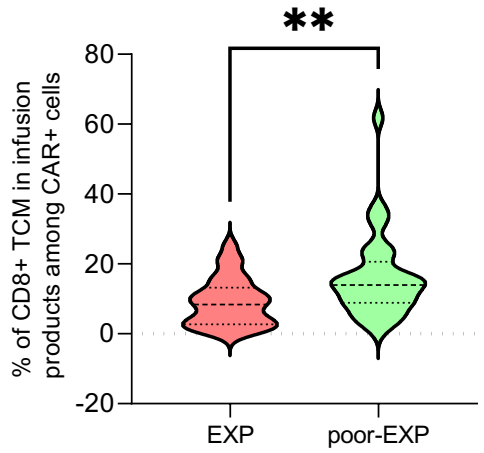
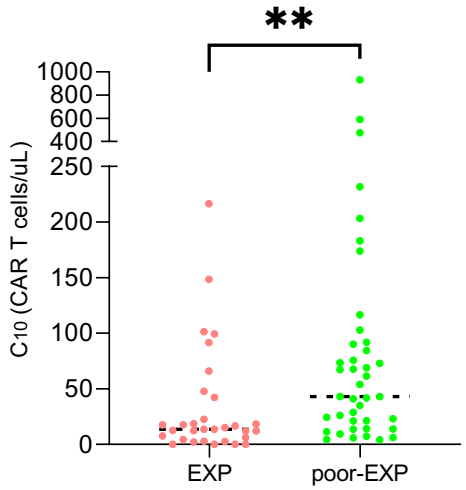
The 4-gene signature in LK correlates with CD8T$_{CM}$ cells in IP and with in vivo CAR T expansion







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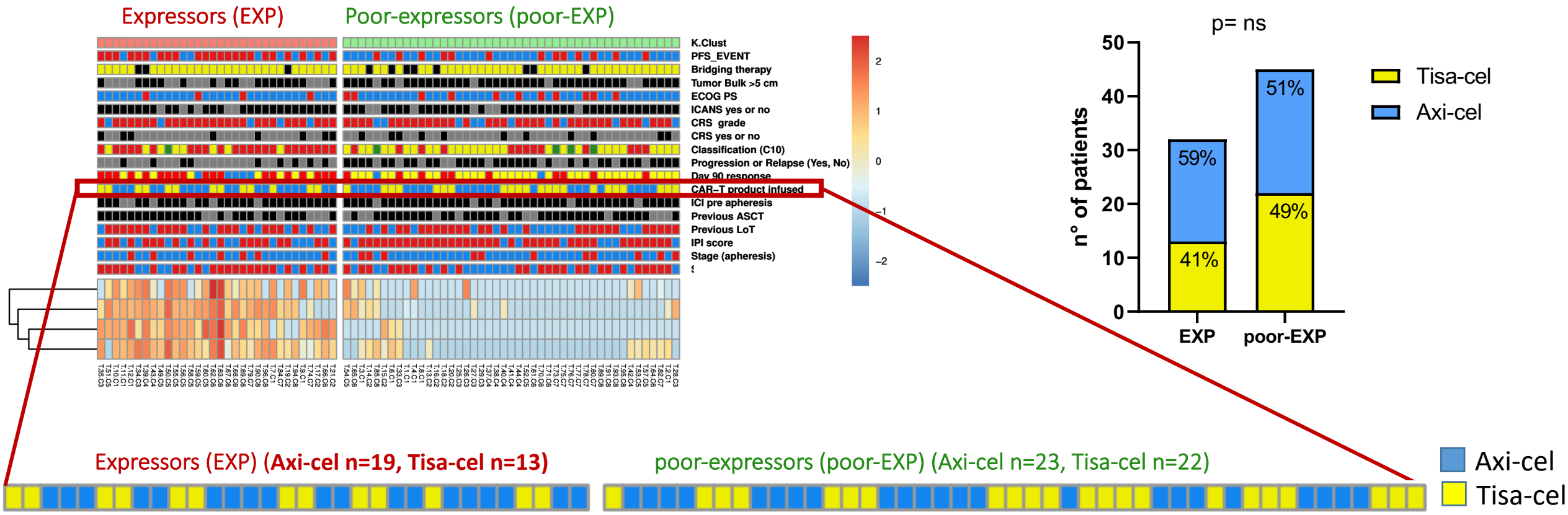
No correlation between the signature and IPs



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Summary:

We have defined novel correlates of response to Tisa-cel and Axi-cel in leukapheresis:

- A less-differentiated status of T cells at leukapheresis is associated to IP features and to *in vivo* CAR T expansion and thus response and survival of LBCL pts
- Concomitantly, a 4-gene signature in the leukapheresis affect IP features, in vivo expansion and segregates pts with different PFS probabilities