Second generation auto-CAR-T

Stephen J. Schuster, M.D.

Professor of Medicine, Perelman School of Medicine
University *of* Pennsylvania, Philadelphia, PA, USA

Disclosures: Stephen J. Schuster

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
AbbVie						X	
ADC Therapeutics						X	
AstraZeneca	X		X			X	
BeiGene						X	
BioNTech			X				
BMS	X					X	
Caribou Bio			X			X	
Genentech/Roche	X					X	
Genmab	X		X			X	
Incyte			X				
Janssen						X	
Novartis	X		X			X	
Vittoria Bio						X	

Session II Outline: CAR-T

Second generation autologous CAR-T

- *Do we need a new CAR or just a tune up?*

Potential approaches to improving auto-CAR T

- T cell "fitness" and its impact on T cell-based therapies
- Potential approaches to improving T cell fitness
  - ✓ *"The sooner the better:"* earlier application of auto-CAR T? Rapid CAR-T manufacturing?
  - ✓ *"A preemptive strike:"* apheresis at diagnosis?
  - ✓ *"Build better bridges:"* tumor control with new agents (*e.g.*, *BTKi, CELMoD, BsAb or ADCs?*)
  - ✓ *"The more, the merrier:"* Dual tumor antigen targeting approaches?
  - ✓ Armored CARs

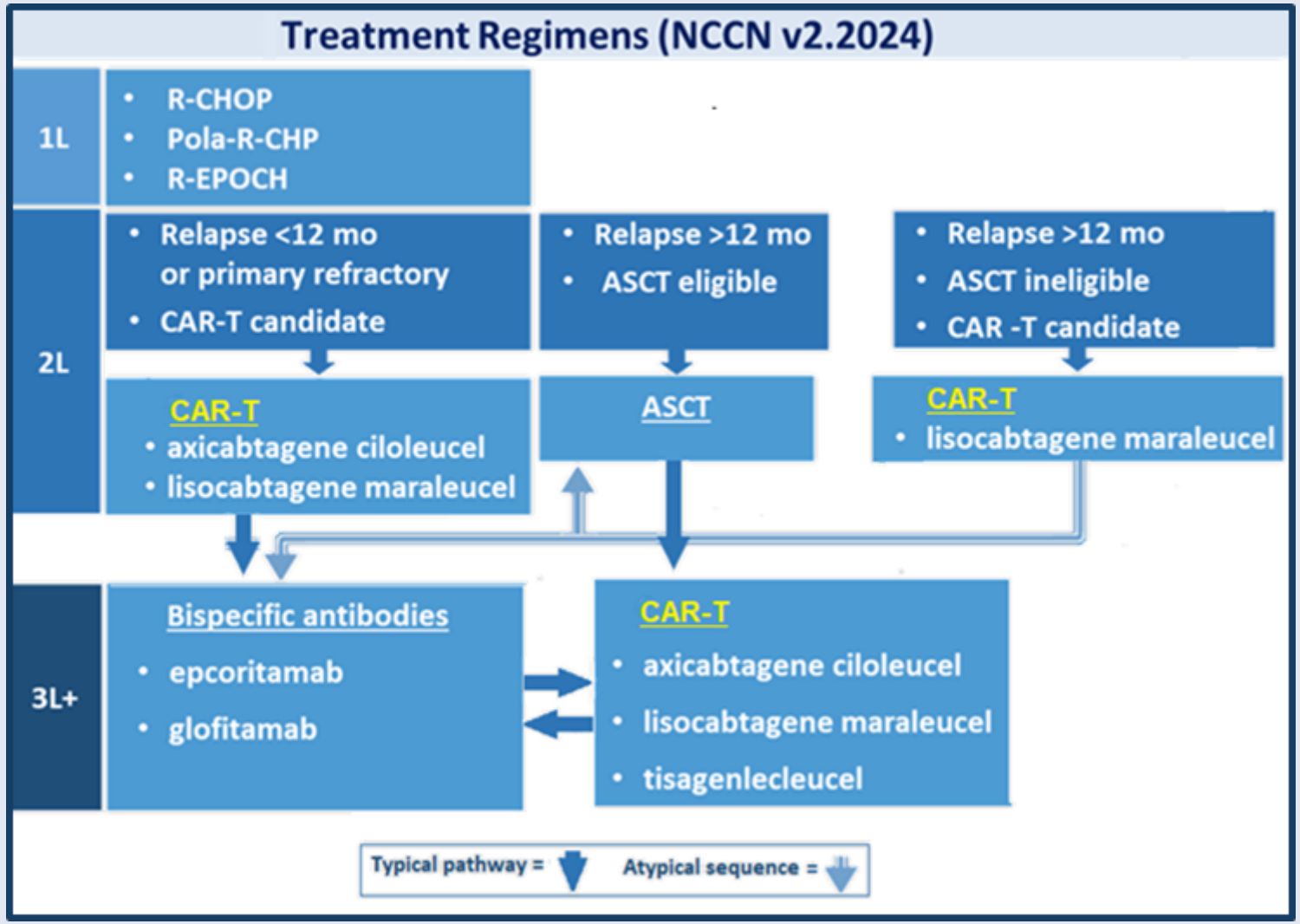
Non-auto-CAR T approaches

- ✓ *"You can't teach an old dog new tricks:"* allo-CAR T ***(Dr. Neelapu will cover)***
- ✓ *in vivo* CAR T ***(Dr. Siddiqi will cover)***

Conclusions

- *Where are we headed?*

CAR-T is a current standard of care (SOC) for LBCL



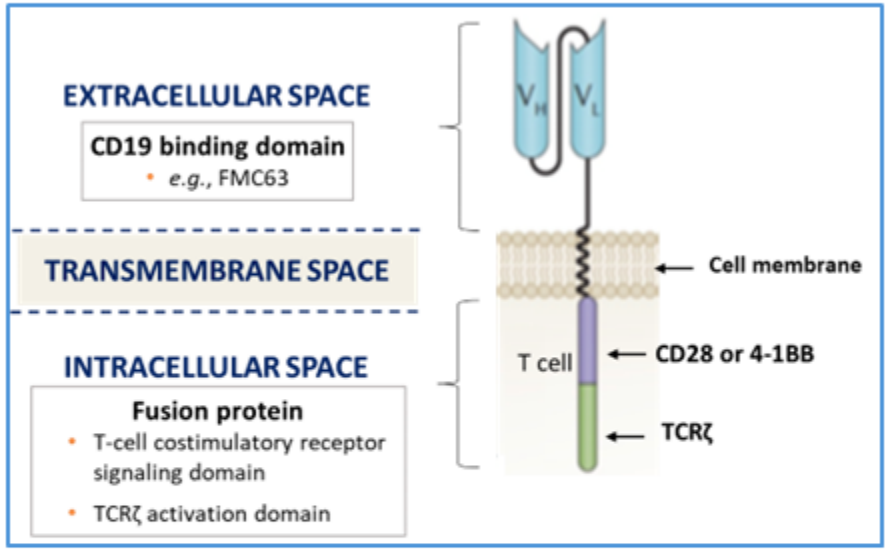
L, line of therapy; CAR-T, chimeric antigen receptor modified T cells; ASCT, autologous stem cell transplant

Based on "Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for B-Cell Lymphomas" V.3.2024.

CAR-T: a current standard of care (SOC) for r/r LBCL

- **Chimeric antigen receptor (CAR) modified autologous T cell therapies (CAR-T) for r/r LBCL**

CAR-T Product	FDA Approved	EMA Approved
Tisagenlecleucel		
• ≥3rd line therapy of r/r LBCL	2018	2018
Axicabtagene ciloleucel		
• ≥3rd line therapy of r/r LBCL	2017	2018
• 2nd line therapy of r/early relapse LBCL	2022	2022
Lisocabtagene maraleucel		
• ≥3rd line therapy of r/r LBCL	2021	2022
• 2nd line therapy of r/early relapse LBCL	2022	2023



- **All available CAR-T cell therapies share *antigen-specificity for CD19* using the same scFv derived from FMC63**
  - ***Efficacy:*** *efficacy in r/r LBCL similar in ≥ 3rd-line trials*
  - ***Toxicities:*** *similar class effects, but differ in degree*
  - ***Logistics of administration:*** *somewhat different*

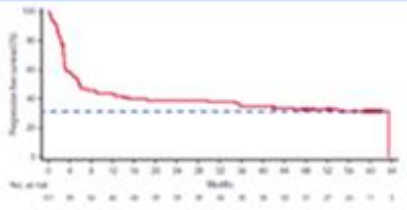
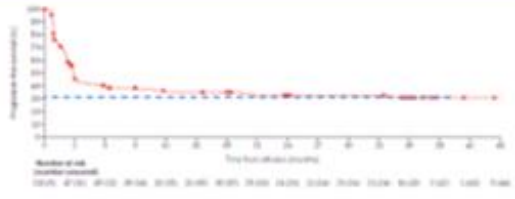
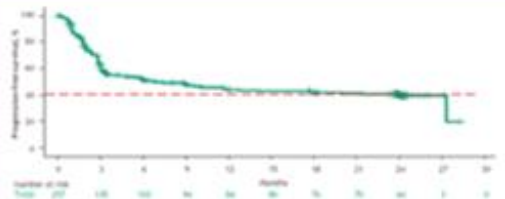
r/r, relapsed or refractory; LBCL, large B-cell lymphomas; FDA, US Food and Drug Administration; EMA, European Medicines Agency

CAR-T for large B-cell lymphomas: Registrational trials

≥ 3rd line therapy for LBCL			
	axi-cel	tisa-cel	liso-cel
CR rate	58%	39%	53%

as ≥ 3rd-line therapy

~ 2/3 of patients ultimately fail

Axicabtagene ciloleucel[1]	Tisagenlecleucel[2]	Lisocabtagene maraleucel[3]
ZUMA-1[1]: axi-cel as ≥ 3rd-line therapy for LBCL N = 101 Median follow-up: 63.1 months **Estimated 5-year EFS: 30.3% (95% CI, 21.5-39.6)**	**JULIET**[2]: tisa-cel as > 3rd-line therapy for LBCL N = 115 Median follow-up: 40-3 months **Estimated 40-month PFS: ~30%**	**TRANSCEND**[3]: liso-cel as ≥ 3rd-line therapy LBCL N = 257 Median follow-up: 23.9 months **Estimated 24-month PFS: 40.6% (95% CI, 34.0-47.2)**
		

*The studies summarized above differ in their designs, patient characteristics and follow-up; therefore, direct comparisons are precluded.

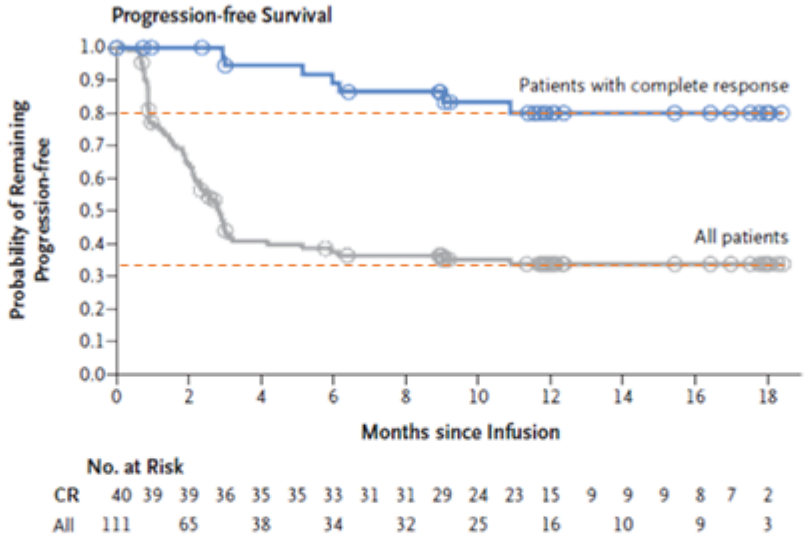
[1]Neelapu SS, et al. Blood 2023; 141(19):2307-2315; [2]Schuster SJ, et al. Lancet Oncol 2021;22(10):1403-1415; [3]Abramson J, et al. Blood 2024;143(5):404-416.

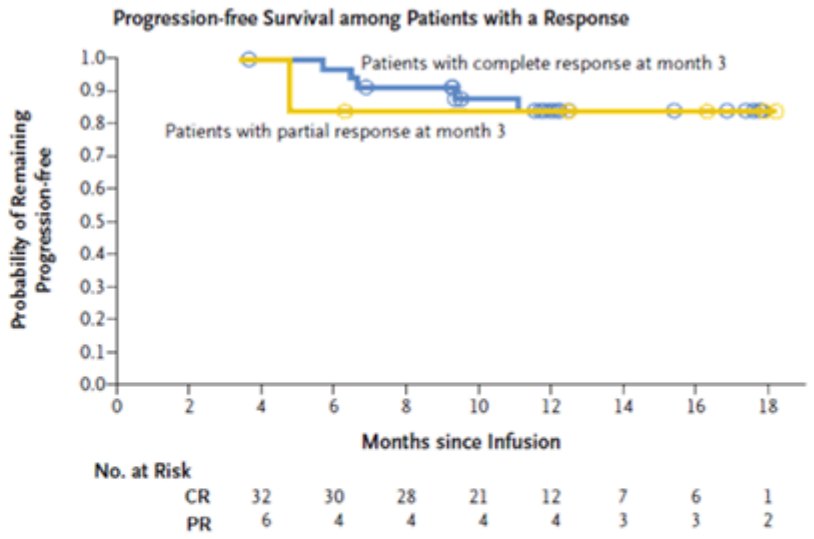
Optimizing auto CAR-T outcomes: Complete responses are often durable

- **Achieving a CR at any time post-infusion is required for a durable response**
- **Data from the JULIET trial: Phase 2 trial of tisagenlecleucel in r/r large B-cell lymphomas**

JULIET trial: 40-month median follow-up	Patients (n=115)
Best overall response	
Complete response	45 (39%)
Partial response	16 (14%)
Stable disease	15 (13%)
Progressive disease	30 (26%)
Unknown*	9 (8%)
Overall response rate	61 (53·0%, 43·5–62·4)
Median time to first response, days	29·0 (28·0–31·0)

Data are n (%), n (%, 95% CI), or median (IQR). *Data for the last assessment not available.



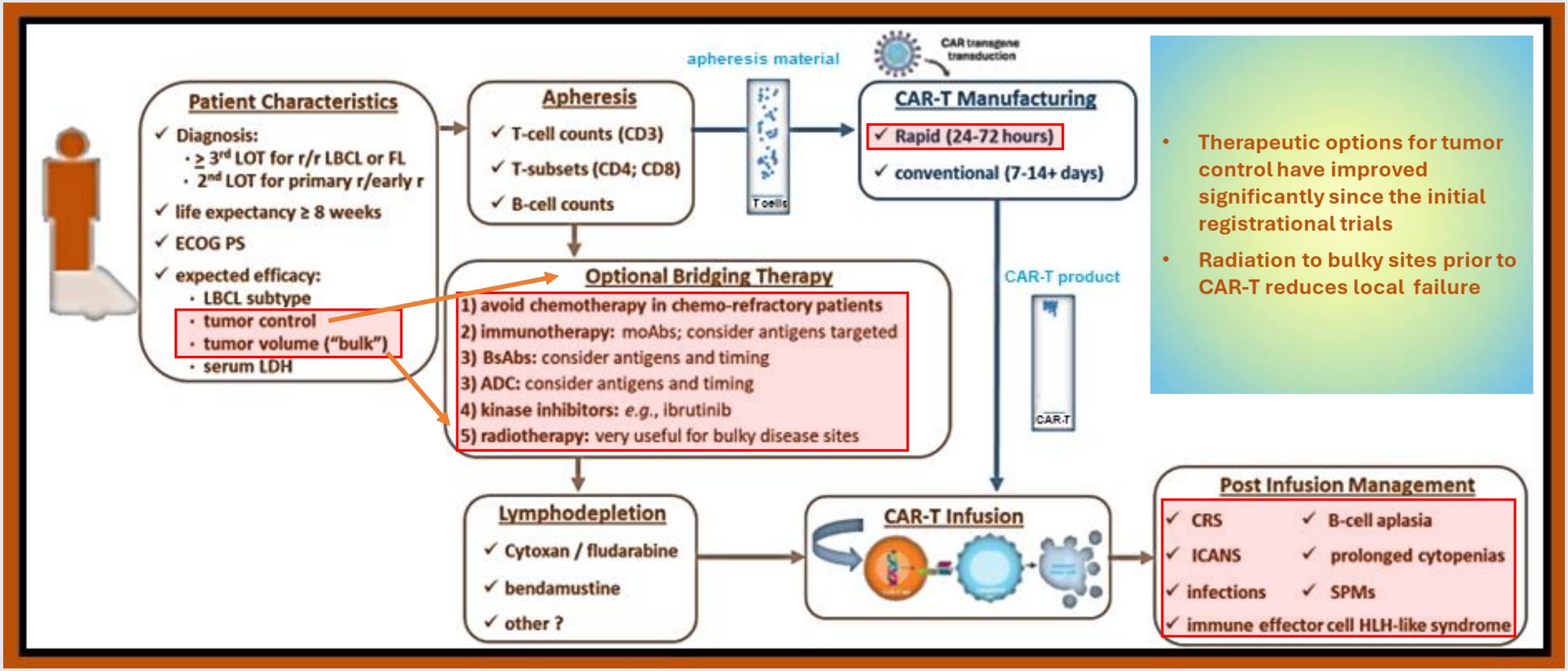


Complete responses can occur late[2]

- **At 1 month after infusion, 16 patients with SD (n = 4) or PR (n = 12) improved to a CR between months 1 to 17**
- At 3 months after infusion, 5/8 (62%) patients with a PR or SD by PET/CT converted to a CR (1 at 6 months, 1 at 9 months, and 3 >12 months)

[1]Schuster SJ, et al. N Engl J Med. 2019;380(1):45-56.
[2]Schuster SJ, et al. Lancet Oncol. 2021;22(10):1403-1415.

CAR-T for LBCL: Factors impacting outcome beyond cellular engineering



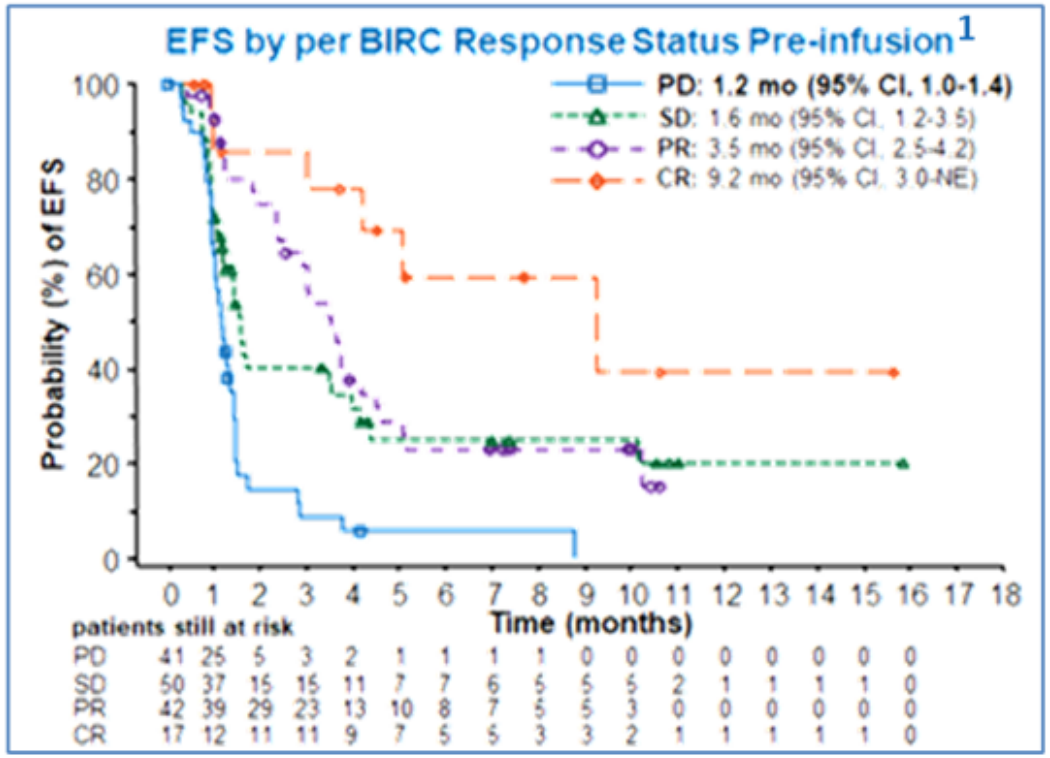
"Focus on what is within your control and let go of the rest."

- Marcus Aurelius

Patient characteristics impact outcome: Tumor Control

• Disease status at the time of CAR-T infusion impacts best response and EFS

- Belinda trial: tisagenlecleucel vs SOC as 2nd LOT in r/r LBCL

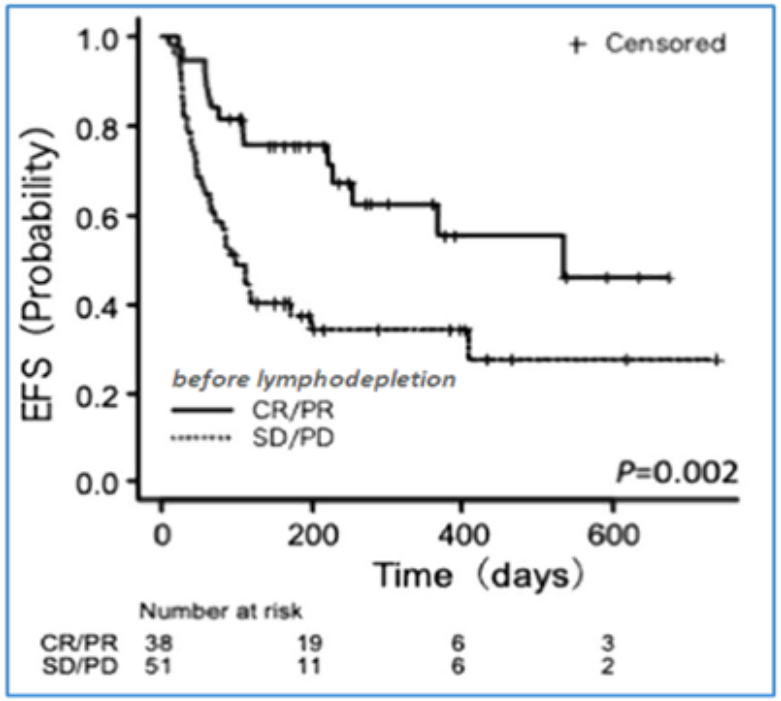


EFS time is relative to date of tisagenlecleucel infusion
EFS events defined as PD/SD after day 71 from randomization or death at any time.
PD = progressive disease; SD = stable disease; PR = partial response; CR = complete response

[1]Bishop *et al.* N Engl J Med. 2021 Dec 14. Epub

- Real-world data from Japan for tisagenlecleucel in r/r LBCL[2]

EFS after tisa-cel by disease status *after* bridging therapy and *before* LD

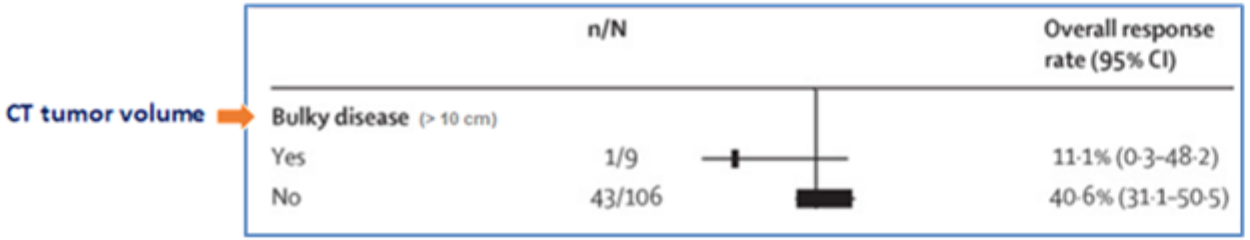


*EFS defined as the period from infusion to either progression or death

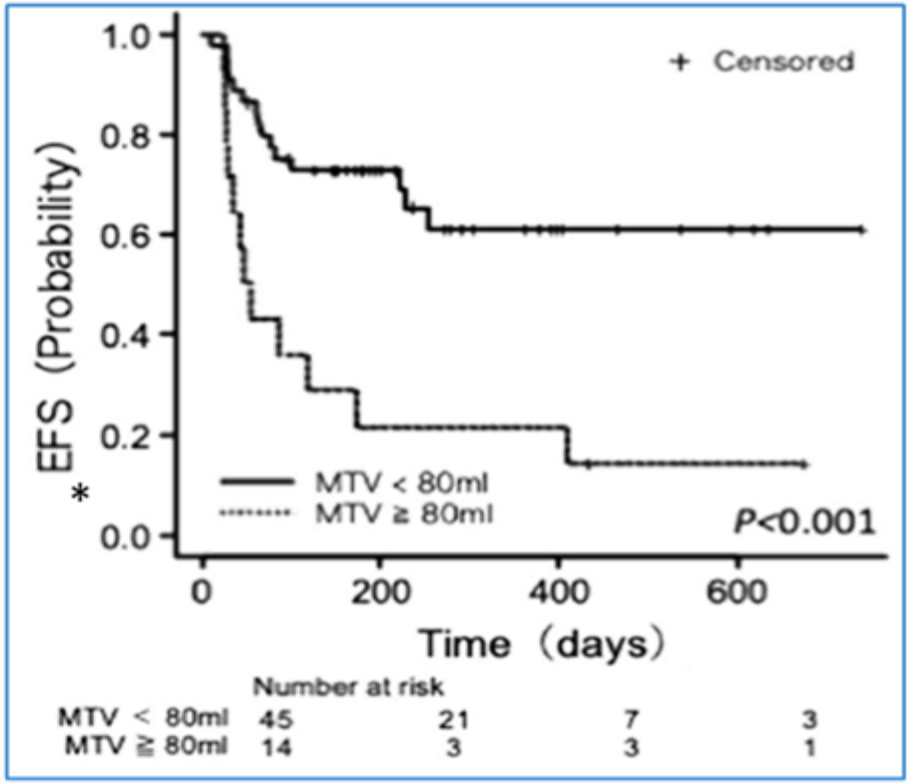
[21]Goto H, et al. Int J Clin Oncol. 2023;28:816–826.

Patient characteristics impact outcome: Tumor Volume

- **Tumor bulk and its impact on response *("size matters")*[1]**
  - **Data from JULIET trial: Phase 2 trial of tisagenlecleucel in r/r LBCL**



- **MTV Data for tisagenlecleucel in r/r LBCL[2]**
  - **Real-world evidence from Japan**



*** EFS defined as the period from infusion to either progression or death**

MTV, metabolic tumor volume, EFS, event-free survival

[1]Schuster SJ, *et al*. Lancet Oncol. 2021;22(10):1403-1415.

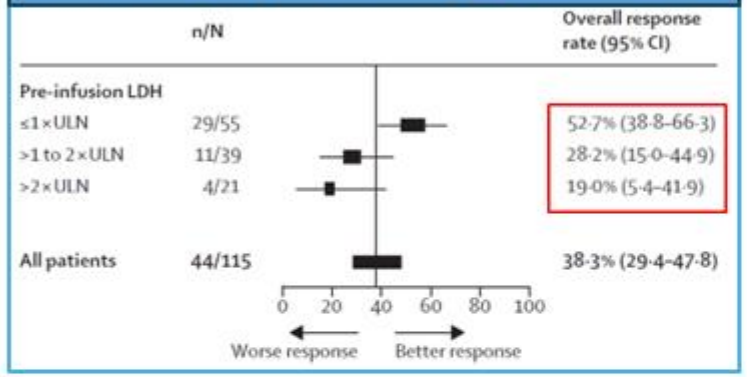
[2]Goto H, et al. Int J Clin Oncol. 2023;28:816–826.

Patient characteristics impact outcome: Serum LDH

- **Pre-infusion serum LDH impacts response to CAR-T and survival outcome**
  - **Data from the JULIET trial: Phase 2 trial of tisagenlecleucel in r/r LBCL**

Response

Overall best response rates by pre-infusion LDH

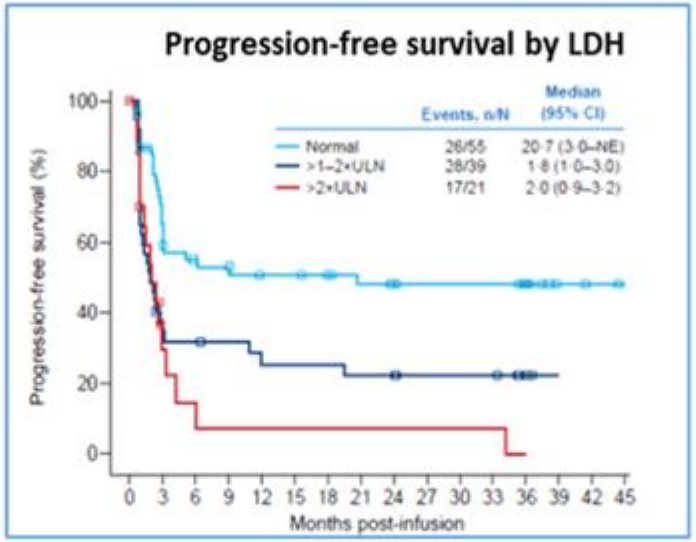


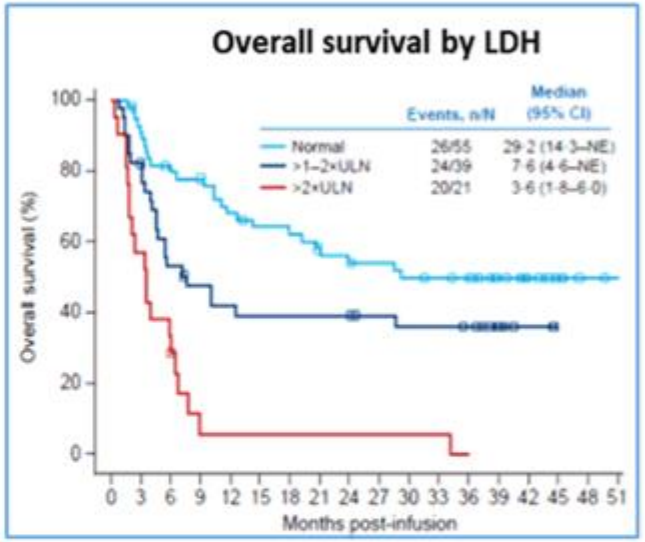
Multivariable analysis *

Predictive Factors from Univariable Analysis	Responders/Patients	Odds Ratio (95% CI)
LDH		
≤ x ULN	29/55 (53%)	2.74 (0.71-10.56)
>2 x ULN	4/21 (19%)	
>1 - 2 x ULN	11/39 (28%)	0.97 (0.23-4.06)
>2 x ULN	4/21	

*Lab analytes are defined as the closest time before or on the day of infusion (93% of values were obtained on the day of infusion)

Survival





Schuster SJ, *et al.* Lancet Oncol. 2021;22(10):1403-1415.

CAR-T for large B-cell lymphomas: CAR-T as an Earlier LOT

≥ 3rd line therapy for LBCL			
	axi-cel	tisa-cel	liso-cel
CR rate	58%	39%	53%

as ≥ 3rd-line therapy

~ 2/3 of patients ultimately fail

Axicabtagene ciloleucel[1]	Tisagenlecleucel[2]	Lisocabtagene maraleucel[3]
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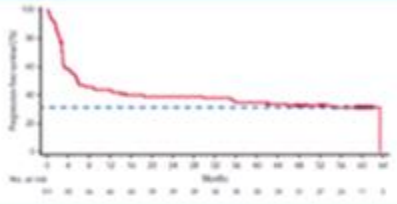
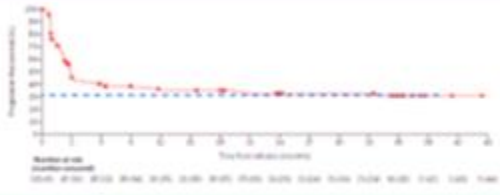
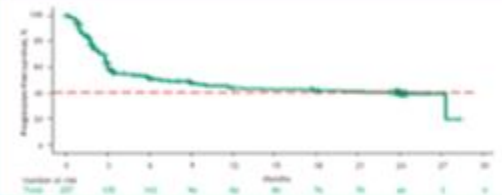
		

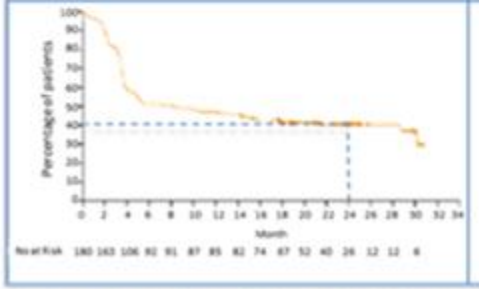
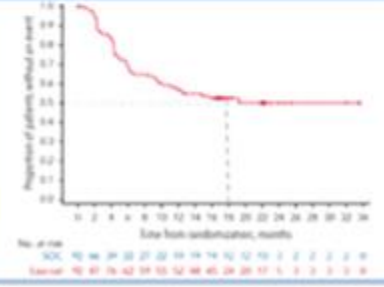
*The studies summarized above differ in their designs, patient characteristics and follow-up; therefore, direct comparisons are precluded.

[1]Neelapu SS, et al. Blood 2023; 141(19):2307-2315; [2]Schuster SJ, et al. Lancet Oncol 2021;22(10):1403-1415; [3]Abramson J, et al. Blood 2024;143(5):404-416.

2nd line therapy for LBCL		
	axi-cel	liso-cel
CR rate	65%	74%

as 2nd-line therapy

~ 1/2 of patients fail by 2 years

Axicabtagene ciloleucel[1]	Lisocabtagene maraleucel[2]
ZUMA-7[1]: axi-cel as ≥ 2nd-line therapy for r/r LBCL N = 180 Median follow-up: 24.9 months **Estimated 24-month EFS: 41% (95% CI, 33-48)**	**TRANSFORM**[2]: liso-cel as ≥ 2nd-line therapy for r/r LBCL N = 92 Median follow-up: 17.5 months **Estimated 18-month EFS: 52.6% (95% CI, 42.3-62.9)**
	

[1]Locke FL, et al. N Engl J Med. 2022;386(7):640-654; [2]Abramson, et al. Blood. 2023;141(14):1675-1684.

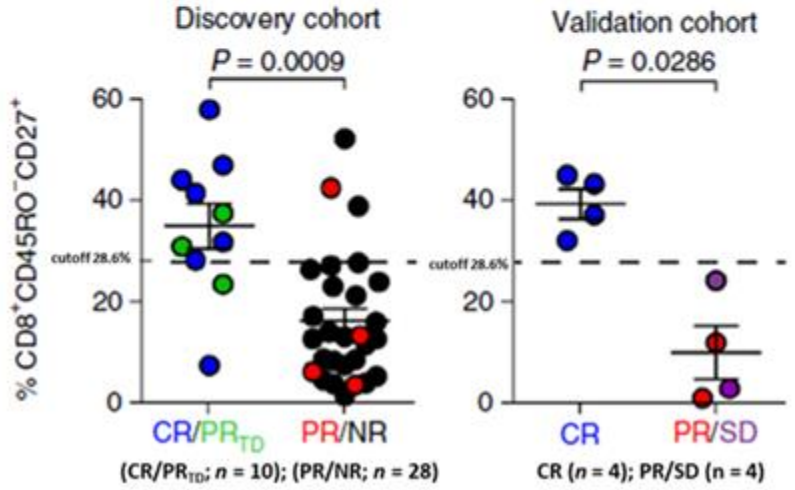
Patient characteristics impact outcome: T-cell fitness

T-cell fitness refers to the functional capacity and metabolic vigor of T cells, reflected by their ability to effectively *recognize antigens*, *respond to co-stimulation*, *proliferate*, *produce cytokines*, *differentiate into effector cells*, *resist exhaustion*, and *provide* immunologic memory.

Patient characteristics impacting T cell fitness: Considerations

- Age-related immunosenescence
- Lymphoma-related immunosuppression
- Therapy-related (iatrogenic) immunosuppression

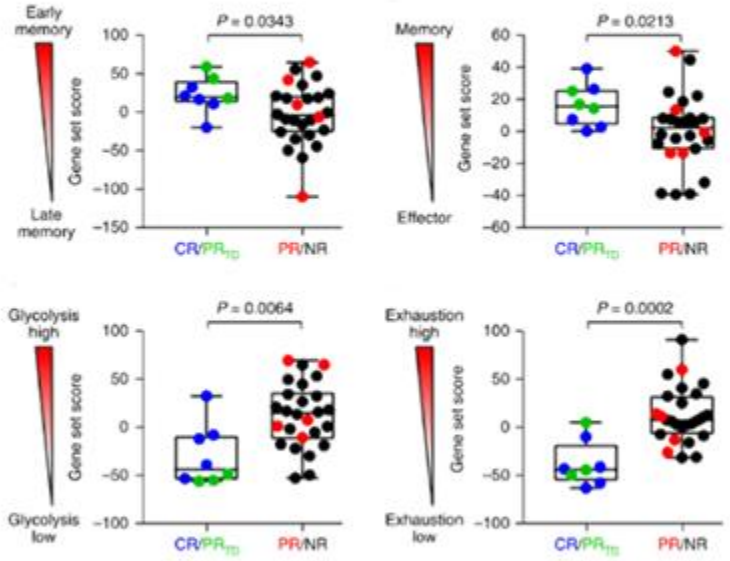
Naïve and memory CD8⁺ T cell content (CD45RO⁻CD27⁺cells) in *leukapheresis material* contribute to response to CAR-T in CLL



Genomic evaluation of CLL patient-derived *CAR-T cell products* and response to CAR-T cells

Genes Significantly Up- or Down-regulated

- Early memory T cell
- Nonexhausted T cell
- Naive vs. activated T_H2 Cd4⁺ T cell
- Unstimulated vs. stimulated memory T cell
- Resting vs. bystander activated CD4⁺ T cel
- Conventional vs. effector memory T cell
- Multipotent vs. progenitor CD4⁺ T cell
- Memory vs. effector CD8⁺ T cell
- Exhausted vs. effector T cell
- Exhausted T cell
- Activated T_H2 vs. naive CD4⁺ T cell
- Stimulated vs. unstimulated memory T cell
- Glycolysis
- Hypoxia
- Effector vs. memory CD8⁺ T cell
- Apoptosis



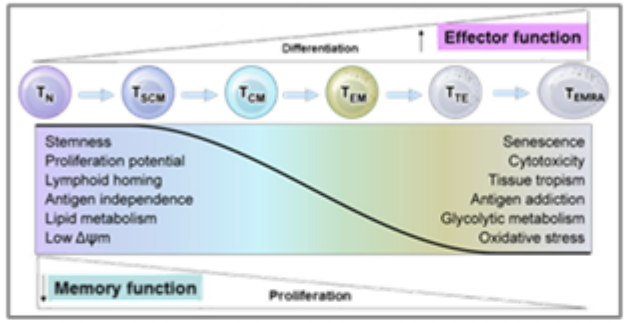
CR, complete remission; PR_{TD}, partial remission with late relapse of transformed disease; PR, partial response; NR, no response

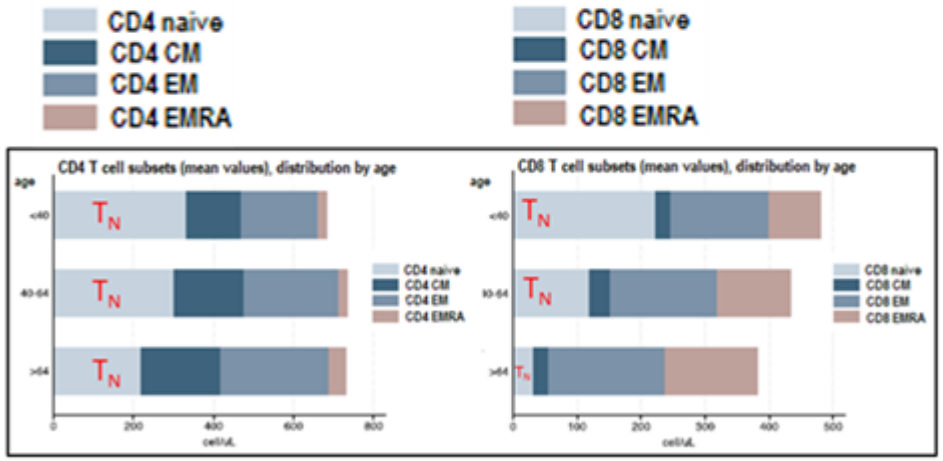
Fraietta, *et al*. Nat Med 2018; 24:563–571.

Patient characteristics impact outcome: T-cell fitness

Age-related immunosenescence impacts naïve and memory T cells

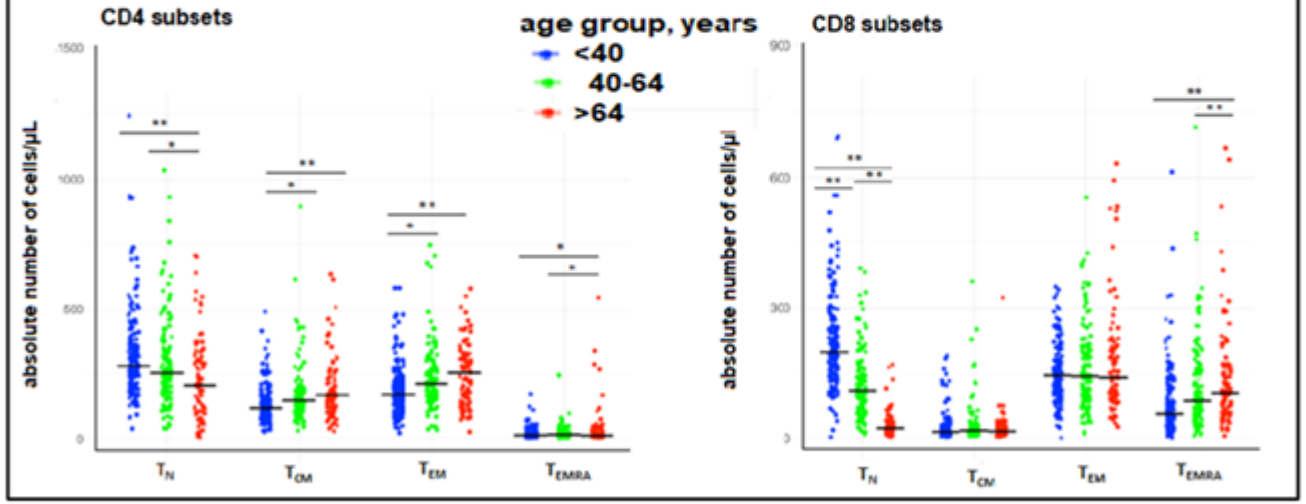
- Study of healthy adults (n = 363) established *age-specific, immune cell reference ranges*;
  A systematic review and meta-analysis validated these findings (n = 7,425)[1]





Demographics and immune cell subsets distribution of study population (*n* = 363) by three age groups

	<40 years old (*n* = 158)	40–64 years old (*n* = 127)	>64 years old (*n* = 78)
Age, median (Q1, Q3)	29 (27, 34)	47 (43, 55)	70.5 (67, 76)
Gender; Male, *n* (%)	72 (45.6%)	56 (44.1%)	29 (37.2%)



T_N, naive T cells; T_{SCM}, T stem cell memory cells; T_{CM}, T central memory cells; T_{EM}, T effector memory cells; T_{TE}, T effector cells; T_{EMRA}, CD45RA$^+$ terminal effector memory T cells

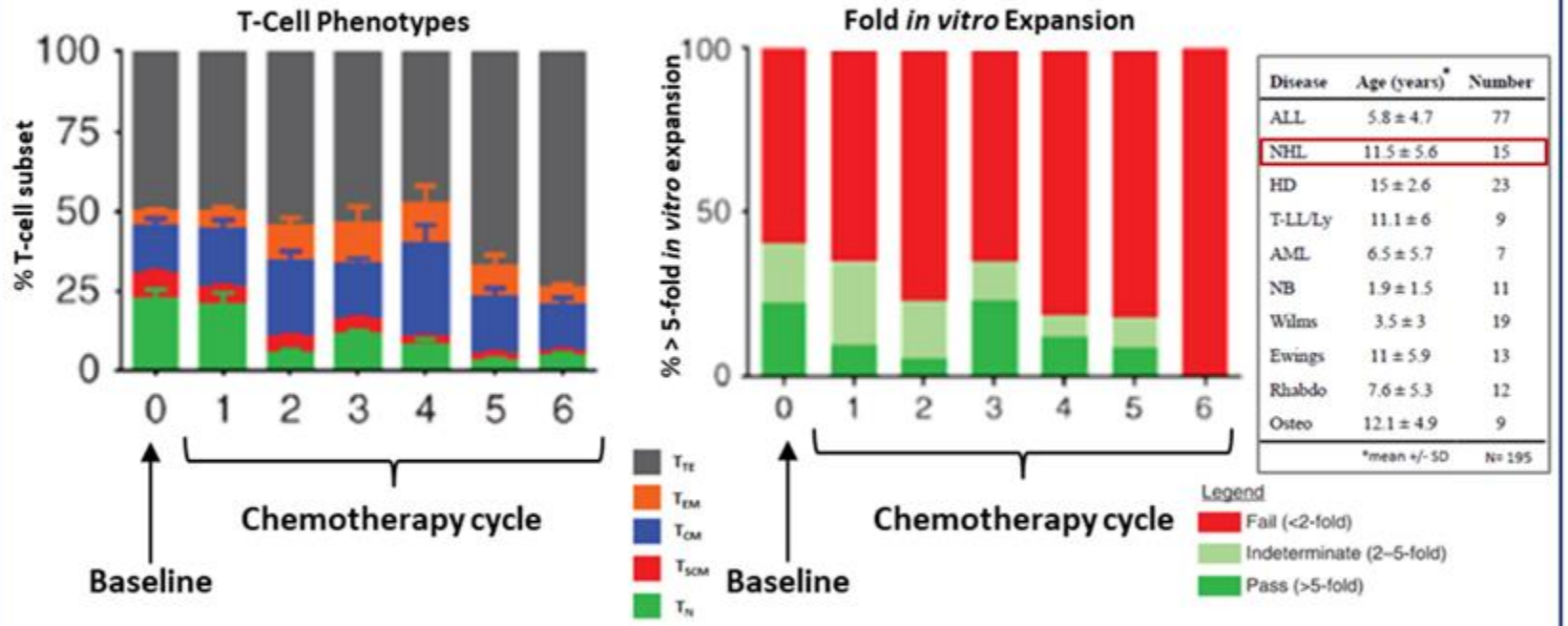
[1]Chang, *et al.* Immunity & Ageing.2024; 21:75

Patient characteristics impact outcome: T-cell fitness

Pre-existing *lymphoma-* and *therapy-related* immunodeficiency impact T cells

- Naïve T-cell deficits *at diagnosis* and *after chemotherapy* may impair cell therapy potential

Pediatric Non-Hodgkin Lymphomas[1]



Disease	Age (years)*	Number
ALL	5.8 ± 4.7	77
NHL	11.5 ± 5.6	15
HD	15 ± 2.6	23
T-LL/Ly	11.1 ± 6	9
AML	6.5 ± 5.7	7
NB	1.9 ± 1.5	11
Wilms	3.5 ± 3	19
Ewings	11 ± 5.9	13
Rhabdo	7.6 ± 5.3	12
Osteo	12.1 ± 4.9	9
	*mean +/- SD	N= 195

T-cell subsets from children with solid tumors and lymphomas *at diagnosis* and *after every cycle of chemotherapy*[1]

% T_N in blood of healthy children: Distribution by age

CD4, n = 805; CD8, n = 807 [2]

Subset	0-3 mo	2-6 y
CD 4+ /45RA + /62L+	89 (61-94)	70 (50-85)
CD 8+ /45RA + /62L +	79 (56-88)	64 (42-81)
	3-6 mo	**6-12 y**
CD 4+ /45RA + /62L+	88 (64-92)	58 (42-74)
CD 8+ /45RA + /62L +	77 (53-88)	58 (39-73)
	6-12 mo	**12-18 y**
CD 4+ /45RA + /62L+	83 (58-91)	51 (31-65)
CD 8+ /45RA + /62L +	72 (47-87)	56 (42-73)
	1-2 y	
CD 4+ /45RA + /62L+	79 (62-90)	
CD 8+ /45RA + /62L +	71 (46-85)	

T_N, naive T cells; T_{SCM}, T stem cell memory cells; T_{CM}, T central memory cells; T_{EM}, T effector memory cells; T_{TE}, T effector cells; T_{EMRA}, CD45RA+ terminal effector memory T cells

[1]Das, *et al.* Cancer Discov. 2019; 9(4):492-499.
[2]Shearer, *et al.* J Allergy Clin Immunol. 2003; 112(5):973-980.

CAR-T manufacturing impacts T-cell fitness

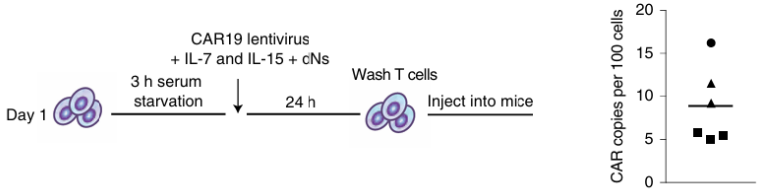
ARTICLES
https://doi.org/10.1038/s41551-021-00842-6
nature biomedical engineering

Rapid manufacturing of non-activated potent CAR T cells

Saba Ghassemi[1,2]✉, ..., Michael C. Milone[1,2]✉

NATURE BIOMEDICAL ENGINEERING | VOL 6 | FEBRUARY 2022 | 118–128 | www.nature.com/natbiomedeng

- Functional CAR T cells can be generated within 24 hours from T cells derived from blood without T-cell activation or ex vivo expansion
- Rapidly generated non-activated CAR T cells exhibited higher anti-leukemic in vivo than activated CAR T cells produced using the standard protocol



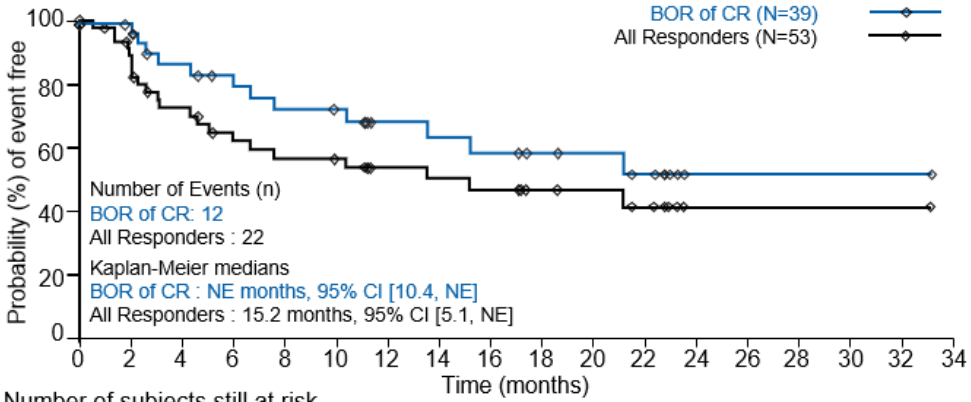
Ghassemi S, ..., Milone MC. Nat Biomed Eng. 2022;6(2):118-128.

Rapcabtagene Autoleucel

NOVARTIS

Rapcabtagene autoleucel, 12.5×10^6 (N = 60)	n (%)
Median follow-up, months (range)	16.4 (0.1-44.1)
Best overall response	
CR, n (%) [95% CI]	39 (65.0) [51.6-76.9]
Complete response rate, n/N (%)	
Month 3	30/55 (54.5)
Month 6	25/44 (56.8)
Month 12	18/38 (47.4)

Duration of Response



Riedell, P. A. et al. 2024 ASH Annual Meeting

Pharmacologic enhancement of T-cell fitness

- **Phase 1b study of tisagenlecleucel combined with ibrutinib in adult patients with r/r large B-cell lymphomas**
- **Ibrutinib started ≥ 21 days *before apheresis* or *after apheresis* for ≥ 21 days before tisagenlecleucel infusion**

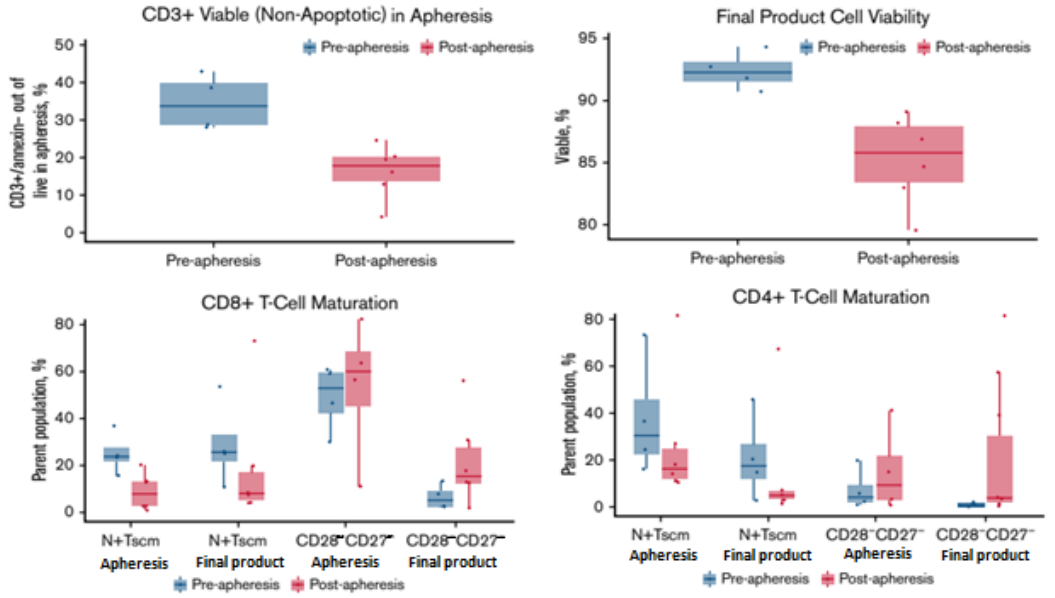
REGULAR ARTICLE

blood neoplasia

Tisagenlecleucel in combination with ibrutinib in adults with relapsed and/or refractory large B-cell lymphomas

Key Point

- Administration of ibrutinib before apheresis may reduce the proportion of senescent T cells in the final manufactured product.

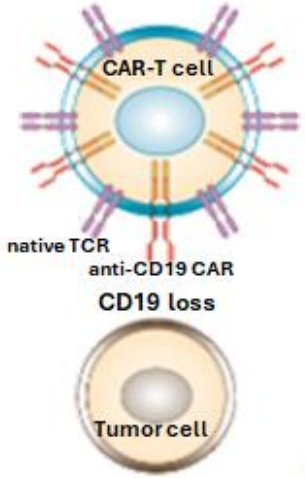


- **Ibrutinib before leukapheresis may modify T-cell characteristics in the apheresis material, improving final CAR-T product quality and clinical outcomes**

Chavez JC, ..., Schuster SJ. Blood Neoplasia 2026;3(1):1-12.

CAR-T- and/or *disease-specific* mechanisms of CAR-T failure

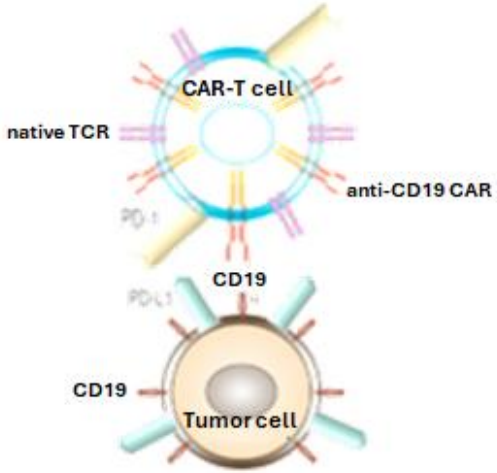
- *Most developmental strategies focus on CAR-T cells, tumor antigens, and/or TME rather than intracellular determinants of resistance to treatment*



CD19 antigen loss

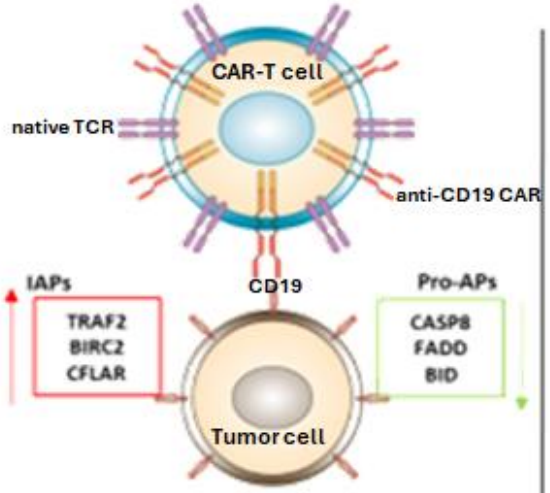
- acquired mutations and alternative splicing of CD19

(Sotillo...Thomas-Tikhonenko Cancer Disc. 2015)



T-cell exhaustion/hypofunction

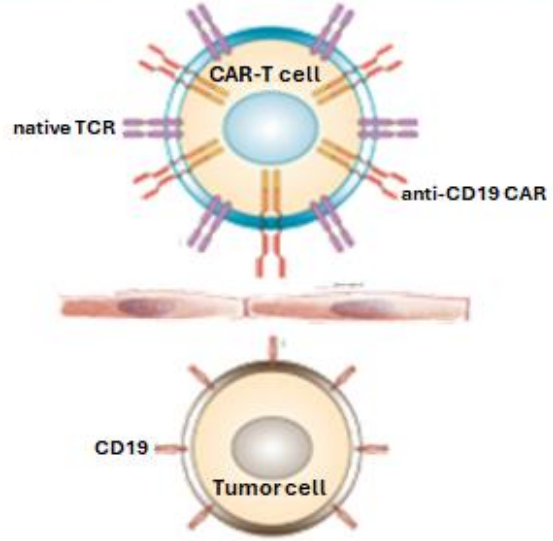
- mediated by inhibitory CAR T receptors and ligands in the tumor microenvironment
- peripheral self-tolerance (B cell recovery? late relapses?)
- TME-induced T cell hypofunction (reversible)



Intrinsic tumor resistance

- loss of death receptor signaling molecules causes resistance to CAR T in vitro + in vivo
- failed CAR T assoc./w lower death receptor-assoc. gene expression by tumor cells

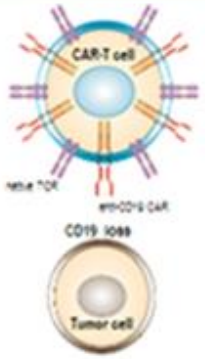
(Singh, et al. Cancer Disc. 2020)

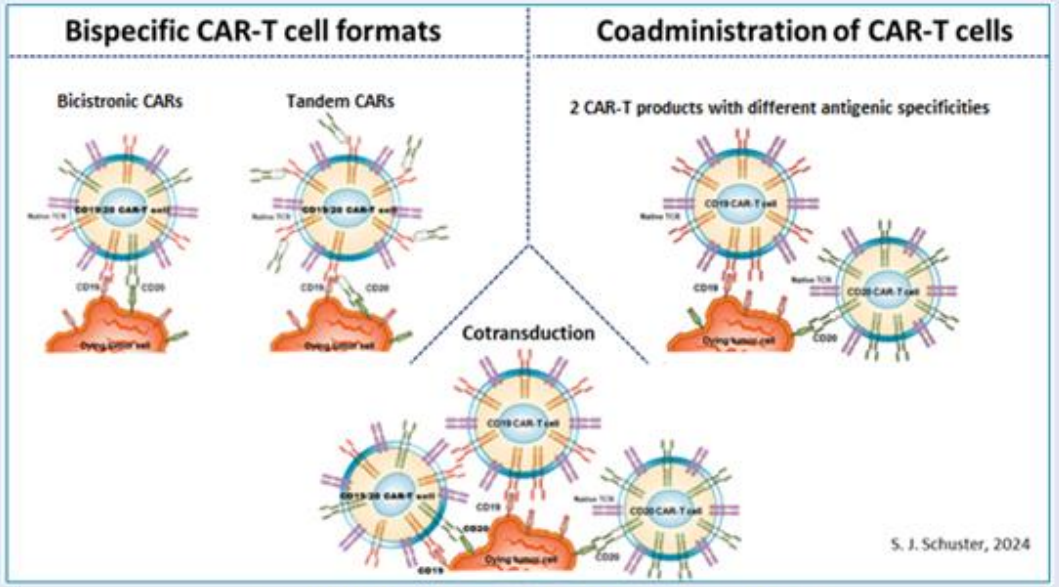


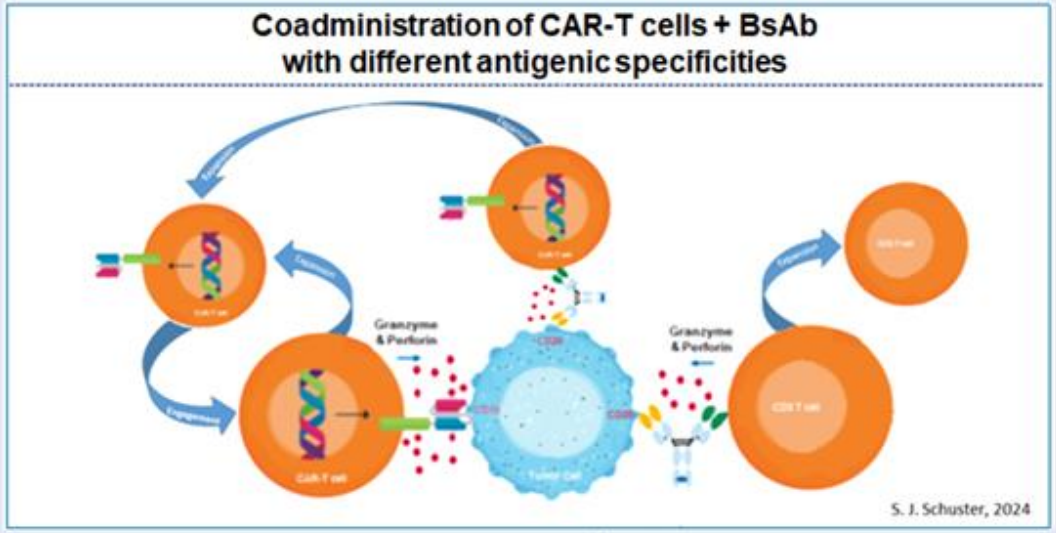
Insufficient T-cell infiltration

- T cells paralysis
- physiologic factors (high interstitial fluid pressure, hypoxia, pH)

Tumor-specific determinants of CAR-T failure: CD19 Antigen Loss

Problem	Potential Solutions
 **CD19 antigen loss** • acquired mutations and alternative splicing of CD19 (Sotillo...Thomas-Tikhonenko Cancer Disc. 2015)	1. **Manufacture CAR-T cells with dual specificity** to target more than one tumor antigen (*i.e.*, "bispecific" CAR-T cell) *a)* ***bicistronic*** CARs on a single cell's surface *b)* ***tandem*** scFv as a single CAR on a single cell's surface 2. **Coadministration of monospecific CAR-T cell** products, each with different tumor antigen specificities (so-called, "cocktail" approach) *a) sequential* administration *b) simultaneous* coadministration 3. **Combine T cell-engaging bispecific antibodies (BsAb) and monospecific CAR-T cells,** with each product, BsAb and CAR-T, having different tumor antigen specificities





Tumor-specific determinants of CAR-T failure: CD19 Antigen Loss

Dual antigen targeting CAR-T approaches

- 8 Phase 1/2, single-arm, noncomparative, prospective, open-label clinical trials

	Study	CAR targets	CAR design	N (% DLBCL)	Prior CAR-T	Prior transplant	LD chemo	CAR T cell dose	Response (OR/CR)	PFS - median - rate	Follow-up - median
CD19/CD22	Zhang, et al.[1]	CD19/CD22	bispecific	32 (84%)	No	Auto, 4 (12.5%)	FC	3.7-32.8 x 10^8 total	79%/**34%**	6.8 mo. **40%, 12-mo.**	8.7 mo.
	Wei, et al.[2]	CD19/CD22	bispecific, tandem	16 (81%)	No	Auto, 1 (5%)	FC	4.9-9.4 x 10^6/kg	87%/**62%**	8.1 mo. **40%, 24-mo.**	13 mo.
	Roddie, et al.[3]	CD19/CD22	bispecific, bicistronic	52 (69%)	No	Auto, 16 (31%)	FC	50-450 x 10^6 total	66%/**49%**	3.3 mo. **26%, 12-mo.**	21.6 mo.
	Spiegel, et al.[4]	CD19/CD22	bispecific, tandem	21 (64%)	No	Auto, 4 (19%)	FC	1.0-3.0 x 10^6/kg	62%/**29%**	3.2 mo. **~23%, 12-mo.**	10 mo.
	Wang, et al.[5]	CD19 + CD22	cocktail	38 (60%)	No	Auto, 6 (15.8%)	FC	CD19: 5.1 +/- 2.1 x 10^6/kg CD22: 5.3 +/- 2.4 x 10^6/kg	72%/**50%**	9.9 mo. **50%, 12-mo.**	14.4 mo.
CD19/CD20	Zhang, et al.[6]	CD19/CD20	bispecific, tandem	87 (66%)	9 (10%)	Auto, 12 (14%)	FC	0.5-8 x 10^6/kg	78%/**70%**	27.6 mo. **61%, 12-mo.**	27.7 mo.
	Shah, et al.[7]	CD19/CD20	bispecific, tandem	16 (56%)	1 (6%)	Auto, 5 (31%); Allo, 1 (6%)	FC	2.5 x 10^6/kg	82%/**64%**	**44%, 24-mo.**	31 mo.
	Sang, et al.[8]	CD19 + CD20	cocktail	21 (100%)	No	Auto, 1 (5%)	FC (n=19) or ifosfamide	CD19: 0.2-4.0 x 10^6/kg CD20: 0.1-4.0 x 10^6/kg	81%/**52%**	5.0 mos. **~24%, 12-mo.**	6.6 mo.
	Summary		**Total N = 283**		**CR rate, median (range) = 51% (29-70)**			**≥12-mo PFS rate, median (range) 40% = (23-61)**			

[1]Zhang, *et al.* Front Oncol 2021;11:664421; [2]Wei, *et al.* Cancer Immunol Res 2021;9(9):1061–1070; [3]Roddie, *et al.* Blood 2023; 141(20):2470-2482; [4]Spiegel, *et al.* Nat Med 2021;27(8):1419–1431; [5]Wang, *et al.* Blood 2020;135(1):17–27; [6]Zhang, *et al.* Leukemia 2022;36(1):189–196; [7]Shah, *et al.* Am J Hematol 2022;97(12):1580–1588; [8]Sang, *et al.* Cancer Med 2020;9(16):5827–5838.

Tumor-specific determinants of CAR-T failure: CD19 Antigen Loss

Bivalent CAR-T: the short story

1. Across studies, bivalent CAR-T constructs targeting two B-cell antigens report **CR rates from 29% to 92%**, with acceptable toxicities
   - ***only 3 products are currently advancing to registrational (Phase 2/3) trials*** (see below)
   - *all 3 target CD19 and CD20*
2. Across studies, ***dual antigen loss has not been observed*** in patients relapsing after bivalent CAR-T
   - This suggests alternative mechanisms of relapse
3. **TanCAR7** (NCT03097770) has the **largest cohort (87 patients)** and **longest follow-up (5 years)**
   - **40% of patients with CR at 12 months remain relapse-free**
   - 5-year OS is 60%

Bivalent CAR-T products currently advancing to registrational (Phase 2/3) trials: Summary

Product	Sponsor	Targets	Phase	N (*treated*)	CAR Construct	CR rate (%)	≥Gr3 CRS (%)	≥Gr3 ICANS (%)	Median follow-up	Survival	Refs
TanCAR7	Beijing Immunochina / PLA General Hospital	CD19/CD20	I/II	**87** (58 LBCL)	**Tandem** scFv, 4-1BB-CD3ζ	**70%** (LBCL / tFL, 68%)	10%	2%	**63.4 months**	mPFS, 33 mo. 5-yr. DOR, 40% 5-yr OS, 60%	[1-2]
Zamto-cel	Miltenyi Biomedicine / Univ. Würzburg	CD20/CD19	I	**12** (8 LBCL)	**Tandem**, non-cryopreserved	**42%**	0	0	**5 years**	12-mo. CR, relapse-free at 5 yr	[3]
KITE-363	Kite / Gilead	CD19/CD20	I	**37** (34 LBCL)	**Bicistronic**	**78%**	3%	8%	**7.3 months**	mDOR, not reached	[4]

[1]Zhang, et al., Leukemia (2022) 36:189–196; [2]Han, et al., Am J Hematol (2026) 1–13; [3]Balke-Want, et al., Blood Adv (2026) 10 (7): 2395–2405; [4]Dahija, et al., J Clin Oncol (2025) 43(16_suppl), abstract 7003.

CAR-T-specific determinants of CAR-T failure: T-cell exhaustion

- ***UPenn trial addressing T-cell exhaustion: an armored CAR***

Phase I Trial of huCART19-IL18 Cells in Patients With Relapsed or Refractory CD19+ Cancers

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Enhanced CAR T-Cell Therapy for Lymphoma after Previous Failure

Jakub Svoboda, M.D.,[1] et al.

N Engl J Med 2025;392:1824-35.
DOI: 10.1056/NEJMoa2408771

Rationale

to utilize IL-18 as a pro-inflammatory cytokine to:

- enhance CAR T cell proliferation
- recruit additional immune cells into the TME to mediate antitumor effects toward CAR-T resistant tumor cells
- mitigate the potential impact of CAR T cell exhaustion

Results

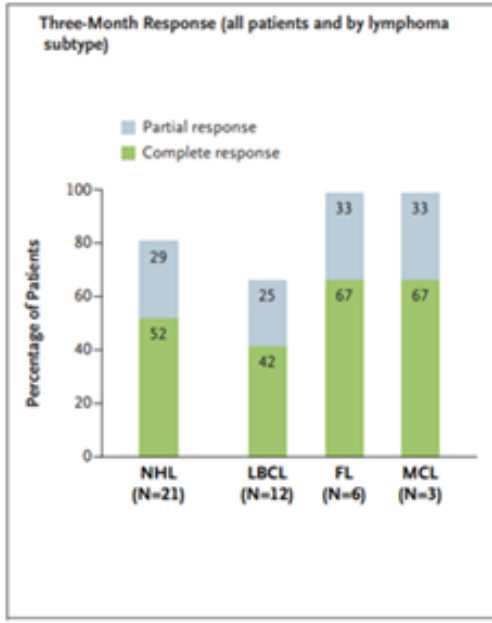
N = 21 received huCART19-IL18

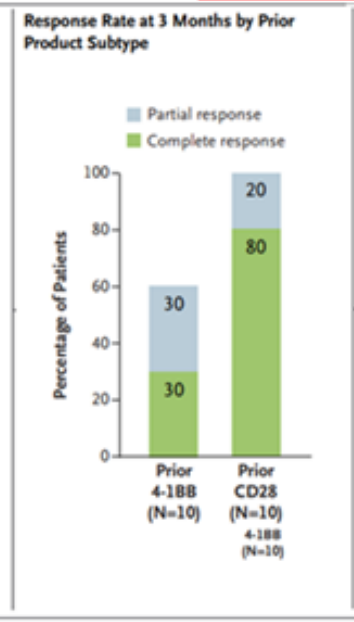
Median follow-up: 17.5 months (range 3 - 34)

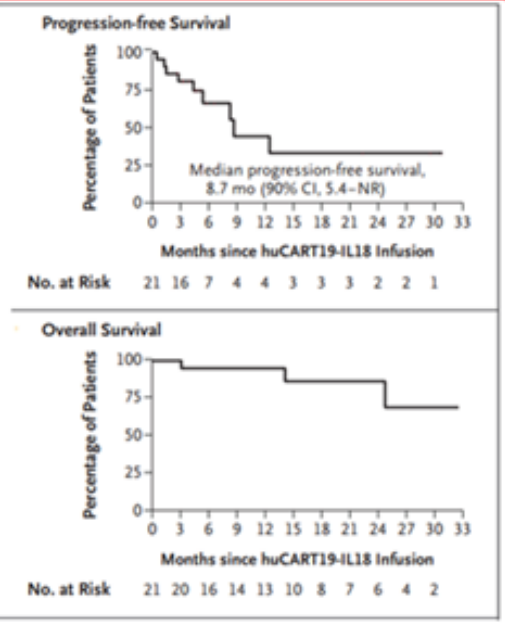
- 3-months ORR: 81% (90%CI, 62-93)
- 3-months CRR: 52% (90% CI, 33-71)
- Median DOR: 9.6 months (90% CI, 5.5-NR)

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Patients (N=21)
Median age (range) — yr	64 (47–74)
Male sex — no. (%)	16 (76)
ECOG performance-status score — no. (%)†	
0	2 (10)
1	19 (90)
Lymphoma subtype — no. (%)	
Large B-cell lymphoma	12 (57)
Diffuse large B-cell lymphoma, not otherwise specified	8 (38)
Transformed follicular lymphoma	2 (10)
High-grade B-cell lymphoma	1 (5)
T-cell histiocyte-rich large B-cell lymphoma	1 (5)
Follicular lymphoma	6 (29)
Mantle-cell lymphoma	3 (14)
Previous CAR therapy — no./total no. (%)	
CD28-based product	10/20 (50)
Axicabtagene ciloleucel	8/20 (40)
Brexucabtagene autoleucel	2/20 (10)
4-1BB–based product	10/20 (50)
Tisagenlecleucel	8/20 (40)
Lisocabtagene maraleucel	2/20 (10)
Response to previous therapy	
Progressive disease — no./total no. (%)	7/20 (35)
Median progression-free survival — mo (90% CI)	6.7 (3.1–10.2)







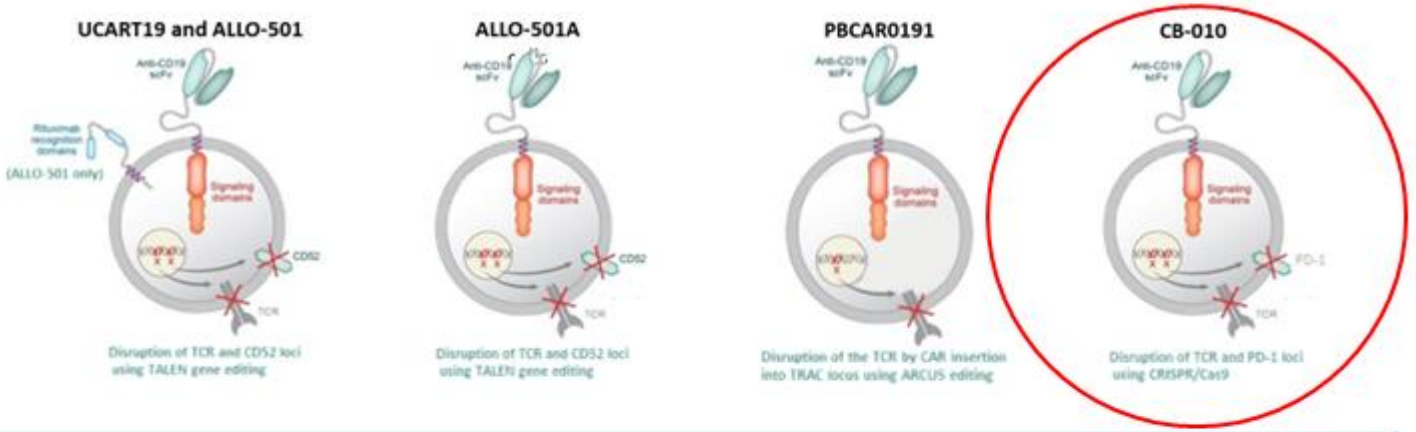
PI: J. Svoboda

CAR-T-specific determinants of CAR-T failure: T-cell exhaustion

- an *"Off-the-Shelf"* allogeneic anti-CD19 CAR-T therapy using young, *healthy, partially HLA-matched* donors

Clinical trials of 'off-the-shelf 'allogenic CAR-T cells for B Cell Lymphomas

Title	NCT	Sponsor	Target	Indication
ALLO-501 ALLO-501A	NCT03939026 NCT04416984	Allogene Therapeutics	CD19	r/r Large B Cell Lymphoma, r/r Follicular
PBCAR0191	NCT03666000	Precision BioSciences	CD19	Non-Hodgkin Lymphoma, B-cell ALL
CB-010	NCT04637763	Caribou BioSciences	CD19	r/r Large B Cell Lymphoma



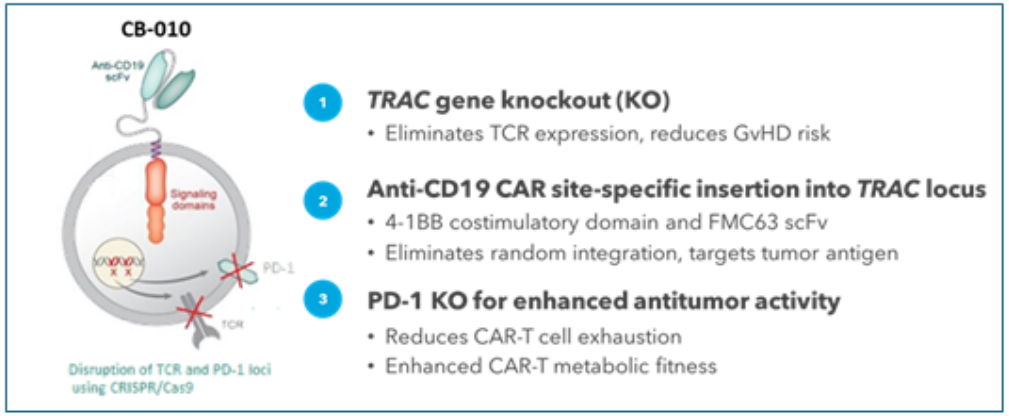
Studies and meta-analysis	N, efficacy/safety evaluable	CR (%)	GvHD (%)	Severe CRS (%)	Severe ICANS (%)
Relapsed/Refractory Large B-cell or Follicular NHL					
- ALLO-501A[3]	12/9	6 (50)	0 (0)	0 (0)	0 (0)
- ALLO-501[4]	36/46	18 (50)	0 (0)	1 (2)	0 (0)
- PBCAR0191[2]	13/16	8 (62)	0 (0)	0 (0)	1 (6)
Pooled results (95 % CI)	**61/71**	**52 % (39–65)**	**0 % (0–5)**	**0 % (0–5)**	**0 % (0–6)**
I^2		**0 %**	**0 %**	**0 %**	**19 %**

CR, complete remission. GvHD, graft versus-host disease. CRS, cytokine release syndrome. ICANS, immune effector cell-associated neurotoxicity syndrome. NA, not available.

Chen S, et al. Crit Rev Oncol Hematol. 2022;179:103807; [1]Benjamin, et al. Lancet 2020;396(10266):1885-1894; [2]Shah, et al. Blood 2021; 138 (Suppl 1): 302; [3]Lekakis, et al. Blood 2021; 138 (Suppl 1): 649; [4]Neelapu, et al. Blood 2021; 138 (Suppl 1): 3878.

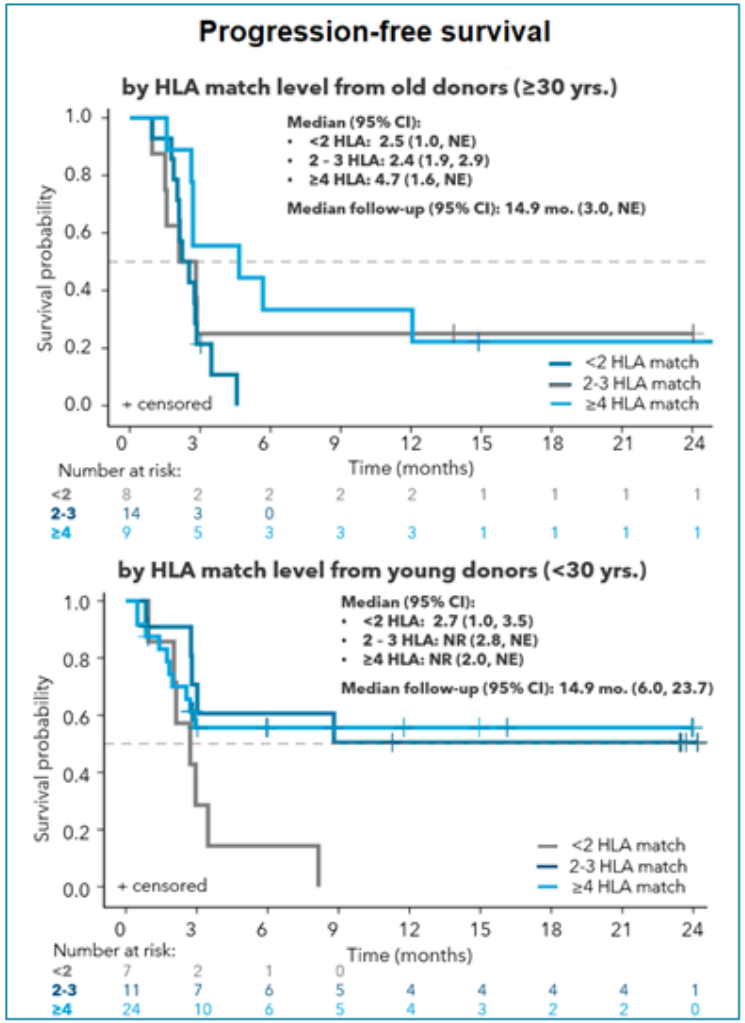
CAR-T-specific determinants of CAR-T failure: T-cell exhaustion

- **CB-010, an allogeneic anti-CD19 CAR-T using young, *healthy, partially HLA-matched* donors**
- **Optimized profile: *donor age < 30 yrs*; ≥ *2 HLA antigens matched***



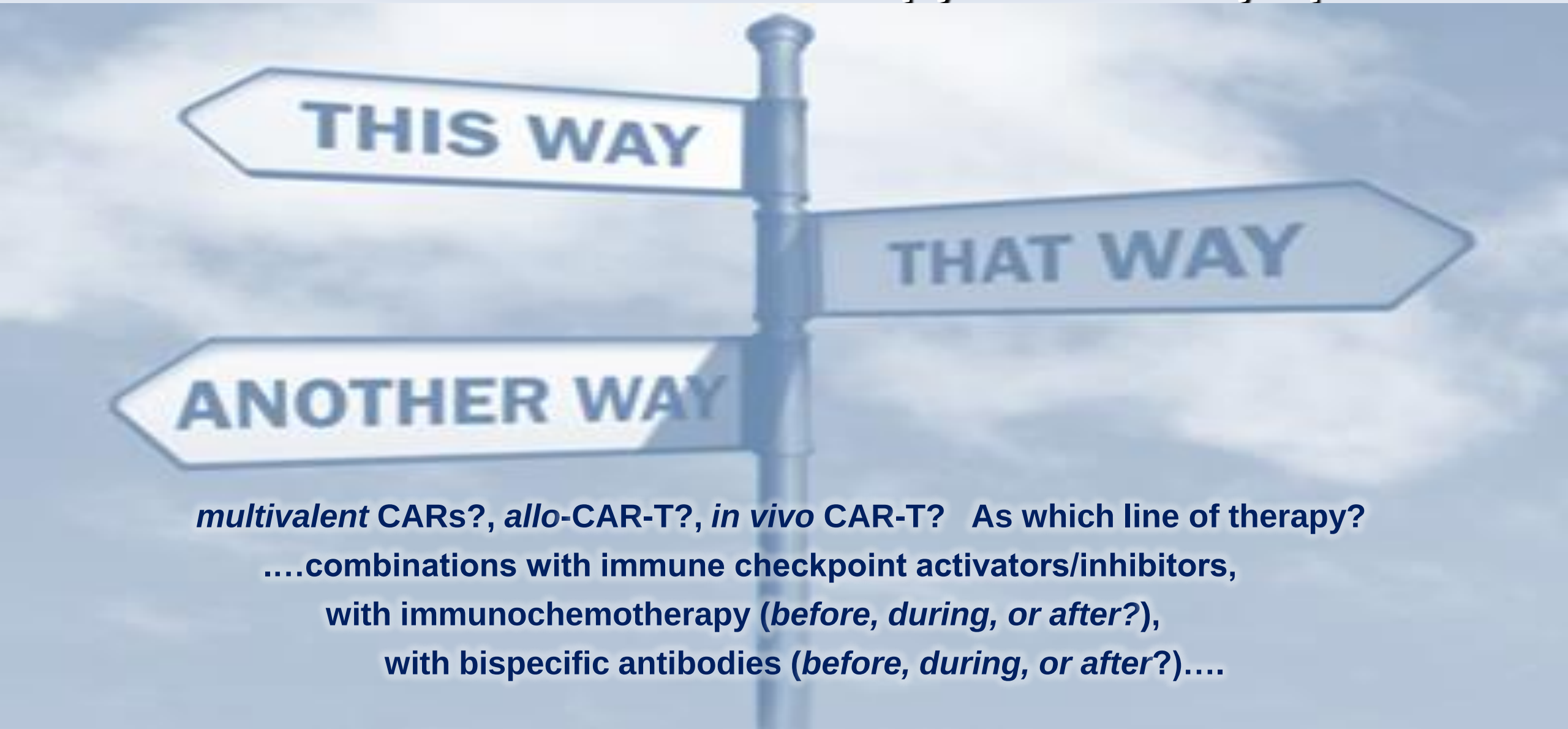
Patient and disease characteristics	All patients[1] N=84	2L LBCL patients N=67	Confirmatory cohort[2] N=22	Optimized profile[3] N=35
Age, years, median (range)	66 (20-86)	66 (20-86)	61 (20-83)	63 (20-86)
Age ≥ 70 years, n (%)	23 (27)	19 (28)	8 (36)	10 (29)
Male, n (%)	64 (76)	51 (76)	16 (73)	25 (71)
NHL subtype, n (%)				
DLBCL, NOS	48 (57)	40 (60)	14 (64)	21 (60)
HGBL	13 (15)	13 (19)	4 (18)	5 (14)
tFL	14 (17)	12 (18)	4 (18)	7 (20)
tMZL	1 (1)	1 (2)	-	1 (3)
PMBCL	2 (2)	1 (2)	-	1 (3)
MCL	3 (4)	-	-	-
FL	2 (2)	-	-	-
MZL	1 (1)	-	-	-
Primary refractory, n (%)	-	33 (49)[4]	11 (50)	17 (49)
Baseline LDH status (%)				
> ULN	46 (55)	50 (75)	11 (50)	18 (51)
> 2x ULN	13 (15)	11 (16)	1 (5)	2 (6)
Bulky disease (≥ 7.5 cm)	17 (20)	13 (19)	2 (9)	4 (11)

[1]Includes 5 patients with exposure to prior CD19-targeting therapy; [2]2L LBCL 4+ HLA matched, dosed with 80M cells;
[3]2L (N=32) and 3L+ (N=3) LBCL patients treated with 40M, 80M, or 120M cells optimized for multiple factors, inc. 2+ HLA matched + young donor



Data cutoff: 29 Sept. 2025; presented at iwCAR-T 2026

The Next Direction of CAR-T Therapy for B-Cell Lymphomas



multivalent **CARs?, *allo*-CAR-T?, *in vivo* CAR-T? As which line of therapy?**

….combinations with immune checkpoint activators/inhibitors,

with immunochemotherapy (*before, during, or after?*),

with bispecific antibodies (*before, during, or after*?)….

Grazie / Thank You!