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Precision at the Edge: Optimizing CAR T-Cell Therapy for R/R DLBCL

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Disclosures

Prof. Stephen J. Schuster's Disclosures

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

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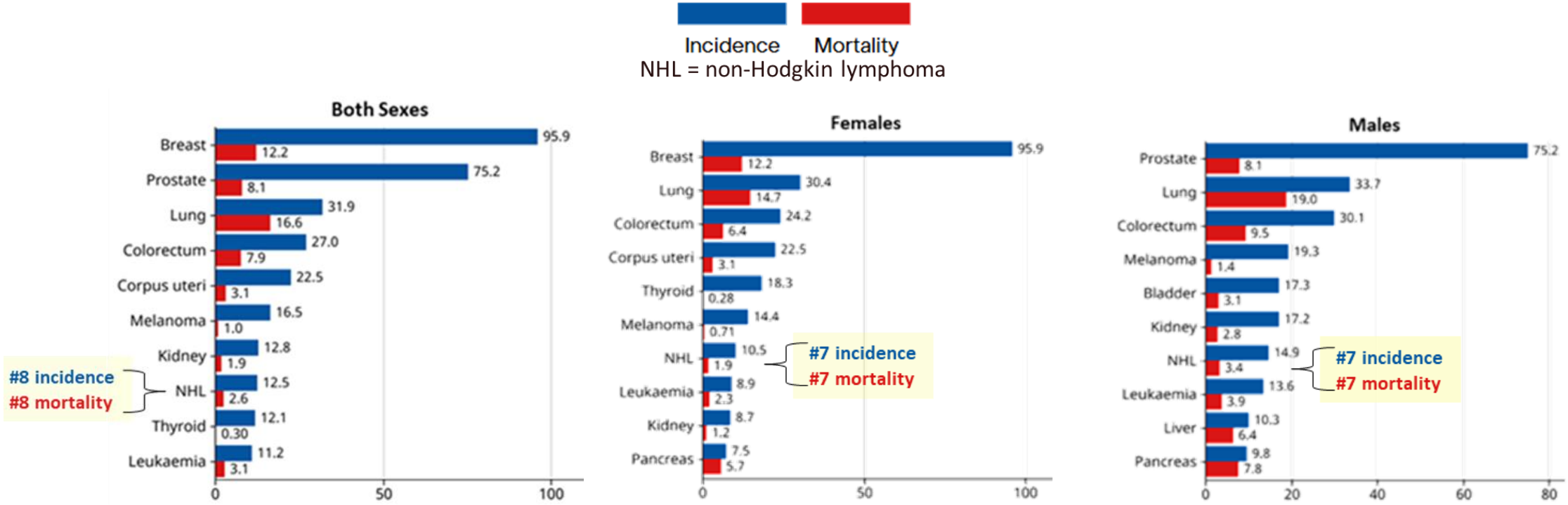
Top 10 Cancers in the United States Include Non-Hodgkin Lymphomas

- **Incidence and Mortality by Age-Standardized Rates (World) per 100,000 per Year**
- **Data: 2022, excludes non-melanoma skin cancers**



Age-Standardized Incidence/Mortality per 100,000 per Year

Cancer TODAY | IARC - https://gco.iarc.who.int/today
Data version : Globocan 2022 (version 1.1)
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International Agency for Research on Cancer (IARC) Global Cancer Observatory (Globocan) Cancer Today. https://gco.iarc.who.int/today/en.

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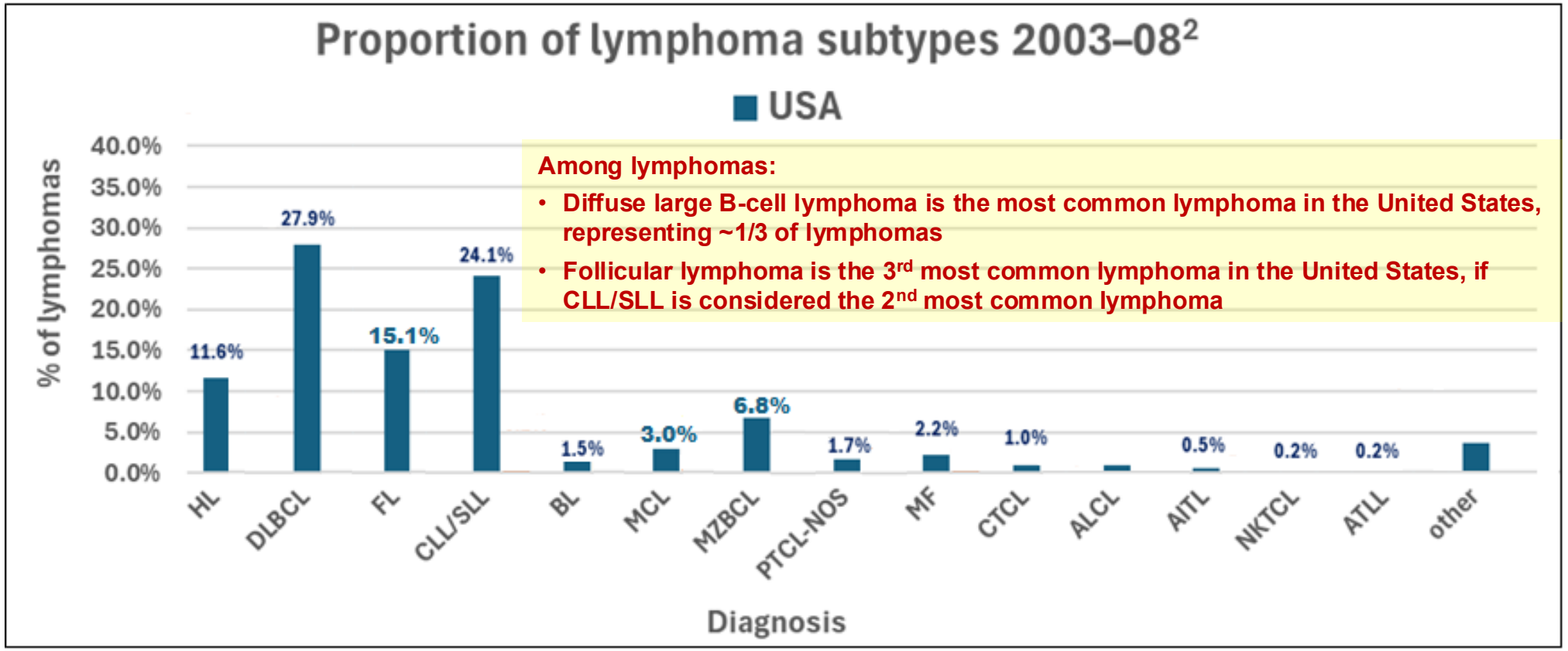
Incidence and Distribution of Lymphoma Subtypes in the United States

Number of lymphomas diagnosed in US (2024)[1] = 80,620 cases; deaths (est.) = 20,140



HL = Hodgkin lymphoma; DLBCL = diffuse large B-cell lymphoma; CLL = chronic lymphocytic leukemia; SLL = small lymphocytic lymphoma; BL = Burkitt lymphoma; MCL = mantle cell lymphoma; MZBCL = marginal zone B-cell lymphoma; PTCL-NOS = peripheral T-cell lymphoma, not otherwise specified; MF = mycosis fungoides; CTCL = cutaneous T-cell lymphoma; ALCL = anaplastic large cell lymphoma; AITL = angioimmunoblastic T-cell lymphoma; NKTCL = NK/T-cell lymphoma; ATLL = adult T-cell leukemia/lymphoma.

[1]National Institutes of Health (NIH) National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Program [seer.cancer.gov]. https://seer.cancer.gov/statfacts/html/nhl.html. [2]Chihara D, et al. *Br J Haematol*. 2014;164(4):536-545.

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Large B-Cell Lymphoma Pathologic Subtypes Vary in Prognosis

Large B-cell lymphomas, WHO 5th edition
Diffuse large B-cell lymphoma, NOS
T-cell/histiocyte-rich large B-cell lymphoma
Diffuse large B-cell lymphoma/ high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements
ALK-positive large B-cell lymphoma
Large B-cell lymphoma with *IRF4* rearrangement
High-grade B-cell lymphoma with 11q aberrations
Lymphomatoid granulomatosis
EBV-positive diffuse large B-cell lymphoma
Diffuse large B-cell lymphoma associated with chronic inflammation
Fibrin-associated large B-cell lymphoma
Fluid overload-associated large B-cell lymphoma
Plasmablastic lymphoma
Primary large B-cell lymphoma of immune-privileged sites
Primary cutaneous diffuse large B-cell lymphoma, leg type
Intravascular large B-cell lymphoma
Primary mediastinal large B-cell lymphoma
Mediastinal grey zone lymphoma
High-grade B-cell lymphoma, NOS

	INCIDENCE RATE, 2011-2012 Age-adjusted per 100,000	ESTIMATED NEW CASES, 2016
Diffuse large B-cell lymphoma	6.9	27,650
Diffuse large B-cell lymphoma, NOS - Germinal center B-cell type - Activated B-cell type - Unclassified	6.3	25,380
High grade B-cell lymphoma with MYC and BCL2 +/- BCL6 rearrangements		
High grade B-cell lymphoma, NOS		
EBV+ DLBCL, NOS		
Primary DLBCL of the CNS	0.3	1,100
Primary cutaneous DLBCL, leg type	0.1	400
T-cell/histiocyte rich large B-cell lymphoma	0.1	200
Intravascular large B-cell lymphoma	<0.1	60
ALK positive large B-cell lymphoma	<0.1	< 50
Plasmablastic lymphoma	<0.1	180
Large B-cell (plasmablastic) lymphoma arising from HHV-8 associated multicentric Castleman disease	<0.1	< 50
Primary effusion lymphoma	<0.1	< 50
Primary mediastinal (thymic) large B-cell lymphoma	0.1	240

~ 85% of LBCLs are DLBCL, NOS

(HGBCL, NOS ~ 1% - 2% of all B-NHLs)

(HGBCL with MYC/BCL2-R ~ 2% - 3% of all B-NHLs ~ 10% of LBCL)

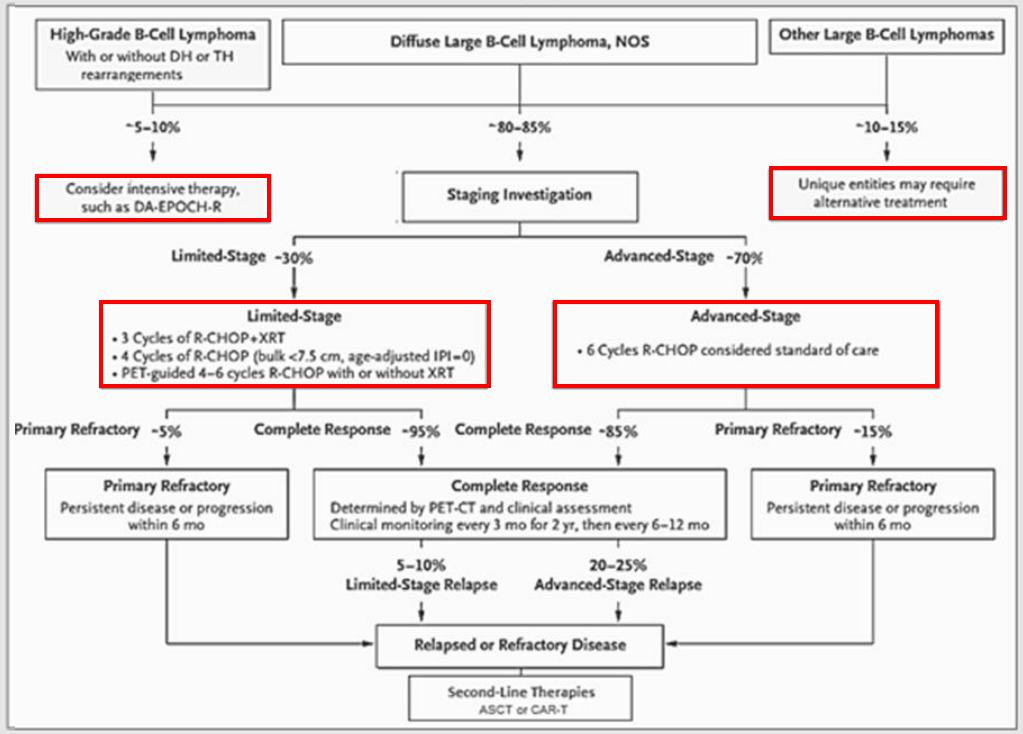
WHO = World Health Organization; LBCL = large B-cell lymphoma; DLBCL = diffuse large B-cell lymphoma; HGBCL = high-grade B-cell lymphoma; NOS = not otherwise specified; MYC = myelocytomatosis viral oncogene homolog; EBV = Epstein-Barr virus; NHL = non-Hodgkin lymphoma.

Alaggio R, et al. *Leukemia*. 2022;36(7):1720-1748. Teras LR, et al. *CA Cancer J Clin*. 2016;66(6):443-459. Swerdlow SH, et al. *Blood*. 2016;127(20):2375-2390.

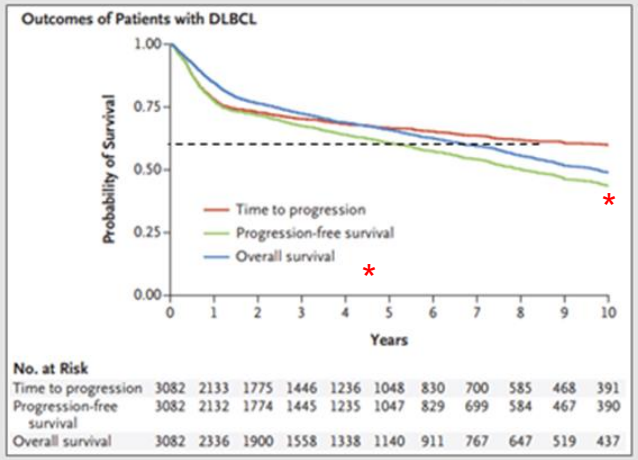
Treatment of Large B-Cell Lymphomas: 1st-Line Therapy

- **First-line combination immunochemotherapy cures about 65% of patients**
- **~30% of patients with refractory or relapsed LBCLs can be cured with 2nd- or 3rd-line therapies**

1st-Line Therapy of DLBCL



Outcomes of 1st-Line Therapy



Clinical Index for Predicting Outcomes in Patients with DLBCL: the International Prognostic Index (IPI)

Prognostic Index, Clinical Factors, and Risk Categories	Proportion of Patients	Estimated 5-yr PFS	Estimated 5-yr OS
		percent	
IPI			
Age, >60 yr; LDH, >ULN; Ann Arbor stage III or IV; ECOG performance status, >1; no. of extranodal sites of disease, >1			
Risk categories			
Low (0 or 1 factor)	34	81	88
Low-intermediate (2 factors)	23	67	76
High-intermediate (3 factors)	23	58	67
High (4 or 5 factors)	20	46	54

Sehn LH, Salles G. *N Engl J Med*. 2021;384:842-58.

DH = double-hit; TH = triple-hit; IPI = International Prognostic Index; PET = positron emission tomography; CT = computed tomography; XRT = radiation therapy; LDH = lactate dehydrogenase; ULN = upper limit of normal; ECOG = Eastern Cooperative Oncology Group; PFS = progression-free survival; OS = overall survival; ASCT = autologous stem cell transplantation; DA-EPOCH-R = dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

Sehn LH, Salles G. *N Engl J Med*. 2021;384(9):842-858.

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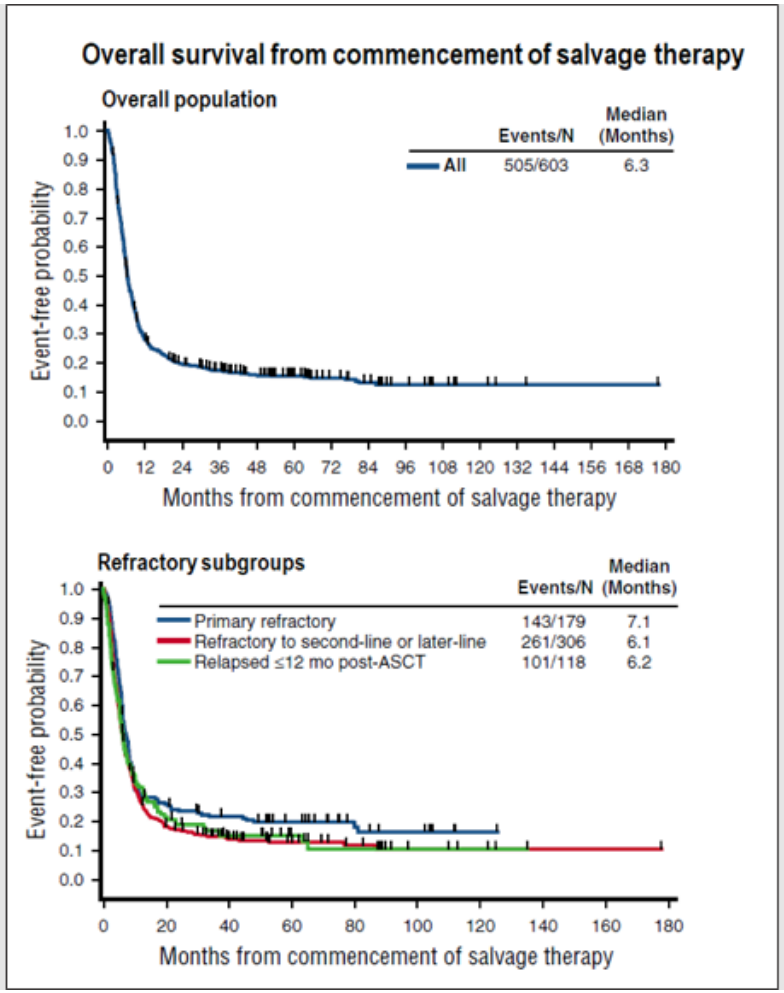
Outcomes of Refractory Large B-Cell Lymphomas before CAR T

SCHOLAR-1 study[1]: *an international, multicohort retrospective study, data from 2001-2014*

Inclusion (N = 636)

- **Diagnoses**
  - diffuse large B-cell lymphoma (87%)
  - transformed follicular lymphoma (4%)
  - primary mediastinal large B-cell lymphoma (2%)
- **Refractory defined as:**
  1) *Progressive disease* (received ≥ 4 cycles of first-line therapy*) or *stable disease* (received 2 cycles of later-line therapy) as best response to chemotherapy
  2) *Relapse* ≤ 12 months after autologous stem cell transplant

  *must have received an anti-CD20 monoclonal antibody and an anthracycline as 1 regimen
- **Outcomes**
  - **response rates to next therapy: ORR = 26%; CR = 7% rate**
  - **median overall survival = 6.3 months**
  - **2-year and 4-year survival estimates 20% and ~15%**



[1]Crump M, et al. *Blood*. 2017;130:1800-1808.

CAR-T, chimeric antigen receptor modified T cell therapy; BsAb, bispecific antibody therapy

ORR = objective response rate; CR = complete response.
Crump M, et al. *Blood*. 2017;130(16):1800-1808.

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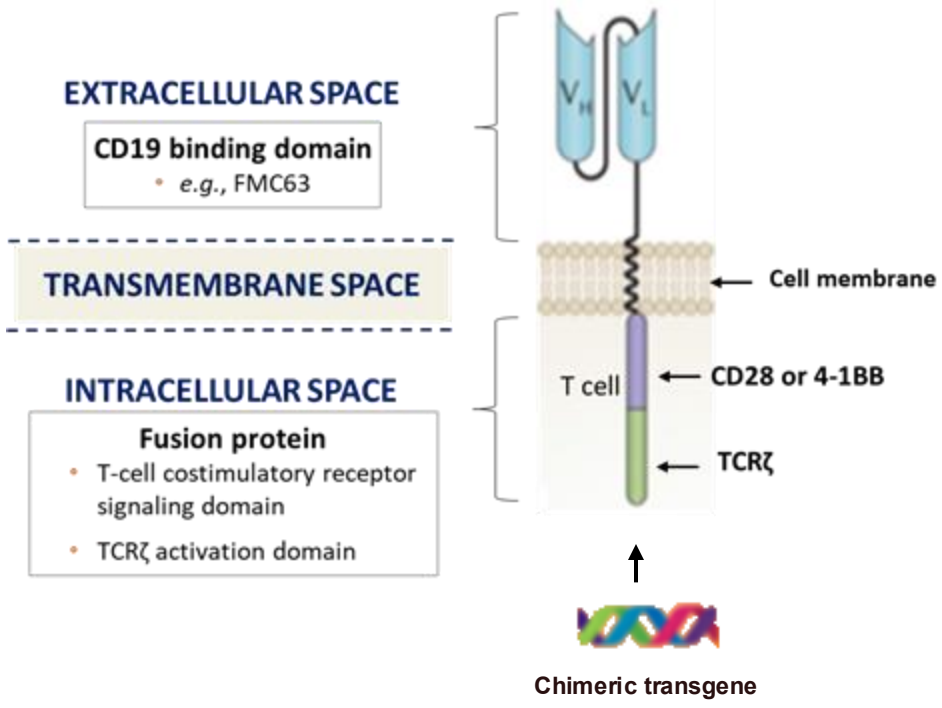
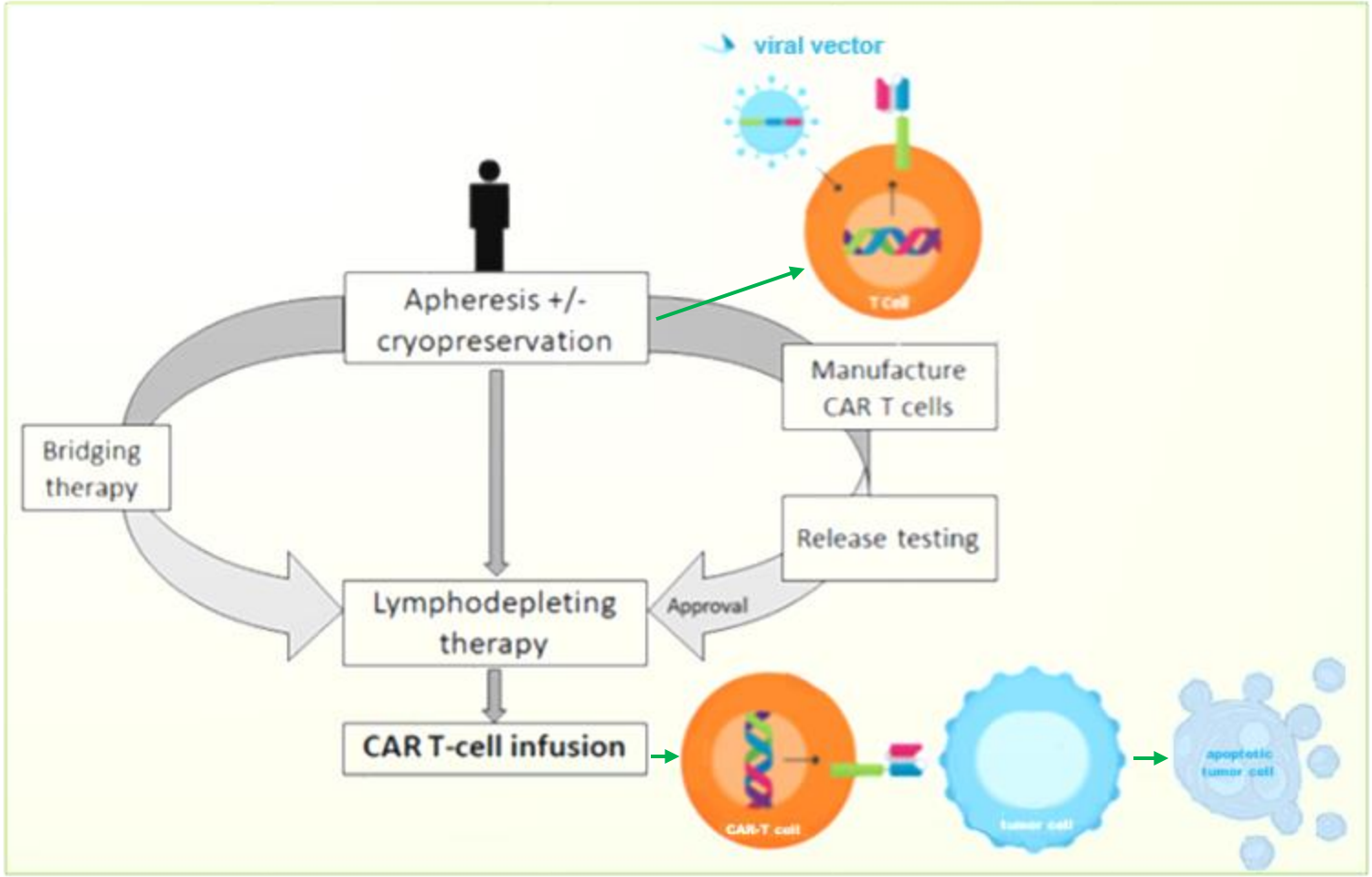
Chimeric Antigen Receptor (CAR)-T: Structure, MOA, and Clinical Process

Generic CD19-directed CAR T cell

Composed of an extracellular CD19 antigen-binding domain and tandem intracellular costimulatory and CD3-ζ signaling domains





MOA = mechanism of action; TCR = T-cell receptor.
Schuster SJ, 2025.

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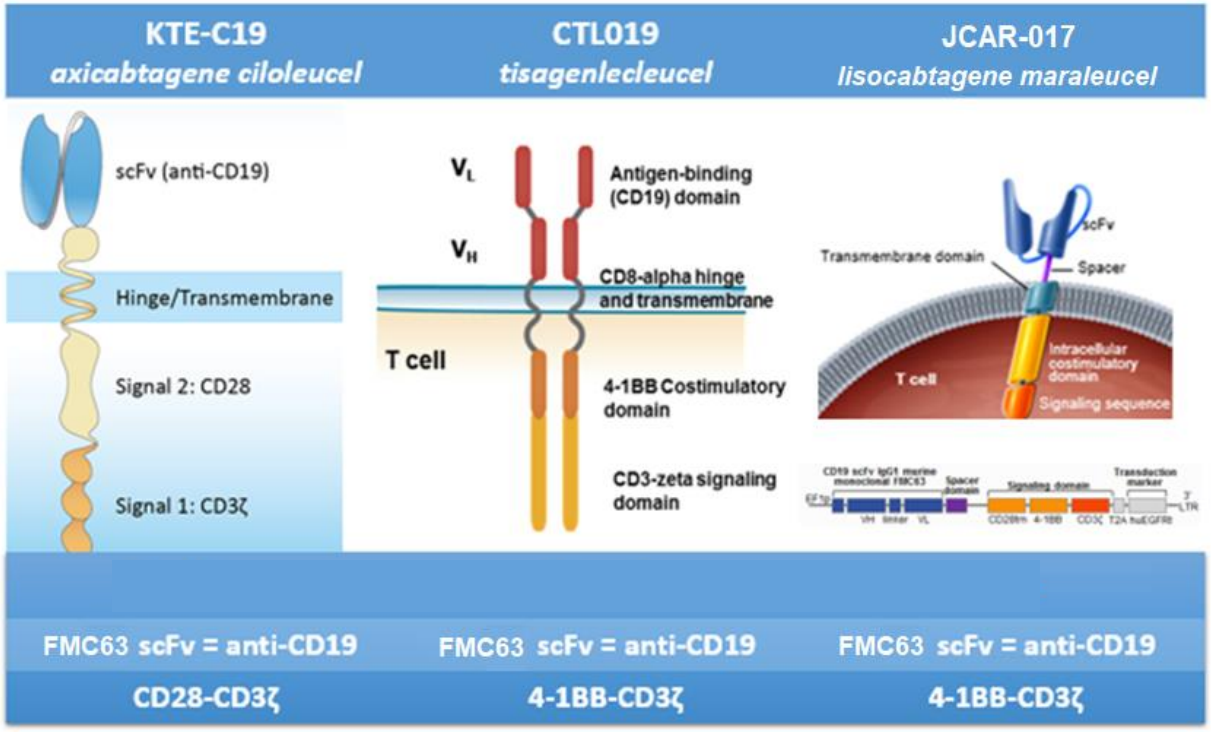
Commercially Available CAR T Products for R/R LBCL

Three commercially available CAR T products and indications



CAR T Products and LBCL Indication	FDA Approval
Tisagenlecleucel[1]	
• ≥3rd-line therapy of r/r LBCL	2018
Axicabtagene ciloleucel[2]	
• ≥3rd-line therapy of r/r LBCL	2017
• 2nd-line therapy of r/early relapse LBCL	2022
Lisocabtagene maraleucel[3]	
• ≥3rd-line therapy of r/r LBCL	2021
• 2nd-line therapy of r/early relapse LBCL	2022
• 2nd line for transplant-ineligible r/r LBCL	2022

- **Efficacy: efficacy similar in 3rd-line trials**
- **Toxicities: similar, but differ in degree**
- **Logistics: somewhat different**

Early relapse is defined as relapse within 12 months of first line of therapy.
r/r = relapsed or refractory; LBCL = large B-cell lymphoma; FDA = US Food and Drug Administration.
[1]US Food and Drug Administration (FDA) [www.fda.gov]. Last updated July 2, 2025. https://www.fda.gov/media/107296/download. [2]US FDA [www.fda.gov]. Last updated June 30, 2025. https://www.fda.gov/media/108377/download. [3]US FDA [www.fda.gov]. Last updated July 31, 2025. https://www.fda.gov/media/145711/download.

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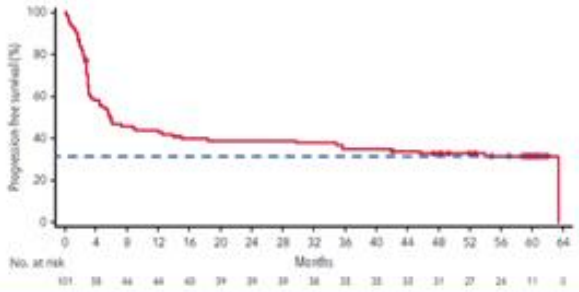
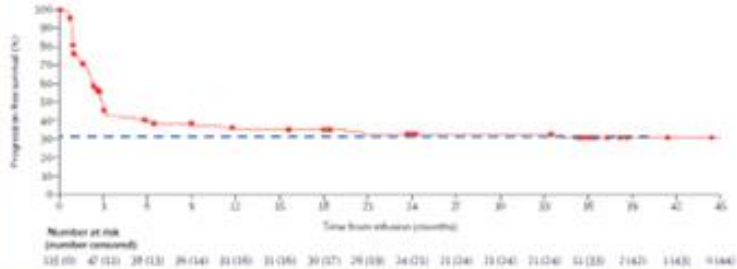
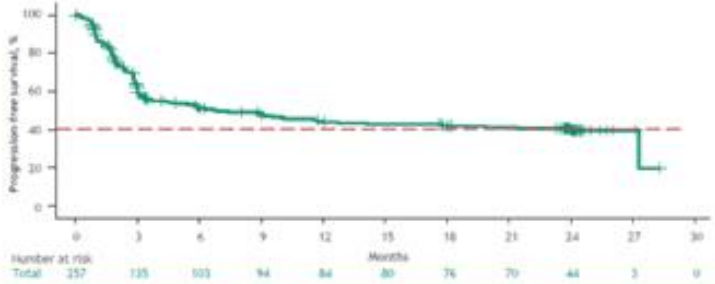
CAR T for >3rd-Line Therapy of Relapsed/Refractory Large B-Cell Lymphomas

Roughly 1/3 of patients with relapsed or refractory large B-cell lymphomas achieve long-term remissions with commercially available CAR T products as ≥3rd-line therapy

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.

Compare with SCHOLAR-1 benchmarks: 2- and 4-year survival estimates 20% and ~15% (estimate)[4]

EFS = event-free survival.
[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 JS, et al. *Blood* 2024;143(5):404-416. [4]Crump M, et al. *Blood* 2017;130(16):1800-1808.

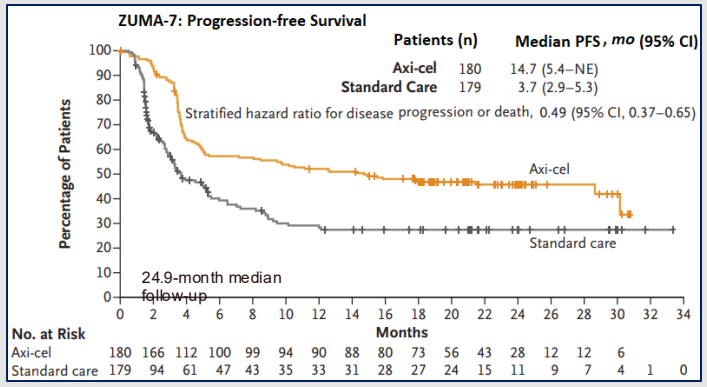
Relapsed/Refractory Large B-Cell Lymphomas: 2nd-Line CAR T

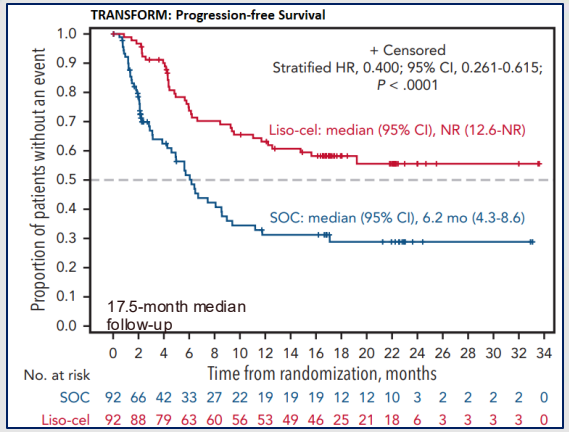
- **Potentially curative approaches: CAR T-cell therapy *or* autologous stem cell transplant**
- **Two randomized trials of CAR T *vs* ASCT**

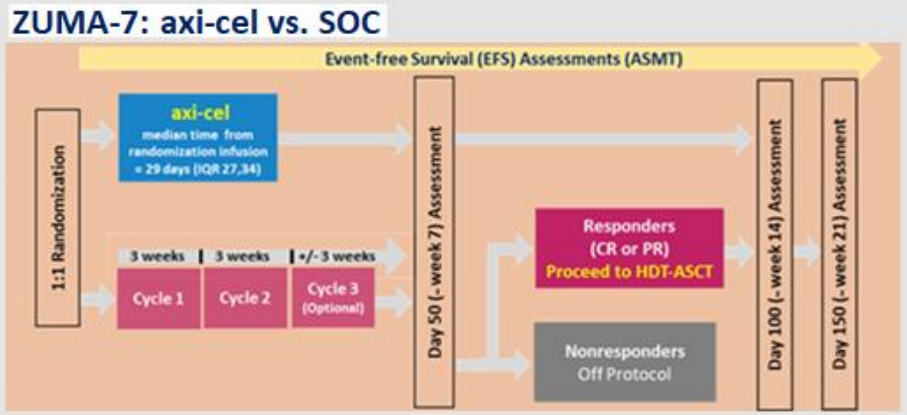
Trial name	Median follow-up	Median event-free survival (95% CI)
ZUMA-7[1] axi-cel vs. SOC	24.9 months	Axi-cel (N=180): 8.3 months (95% CI, 4.5-15.8) SOC arm (N=179): 2.0 months (95% CI, 1.6-2.8) HR 0.398 (95% CI, 0.308-0.514); *P*<0.0001
TRANSFORM[2, 3] liso-cel vs. SOC	6.2 months	Liso-cel (N=92): 10.1 months (95% CI, 6.1-NR) SOC arm (N=92): 2.3 months (95% CI, 2.2-4.3) HR 0.35 (95% CI 0.23-0.53); *P*<0.0001

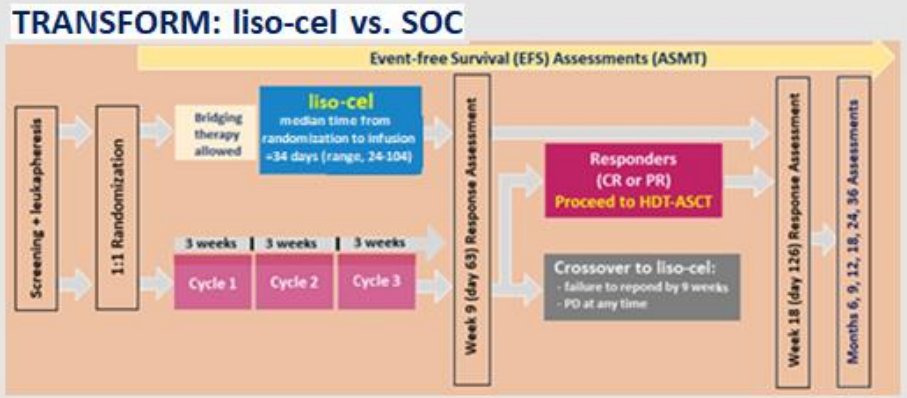
*The studies summarized above differ in their designs, patient characteristics and follow-up; direct comparisons are precluded.

~ 60% risk reduction as 2nd-line therapy compared to SOC in randomized trials



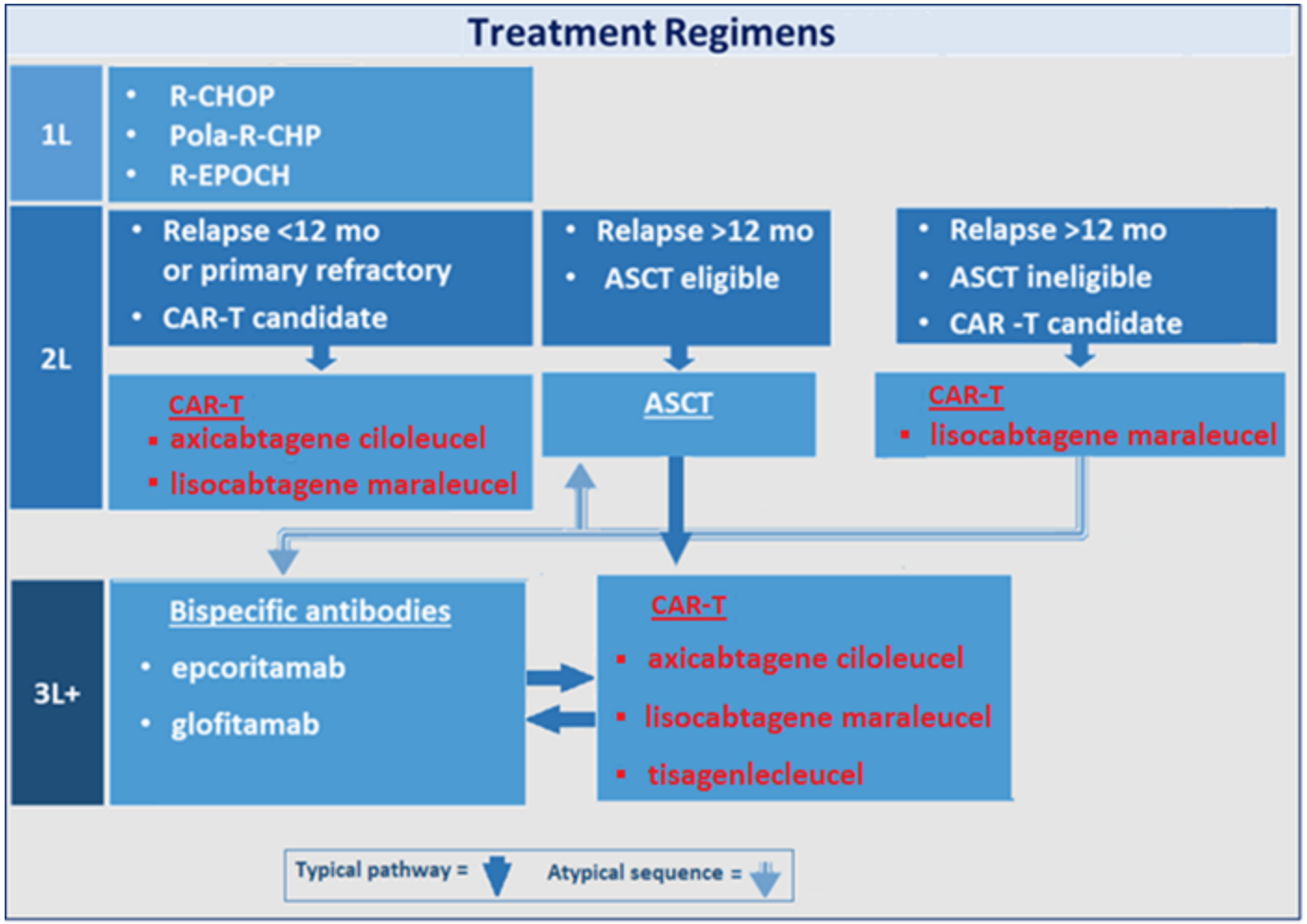






[1]Locke FL, et al. *N Engl J Med*. 2022;386(7):640-654. [2]Abramson JS, et al. *Blood*. 2023;141(14):1675-1684. [3]Karsten IE, et al. *Br J Haematol*. 2024;205(6):2163-2174.

Treatment Approaches to Large B-Cell Lymphomas: 1L, 2L, ≥3L



L = line of therapy; CAR T = chimeric antigen receptor modified T cells; Pola-R-CHP = polatuzumab vedotin, rituximab, cyclophosphamide, doxorubicin, prednisolone.

Referenced from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for B-Cell Lymphomas V.3.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. To view the most recent and complete version of the guidelines, go online to NCCN.org.

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CAR T Therapy: Adverse Events of Special Interest

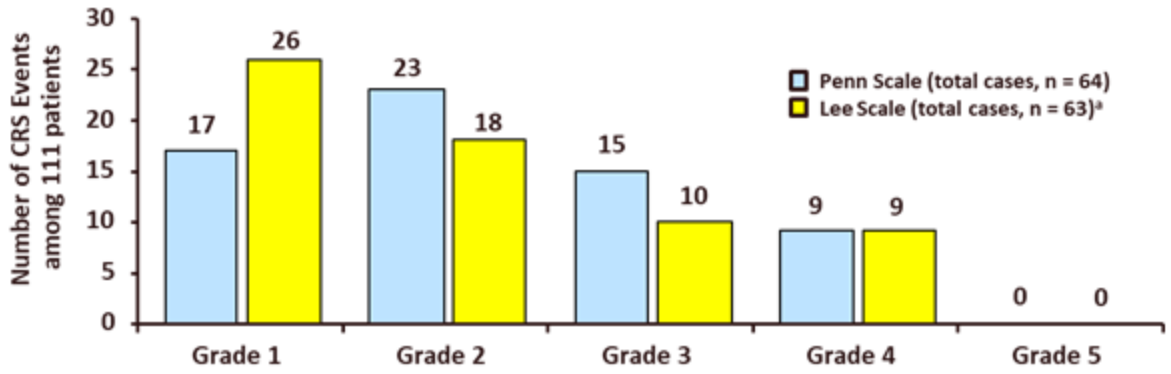
1. **Cytokine release syndrome (CRS)**
2. **Neurotoxicity (ICANS)**
   - Aka, immune effector cell-associated neurotoxicity syndrome
3. **Prologed cytopenias**
4. **"B-cell aplasia"/hypogammaglobulinemia**

CAR-T Product	CRS grade 3/4	Neurotoxicity grade 3/4
Tisagenlecleucel[1*]	22% *	12%
Axicabtagene ciloleucel[2**]	13% **	28%
Lisocabtagene maraleucel[3**]	2% **	10%

Data are from 3 separate trials and are not intended to be a direct comparison of agents. Tisagenlecleucel used a different toxicity grading scale (Penn Scale).[4]

Penn scale; **Lee scale

Regrading of CRS Events on the JULIET Trial: Comparison of Penn and Lee CRS Scales[5]



[a]1 patient was regraded as grade 0 and is not presented in the figure for the Lee scale

Regrading JULIET CRS Events, Grade ≥3	
Penn scale, grade ≥3 events	24/111 (22%)
Lee scale, grade ≥3 events	19/111 (17%)

Fewer grade 2 and 3 CRS events (downgraded to grade 1) by Lee scale

[1]Schuster SJ, et al. *N Engl J Med* 2019;380(1):45-56. [2]Neelapu SS, et al. *N Engl J Med* 2017;377(26):2531-2544. [2]Locke, et al. *Lancet Oncol* 2019;20(1):31-42.
[3]Abramson JS, et al. *Lancet.* 2020;396: 839-582. [4]Porter D, et al. *J Hematol Oncol*. 2018;11:35. [5]Schuster SJ, et al. *Blood Adv.* 2020;4(7):1432-1439.

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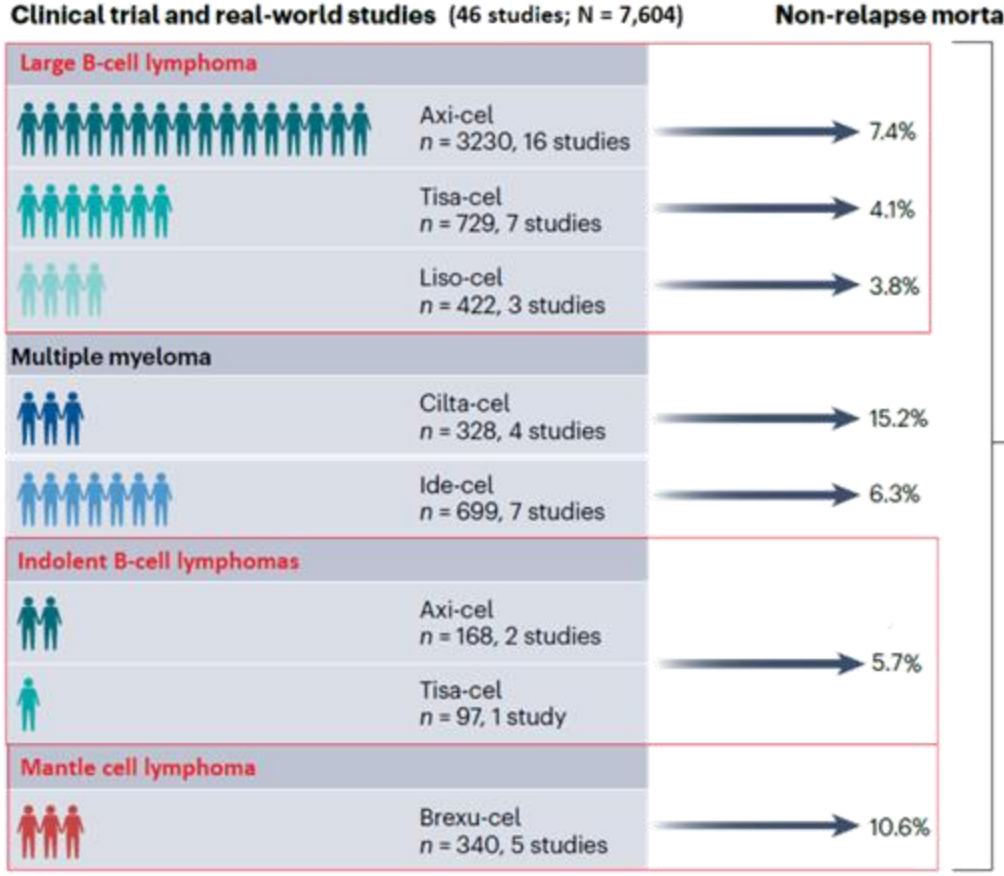
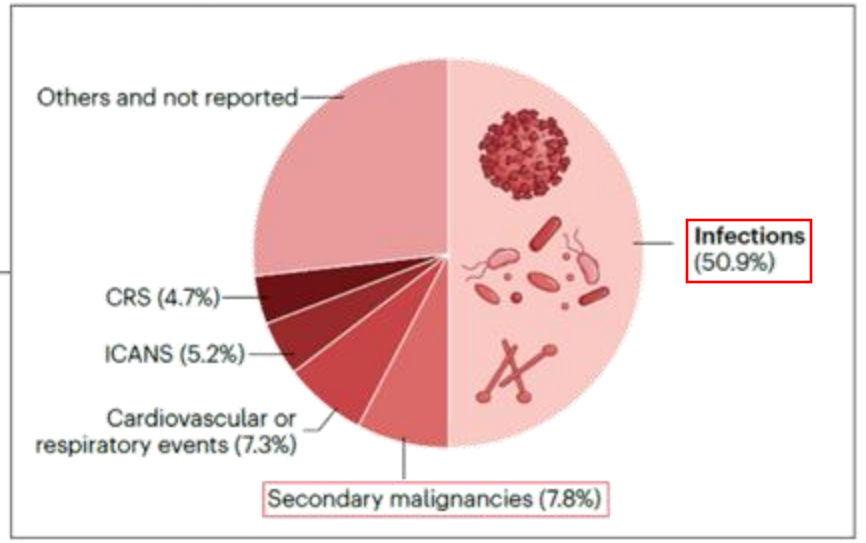
Non-Relapse Mortality after CAR T for B-Cell Lymphomas and Multiple Myeloma

Systematic review and meta-analysis using MEDLINE, Embase, and CINAHL (Cochrane) for reports of non-relapse mortality after CAR T therapy in lymphoma and multiple myeloma up to March 2024[1,2]



NRM point estimates across diseases:
mantle cell lymphoma (10.6%) > multiple myeloma (8.0%) > large B-cell lymphomas (6.1%) > indolent lymphomas (5.7%)

Top five non-relapse-related causes of death



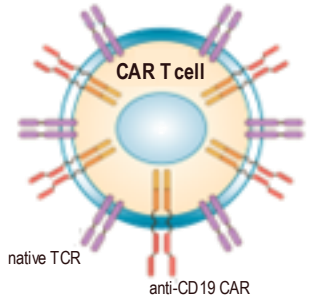
- **SPMs have been reported after CAR T in ~5% of patients**
- **The incidence of SPMs after CAR T does not appear higher than expected: At 30 years from a primary cancer diagnosis, the cumulative incidence of all second cancers is 20.5% (95% CI 19.1%-21.8%)[3]**

These numbers suggest that mortality after CAR T is generally due to failure of therapy.

SPM = second primary malignancy.

[1]Cordas Dos Santos DM, et al. *Nat Med*. 2024;30(9)2667-2678. [2]Blumenberg V, Maus MV. *Nat Med*. 2024;30(9):2413-2414. [3]Morton LM, et al. *Am Soc Clin Oncol Educ Book*. 2014;34(1):e57-e67.

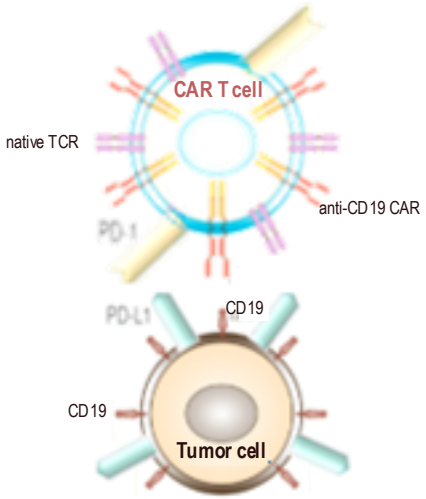
Putative Mechanisms of Resistance to CAR T Therapy in Large B-Cell Lymphomas



CD19 antigen loss

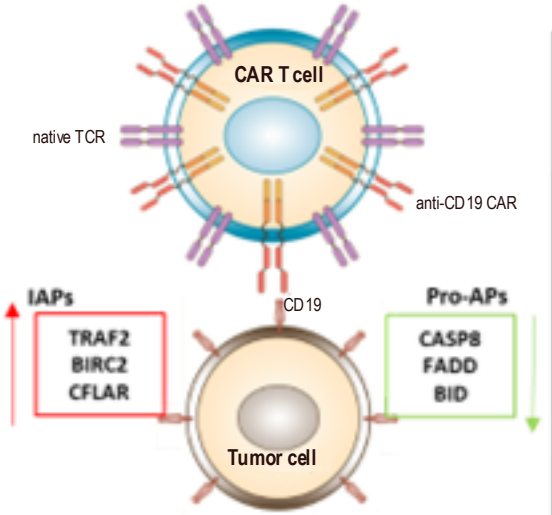
- acquired mutations and alternative splicing of CD19

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



T-cell exhaustion/hypofunction

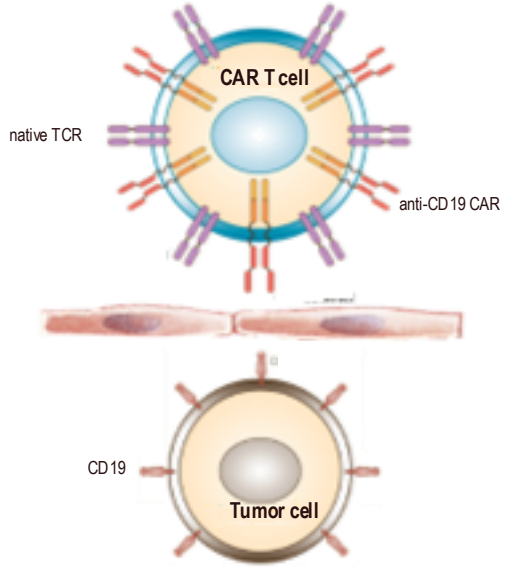
- mediated by inhibitory CAR T receptors and ligands in the tumor microenvironment
- peripheral self-tolerance (B cell recovery? late relapses?)
- TME-induced T cell hypofunction (reversible)



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)

TME = tumor microenvironment.

Schuster SJ, 2024. Sotillo E, et al. *Cancer Discov*. 2015;5(12):1282-1295. Singh N, et al. *Cancer Discov*. 2020;10(4):552-567.

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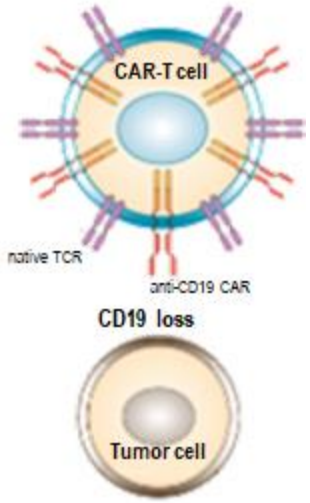
CD19 Antigen Loss-Mediated Resistance to CD19-Directed CAR T

Problem



CD19 antigen loss
Acquired mutations and alternative splicing of CD19

(Sotillo E, et al. *Cancer Discov*. 2015;5(12):1282-1295.)

Potential Solutions

- Manufacture CAR T cells with dual specificity to target more than one tumor antigen (*ie*, "bispecific" CAR T cell)
  - *Bicistronic* CARs on a single cell's surface
  - *Tandem* scFv as a single CAR on a single cell's surface
- Coadministration of monospecific CAR T-cell products, each CAR T with different tumor antigen specificities (so-called "cocktail" approach)
  - *Sequential* administration
  - *Simultaneous* coadministration
- Combine T cell-engaging bispecific antibodies (BsAb) and monospecific CAR T cells, with each product, BsAb and CAR T, having different tumor antigen specificities

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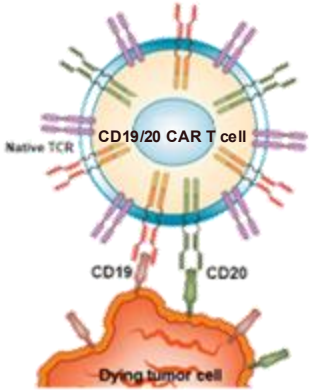
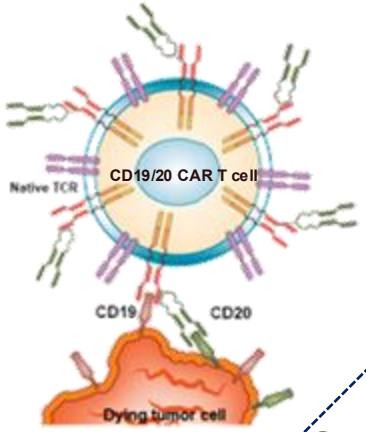
Dual-Antigen Targeted CAR T Approaches

Bispecific CAR T-cell formats

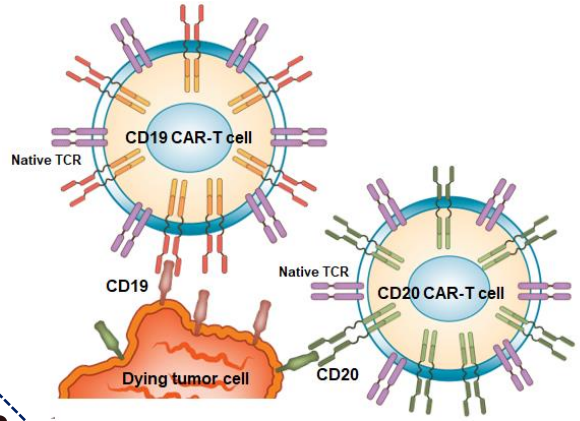
Bicistronic receptors



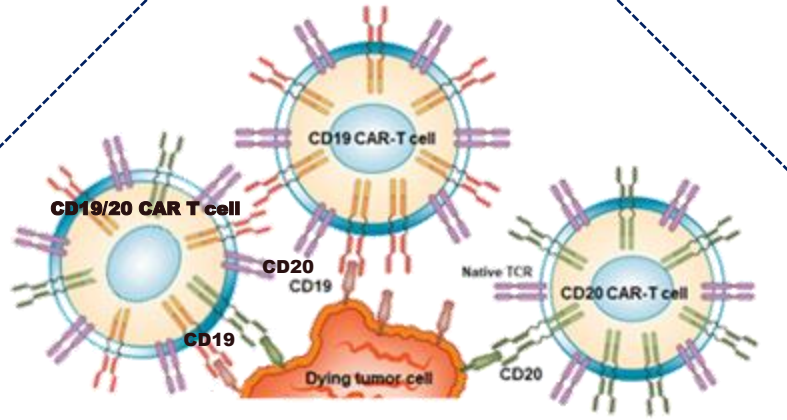
Bivalent tandem receptor



Coadministration of CAR T cells



Cotransduction



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Dual-Antigen Targeted CAR T Approaches for B-Cell Malignancies

Early Phase 1/2, Single-Arm, Noncomparative, Prospective, Open-Label 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)**			

***Possible reasons for unconvincing advantage to dual-antigen targeting*:**

***i)* Do not address mechanisms of resistance other than antigen escape; *ii)* Insufficient pt numbers and follow-up for most trials; *iii)* Wide heterogeneity among products, including different second antigen targets (CD20 or CD22); *iv)* Complex manufacturing optimization with low transduction efficiency and large size of bicistronic and tandem vectors**

LD = lymphodepleting; OR = overall response; FC = fludarabine, cyclophosphamide; pt = patient.

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

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Dual-Antigen Targeted CAR T Approaches for B-Cell Malignancies

KITE-363, a bicistronic anti-CD19/CD20 CD28/4-1BB costimulated CAR T cell phase 1a/1b clinical trial

CD20/CD19 Dual-Targeted CAR T Trial for R/R B-Cell Lymphoma

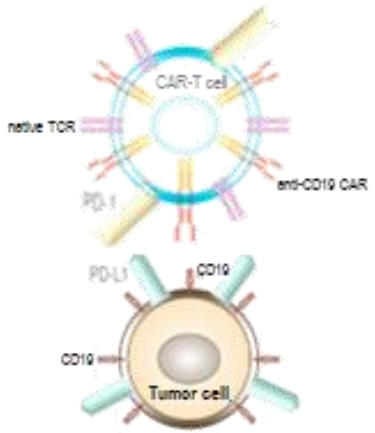
ref. #	Product	CAR Transgene	Cohort	N	Diagnosis n (%)	Prior R+, n (%)	Prior CAR T n (%)	LD	ORR/CRR*	Median F/U mo (range)	Survival	AESI at RP2D (n = 26)	
1	KITE-363	bicistronic CD28 + 4-1BB	*all infused*	37	LBCL, 34 (92) iNHL, 2 (5) NLPHL, 1 (3)	1 = 17 (46) ≥2 = 20 (54)	7 (19)	Cy/Flu 300/30	77%/62%	12 mo (6-39)	Median DOCR, not reached	CRS, any grade: 88% CRS, grade 3/4: 4%	ICANS, any grade: 46% ICANS, grade 3/4: 8%
			RP2D	26	LBCL, 25 (96) iNHL, 0 NLPHL, 1 (4)	1 = 15 (58) ≥2 = 11 (42)	3 (12)		CAR-Naive (n = 23) 87%/78% prior CAR T (n=3) 33%/33%	11 mo (6-22)	6-mo DOCR, 71% (95%CI: 44-87)		

*The first post-infusion response assessment was conducted on day 28.
AESI = adverse events of special interest; B-NHL = B-cell non-Hodgkin lymphoma; Cy/Flu = cyclophosphamide, fludarabine; DOCR = duration of CR; iNHL = indolent non-Hodgkin lymphoma; LBCL = large B-cell lymphoma; mo = month; NLPHL = nodular lymphocyte predominant Hodgkin lymphoma; RP2D = recommended phase 2 dose; F/U = follow-up; CRR = complete response rate.

[1]Dahiya S, et al. *J Clin Oncol*. 2025;43(Suppl 16):7003.

T-Cell Exhaustion/Hypofunction as Mechanisms of Resistance to CD19-Directed CAR T

Problem



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)

Potential Solutions

1. **Combining immunomodulatory drugs with CAR T cells**
   eg, ibrutinib

([1]Miklos…Schuster, et al. *Target Oncol*. 2025;20(2):217-234. [2]Chavez…Schuster, et al. *Blood Neoplasia*. 2025; *accepted for publication*)

2. **Shorten *ex vivo* manufacturing time**
   eg, rapcabtagene autoleucel (YTB323)
3. **Autologous "armored" CAR T cells**
   eg, IL-18 armored CAR T cells
4. **Autologous CAR T cells with alternative receptor costimulatory domains**
   eg, KIR-CAR/Dap12-CAR T cells (NCT06544265: SynKIR-310 for Relapsed/Refractory B-NHL)
5. **Restimulation of CAR T cells with CD19 peptide mimotopes**

([1]Ma L, *et al. Science* 2019;365(6449):162-168. [2]Singh…June. *Science* 2019;365(6449):119. [3]Ma L, et al. *Cell*; 2023;186(15):3148-3165.)

6. **Allogeneic CAR T cells**
   eg, CB-010: allogeneic anti-CD19 CAR T with PD-1 and TRAC gene knockouts

Schuster SJ, 2024.

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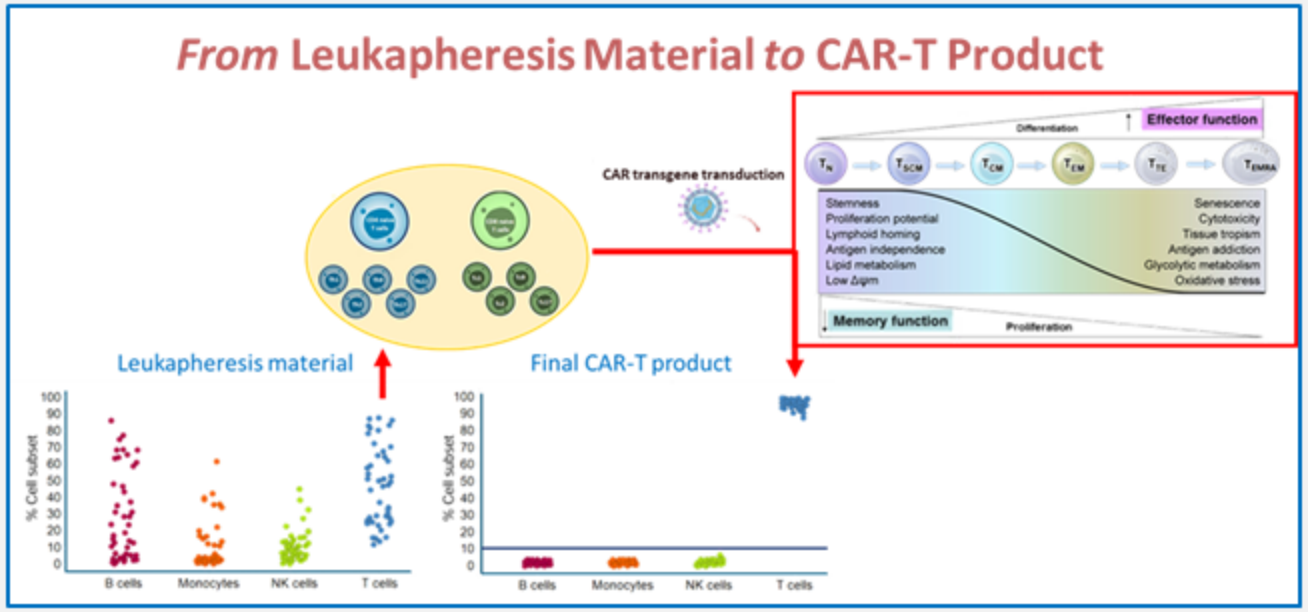
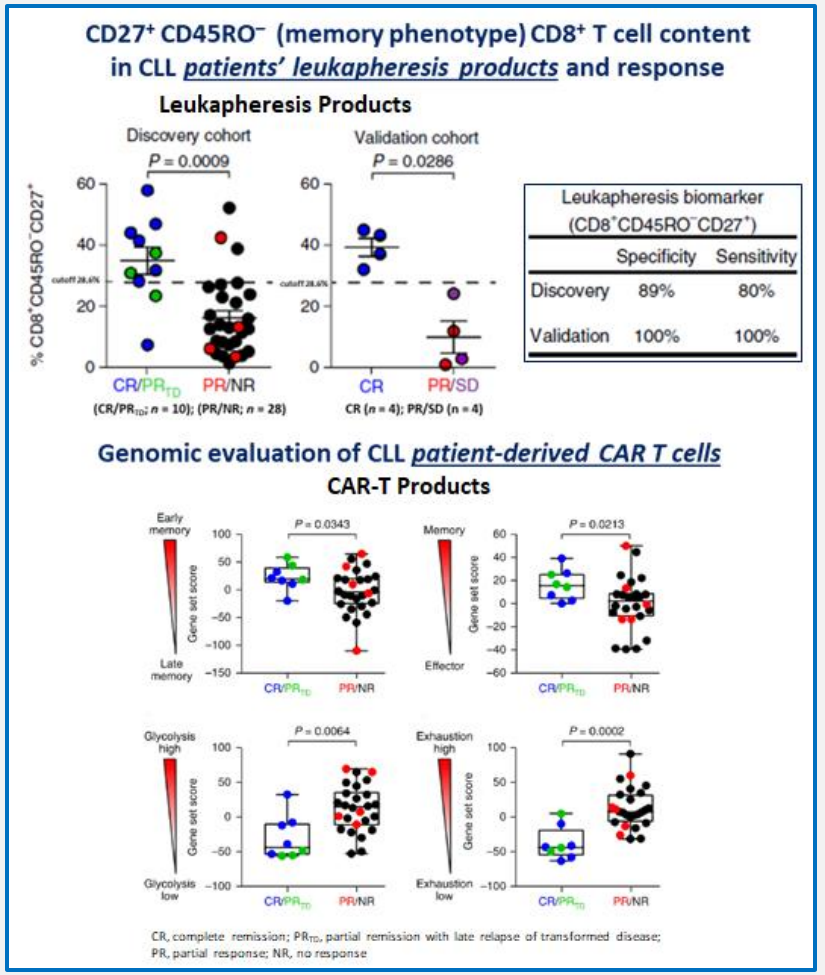
Rapid CAR T Manufacturing to Enhance CAR T "Fitness"

Functional T-cell subsets determine CAR T-cell responses



Data supporting impact of lymphocyte fitness on CAR-T outcomes in lymphomas		
Large B-cell lymphomas	Ibrutinib before apheresis may improve tisagenlecleucel manufacturing in r/r DLBCL *(Phase 1b Study)*	Chavez ... Schuster. ASH 2020. abstract # 1200 poster
Follicular lymphoma	Higher baseline CD8+ naive T cells (>2.14%) is associated with improved long-term clinical outcomes *(ELARA 3-Year Follow-up)*	Schuster, et al. ASH 2023. abstract # 601 oral presentation



Fraietta JA, et al. *Nat Med*. 2018;24(5):563-571.

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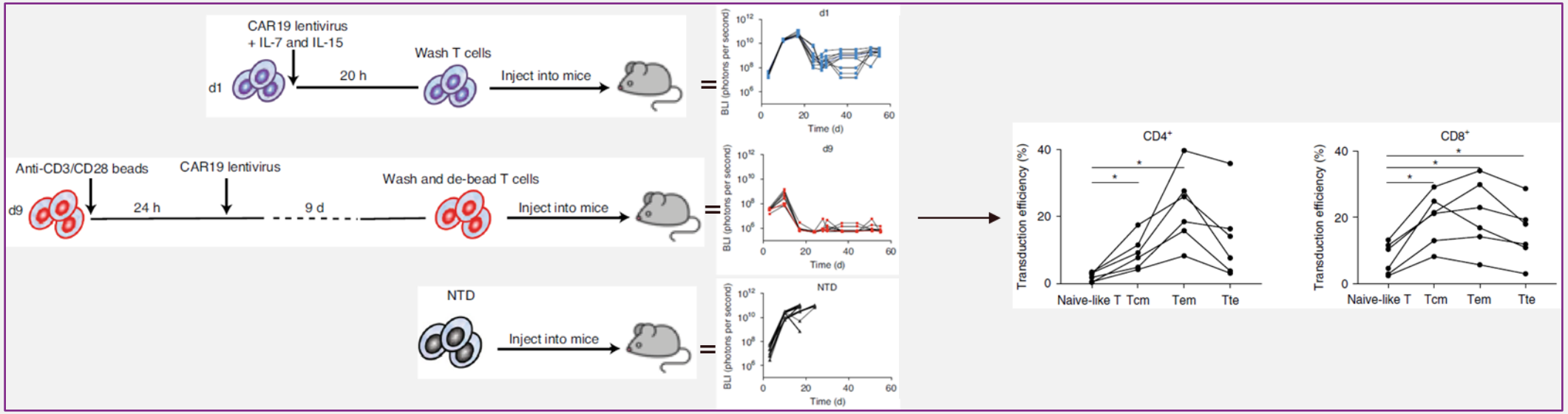
Rapid CAR T Manufacturing to Enhance CAR T "Fitness"

Rapid manufacturing of non-activated potent CAR T cells

Saba Ghassemi[1,2], Joseph S. Durgin[1], Selene Nunez-Cruz[1,2], Jai Patel[1], John Leferovich[1,2], Marilia Pinzone[2], Feng Shen[1], Katherine D. Cummins[1], Gabriela Plesa[1], Vito Adrian Cantu[3], Shantan Reddy[3], Frederic D. Bushman[3], Saar I. Gill[1,4], Una O'Doherty[2], Roddy S. O'Connor[1,2] and Michael C. Milone[1,2]

- Activation and expansion of CAR T cells leads to their progressive differentiation and associated loss of anti-leukaemic activity
- Here we show that functional CAR T cells can be generated within 24 hours from T cells derived from peripheral blood without the need for T-cell activation or ex vivo expansion



Ghassemi S, et al. *Nat Biomed Eng*. 2022;6(2):118-128.

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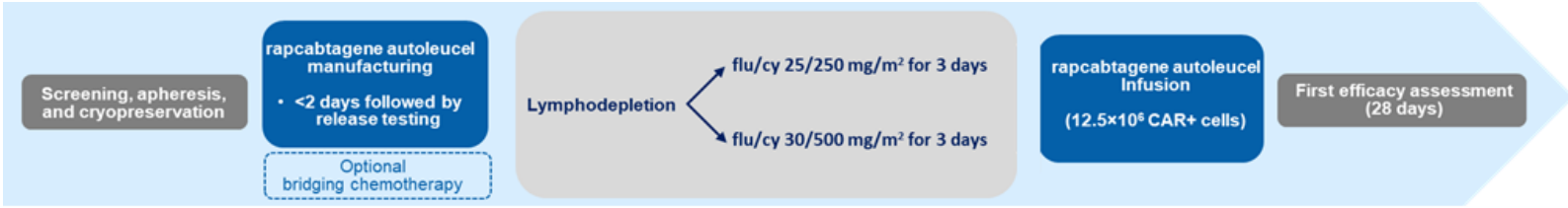
Rapid CAR T Manufacturing to Enhance CAR T "Fitness"

Phase 2 Study of Rapcabtagene Autoleucel in R/R DLBCL

Rapcabtagene autoleucel (YTB323) is an investigational CD19-directed CAR T-cell therapy that uses **rapid CAR T manufacturing,** which *preserves stemness* ("fitness") and is *available in ≤2 days*

Patient Characteristics	N=63, infused
Median age, years (range)	64 (26-81)
≥65 y, n (%)	29 (46.0)
Rearrangements in *MYC/BCL2/BCL6* genes, n (%)	
Double/triple hits	16 (25.4)
Negative	25 (39.7)
Unknown	22 (34.9)
Relapsed/refractory disease status, n (%)	
Refractory to last line of therapy	37 (58.7)
Refractory to all prior lines	13 (20.6)
Relapsed after last line of therapy	26 (41.3)
Histology, n (%)	
DLBCL	52 (82.5)
Transformed lymphoma	8 (12.7)
Elevated LDH (>ULN), n (%)	27 (42.9)
Prior lines of therapy, n (%)	
2	46 (73.0)
≥3	17 (27.0)



Results	N=60, response evaluable n (%)
Median follow-up, months (range)	16.4 (0.1-44.1)
Overall response rate, n (%) [95% CI]	53 (88.3) [77.4-95.2]
CR, n (%) [95% CI]	39 (65.0) [51.6-76.9]
Complete response rate, n/N (%)	
Month 3	30/55[e] (54.5)
Month 6	25/44[e] (56.8)
Month 12	18/38[e] (47.4)

	Responders, N = 53	Patients with CR, N=39
Median DOR, months (95% CI)	15.2 (5.1-NE)	NE (10.4-NE)
12-month DOR, %	53.9	69.1

	N=63, all patients	N=30, Month-3 CR patients
Median OS, months (95% CI)	NE (19.5-NE)	NE (NE-NE)
12-month OS, %	83.0	100

Adverse Events of Special Interest, n=63		
	CRS, n (%)	ICANS, n (%)
Any Grade	28 (44.4)	5 (8%)
Grade 1	17 (27.0)	2 (3.2)
Grade 2	7 (11)	0
Grade 3	2 (3.2)	2 (3.2)
Grade 4	2 (3.2)	1 (1.6)

DOR = duration of response; NE = not estimable.

Riedell PA, et al. *Blood*. 2024;144(Suppl 1):67.

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Armored CAR T to Overcome T-Cell Exhaustion

ORIGINAL ARTICLE

Enhanced CAR T-Cell Therapy for Lymphoma after Previous Failure

Jakub Svoboda, M.D.,[1] Daniel J. Landsburg, M.D.,[1] James Gerson, M.D.,[2] ... Stephen J. Schuster, M.D.,[1] and Carl H. June, M.D.[4,5,7]

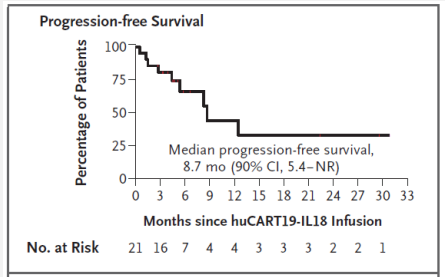
N ENGL J MED 392;18 NEJM.ORG MAY 8, 2025

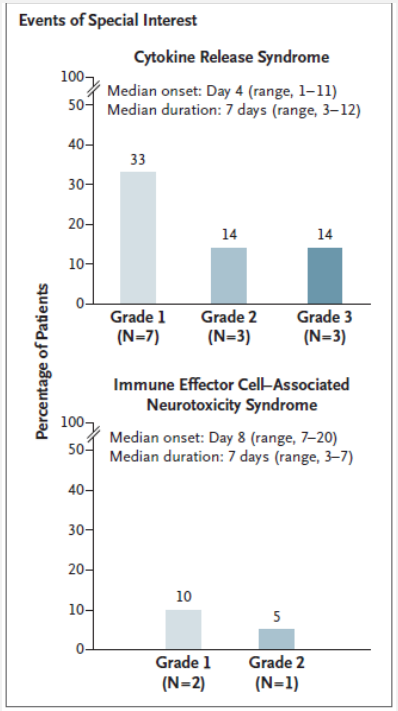
- An interleukin-18 secreting, CD19-directed CAR T cell *(huCART19-IL18)* was designed to enhance antitumor activity
- Assess safety, feasibility, and preliminary efficacy of huCART19-IL18 in patients with r/r B-cell lymphomas after prior anti-CD19 CAR T therapy

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Patients (N=21)
Median age (range) — yr	64 (47–74)
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)
Median no. of previous medications (range)	7 (4–14)
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	6.7 (3.1–10.2)

Results	
Median follow-up, months (range)	17.5 months (3-34)
Overall response rate, n (%) [95%CI]	81% [90%CI, 62-93]
CR, n (%) [95% CI]	52% [95%CI, 33-71]
Median duration of response, months [95%CI]	9.6 months [90%CI, 5.5-NR]





Svoboda J, et al. *N Engl J Med*. 2025; 392(18):1824-1835.

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Allogeneic CAR T to Overcome T-Cell Exhaustion

Potential Advantages

- **Prepared from health donor T cells**
  - Avoids negative impact of prior therapies and/or disease on autologous immune cells
- No manufacturing failures or treatment delay for manufacturing and release testing
- Leukapheresis of patient not required
- Multiple products from a single donor
- Reduced cost per patient

Potential Pitfalls

- Graft-versus-host disease **(GvHD)**
  - Mediated by HLA mismatch between donor and recipient
- Host-versus-graft rejection **(HvG)**
  - Rejection of allogeneic CAR T cells by host immune cells

Ideal "Universal" Allogeneic CAR T Product

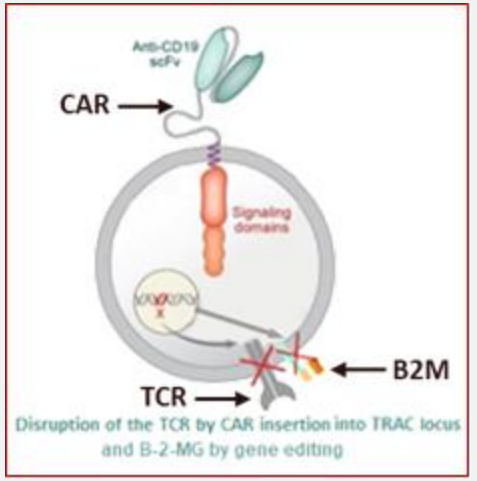
- **Resistant to recognition of host (recipient) tissue by donor CAR T cells**
  - Knock-out of TCR gene (TRAC) in donor T cells blocks expression of TCR
  - **Prevents GvHD—*ie*,** only tumor-targeted cytotoxicity
- **Resistant to rejection of donor CAR T cells by host CD8+ αβ-T cells**
  - Knock-out of donor T-cell β2-microglobulin prevents expression of MHC class I and recognition/elimination by recipient CD8+ αβ-T cells
  - No HvG rejection—*ie*, enhances CAR T-cell persistence for efficacy
- **Engineered for potency and safety**

2022, Systematic Review and Meta-analysis[1]

- **"Off-the-Shelf" Allogeneic Anti-CD19 CAR T for R/R B-Cell Lymphomas**
  - 3 studies; n=71 patients

Study	N, efficacy/safety evaluable	CR (%)	GvHD (%)	Severe CRS (%)	Severe ICANS (%)
R/R B-cell 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.



[1]Chen S, et al. *Crit Rev Oncol Hematol*. 2022;179:103807. [2]Shah BD, et al. *Blood*. 2021;138(Suppl 1):302. [3]Lekakis LJ, et al. *Blood*. 2021;138(Suppl 1):649; [4]Neelapu SS, et al. *Blood* 2021;138 (Suppl 1):3878.

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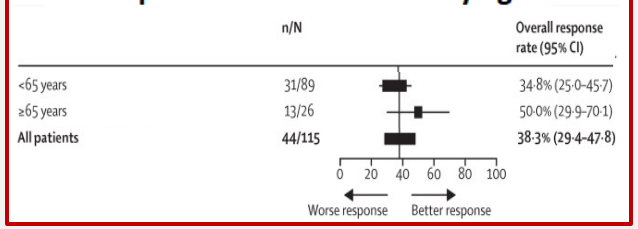
Predictors of Response to CAR T in R/R LBCL

Patient selection

- ✓ Diagnosis: LBCL subtype
- ✓ life expectancy ≥8 weeks with palliative therapy
- ✓ ECOG performance status at screening vs. at infusion*
- ✓ expected efficacy
  - tumor control
  - tumor bulk
  - serum LDH

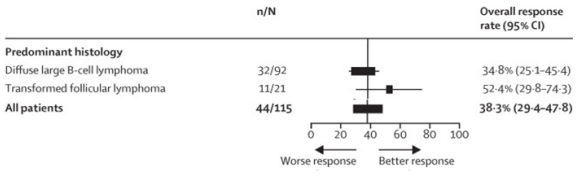
*ECOG PS 0-2; at least ambulatory and capable of self care; generally, up and about more than 50% of waking hours

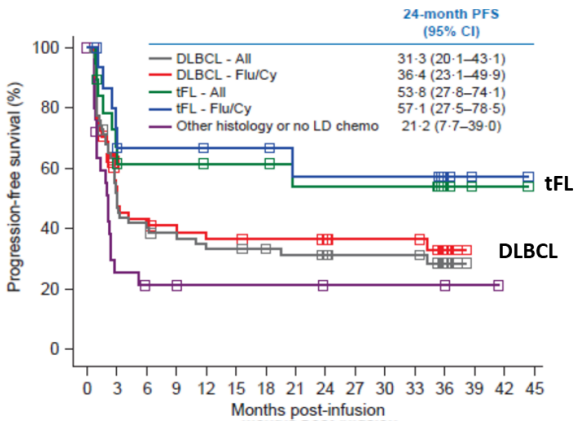
Response rate unaffected by age[1]



LBCL Subtype

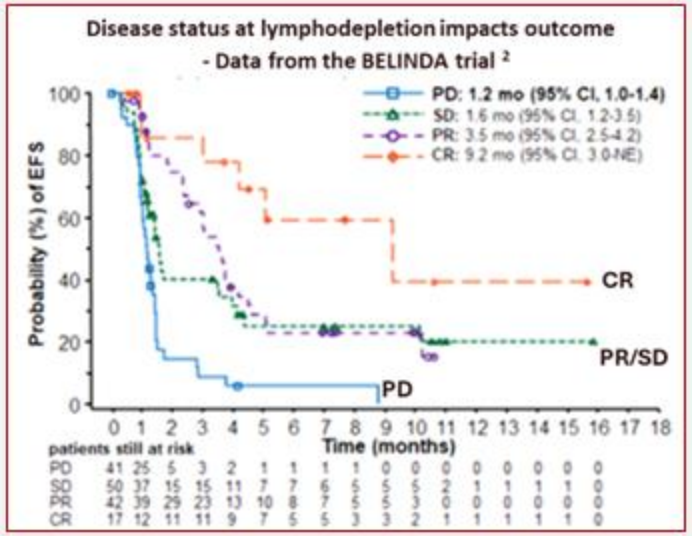
Response rates differ by large B-cell lymphoma subtypes[1]





Disease Control

Disease status at lymphodepletion impacts outcome - Data from the BELINDA trial [2]



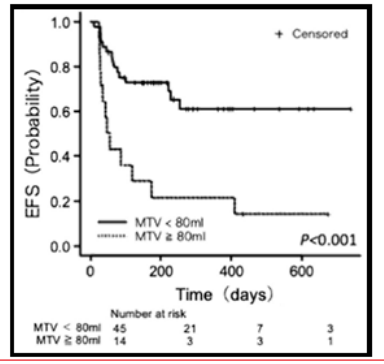
Tumor Bulk

Tumor bulk impacts response [1]

Anatomic tumor volume

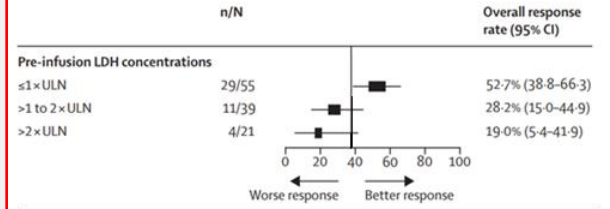
CT tumor volume	n/N	Overall response rate (95% CI)
Bulky disease (> 10 cm)		
Yes	1/9	11·1% (0·3–48·2)
No	43/106	40·6% (31·1–50·5)

Metabolic tumor volume [3]

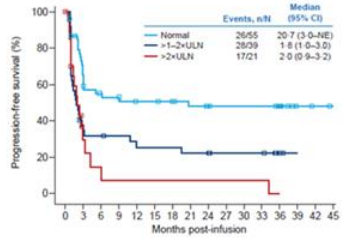


Serum LDH

Overall response rates by LDH[1]



Progression-free survival by LDH



tFL = transformed follicular lymphoma; SD = stable disease; PD = progressive disease.

[1]Schuster SJ, et al. *Lancet Oncol*. 2021;22(10):1403-1415. [2]Bishop MR, et al. *N Engl J Med*. 2022;386(7):629-639. [3]Goto H, et al. *Int J Clin Oncol*. 2023;28(6):816-826.

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Circulating Tumor DNA Assay for Detecting MRD by PhasED-Seq

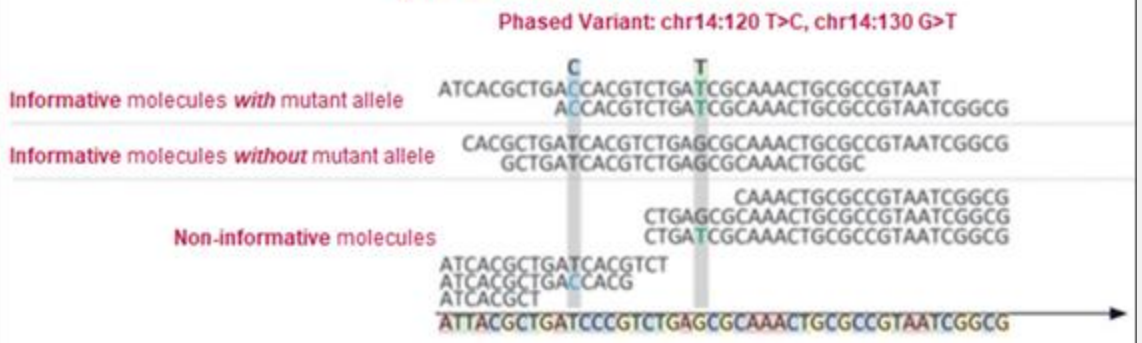
PhasED-Seq Assay

- Requires tumor tissue for baseline DNA sequencing
- Detects "phased variants" (≥2 single nucleotide variants on same DNA fragment)
- By requiring ≥2 single nucleotide variants on same DNA fragment for detection, background noise from sequencing errors is reduced
  - Improves sensitivity
  - Increases accuracy

PhasED-Seq Assay Performance Characteristics[1,2]

- False positive rate: 0.24%
- Background error rate: 1.95E-08 (0.0000000195)
- 95% detection rate with 120 ng DNA input: 0.7 PPM with precision >96%
- Positive agreement with reference assay (PPA): 90.62% (95% CI 74.98%, 98.02%)
- Negative agreement with reference assay (NPA): 77.78% (95% CI 52.73, 93.59)

Informative Molecules[2]



Informative Molecules are cfDNA molecules that span the location of a tumor-specific phase variant; i.e., harbor tumor-specific mutations that include one or more phase variants in a patient's previously identified list of phase variants

PhasED-Seq ctDNA Assay: Clinical Validation[3]

- ctDNA by PhasED-Seq studied for prediction of outcomes of patients receiving axi-cel on ZUMA-7 (n=44)[3]
- Phased variants were identified in biopsies obtained prior to treatment and longitudinally monitored in plasma at ~50, 100, and 150 days post-randomization
- **PPV** and **NPV** assessed **at days 50 and 150**

Landmark Analysis		Day-50	Day-150
PPV =	MRD+ r/r pts / Total MRD+ pts	**50%**	**57%**
NPV =	MRD- CR/PR pts / Total MRD- pts	**70%**	**77%**

PhasED-Seq = phased variant enrichment and detection sequencing; ctDNA = circulating tumor DNA; cfDNA = cell-free DNA; MRD = minimal residual disease; NPV = negative predictive value; PPV = positive predictive value.

[1]Boehm N, et al. *medRxiv*. 2024.08.09.24311742 [Preprint]. [2]Klimova N, et al. *Oncotarget*. 2025:16:329-336. [3]Miles B, et al. *J Clin Oncol*. 2025;43(16 Suppl):e15043.

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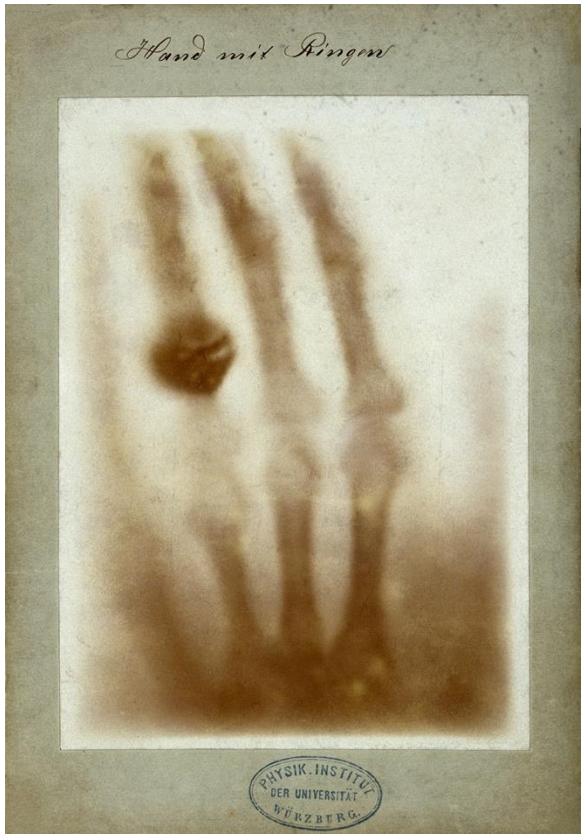
Deep Learning-Based Image Analysis

"*Images are more than pictures, they are data.*" Gillies RJ, et al. *Radiology*. 2016;278(2):563-577.



Röntgen's first "medical" X-ray:
His wife's hand, December 22, 1895[3]

Hypothesis: Radiologic images contain detailed quantitative data beyond that used to reconstruct humanly recognizable anatomic and functional pictures; these additional data may contain prognostic information, which could aid clinical decision-making

Objectives: 1) To **extract *image-agnostic features* (data)** from PET/CT images that correlate with and can predict clinical outcomes using machine learning

2) To **develop a computerized decision support system (program)** by retraining a pretrained neural network model (AlexNet[1]) using transfer and incremental learning

3) To **prospectively validate the utility of this program** for prediction of outcomes of patients undergoing CAR T therapy for relapsed or refractory lymphomas

Results[2]:

- ***Pretreatment*** diagnostic CT, low-dose CT, and ^{18}FDG-PET images from patients with known CAR T treatment outcomes were used to **build a neural network model** to predict ***single lesion-level treatment response***
  - **770 nodal lesions from 39 patients studied in 3,040 experiments performed to develop model**
  - **Binary lesion-level predictions: CR or non-CR at 12 months post-CAR T infusion**
  - **Binary patient-level predictions: CR or non-CR at 12 months post-CAR T, based on single lesion-level predictions from 3 whole slices per lesion as input and ≥60% "majority rule"** (≥60% lesions predicted to respond performed as well as all lesions predicted to respond)

Validation: Investigators blinded to patient outcomes analyzed pre-treatment low-dose CT and ^{18}FDG-PET images using 3 whole image slices per lesion and the ≥60% majority rule to predict patient CAR T treatment outcome

[1]Krizhevsky A, et al. *Communications of the ACM*. 2017;60(6):84-90. [2]Tong Y, et al. *PLoS One*. 2023;18(7):e0282573. [3]Röntgen W, uploaded by Yann [commons.wikimedia.org]. Last updated January 31, 2024. https://commons.wikimedia.org/wiki/File:First_medical_X-ray_by_Wilhelm_Röntgen_of_his_wife_Anna_Bertha_Ludwig%27s_hand_-_18951222.jpg.

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Predicting CAR-T Outcome from Pre-Treatment PET/CT Images

- Pre-infusion images obtained from the JULIET study, a phase 2 study of tisagenlecleucel in adult pts with r/r LBCLs (NCT02445248)
- Investigators were blinded to patient outcomes
- Image datasets were acquired on 15 different model scanners from 3 manufacturers from 27 hospitals in 10 different countries
- Pretreatment PET/CT images from 68 pts treated with tisagenlecleucel were evaluated using DL-based image analysis to predict lesion responses without retraining the previously described model
- Image input from 3 contiguous mid-portion whole-image slices through nodal lesions in FDG-PET and low-dose CT scans (6 image slices input per lesion) analyzed to predict individual lesion responses for each patient, and patient responses predicted based on lesion-level predictions and the ≥60% majority rule
- Patients classified as CR or non-CR after CAR T infusion by 12-month radiologic outcome (or last follow-up if <12 months)
  - Responders met protocol-specified radiologic criteria for CR at month 12; non-responders did not meet CR criteria by month 12
  - Actual patient outcomes per protocol: Responders, n=19; non-responders, n=49 (CR rate at month 12 = 28%)
- DL-based PET/CT image analysis results:

Deep Learning-Based Image Analysis Predicts CR from Pretreatment PET/CT, ≥60% Rule

Input: PET + LD-CT, 3 slices each per lesion n = 68	Sensitivity	Specificity	Balanced Accuracy*	Positive Predictive Value	Negative Predictive Value
	77%	49%	63%	37%	85%

*Balanced Accuracy = (sensitivity + specificity)/2; balanced accuracy reported because of imbalance between number of responders and non-responders in test group
r/r LBCL = relapsed/refractory large B-cell lymphomas; DL = deep learning; pts = patients; CR = complete response; LD-CT = low-dose CT.
Schuster SJ, et al. Presented at: 18th International Conference on Malignant Lymphoma (ICML); 2025.

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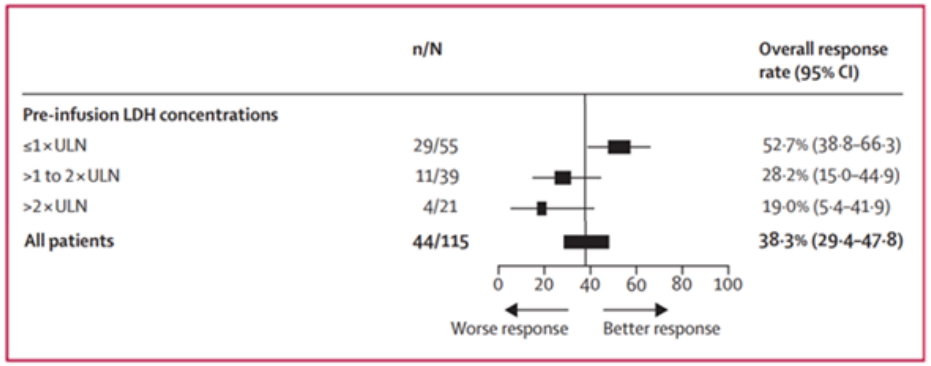
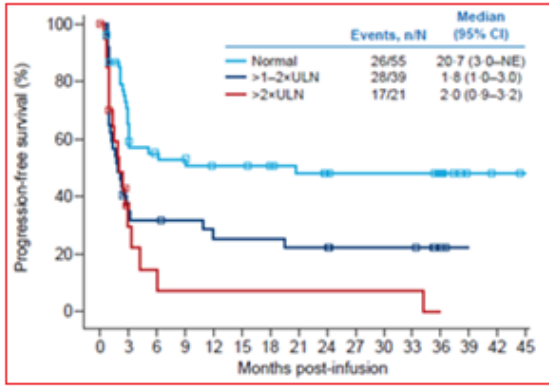
Predicting CAR-T Outcome before Treatment: LDH vs DL-Image Analysis

JULIET trial: Response rates and survival by pre-treatment serum LDH[1]

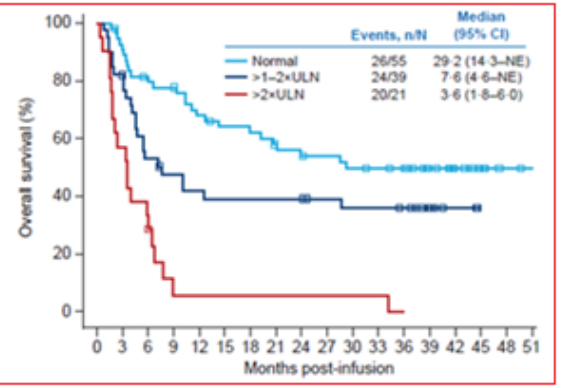
Overall response rates by LDH[1]



Progression-free survival by LDH [1]



Overall survival by LDH [1]



Pretreatment Serum LDH ≥2 x Upper Limit of Normal Predicts CAR T Failure				
Input: LDH ≥ 2 x ULN **n=67** (cohort evaluated by image analysis)	**Sensitivity**	**Specificity**	**Positive Predictive Value**	**Negative Predictive Value**
	17%	**100%**	**7%**	**76%**

Deep Learning-Based Image Analysis Predicts CR from Pretreatment PET/CT, ≥60% Rule[2]				
Input: PET + LD-CT, 3 slices each per lesion **n=68**	**Sensitivity**	**Specificity**	**Positive Predictive Value**	**Negative Predictive Value**
	77%	**49%**	**37%**	**85%**

[1]Schuster SJ, et al. *Lancet Oncol*. 2021;22(10):1403-1415. [2] Schuster SJ, et al. Presented at: 18th ICML; 2025.

Key Learning Points

- First-line immunochemotherapy cures about 65% of patients with DLBCL; ~30% of patients with refractory or relapsed DLBCL can be cured by 2nd- or 3rd-line therapies
- CAR T therapy (axi-cel or liso-cel) is superior to ASCT as 2nd-line therapy of DLBCL
- Non-relapse mortality accounts for about 6% of CAR T failures; so, most failures are due to failure of CAR T therapeutic efficacy
- To date, studies support the potential safety and efficacy of allogeneic CAR T cells for B-cell lymphomas
  - Longer follow-up is needed to establish durability of complete responses
  - Larger multicenter clinical trials are needed to confirm early results
- ctDNA MRD testing, included in NCCN guidelines for PET-positive DLBCL patients post-first-line treatment, can be an alternative to biopsy; while not formally FDA-approved for the post-CAR T setting, MRD monitoring is incorporated into expert consensus guidelines for monitoring of patients post-CAR T therapy
- Prediction of CAR T treatment outcome from pretreatment images using a machine learning method seems feasible, but is a “work-in-progress”
  - Incremental learning using additional patient information (eg, LDH, histology, etc.), together with radiomic features from deep learning, should enhance the predictive accuracy of our model

Crawshay P [www.vastphotos.com]. Last updated October 14, 2019.
https://vastphotos.com/photo/philly-nights-by-phil-crawshay.