Risk of Second Primary Tumors and T-cell lymphoma after CAR-T cell therapy

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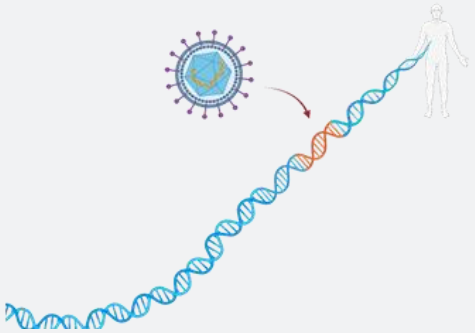


Penn Medicine
Abramson Cancer Center

Disclosures

Disclosures of Prof. Stephen J. Schuster

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

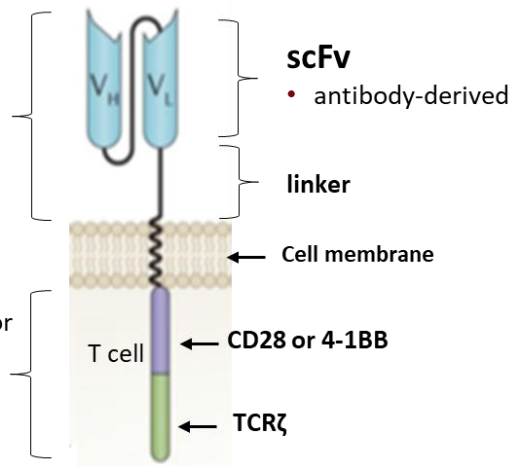
CAR-T cell = T cell with antigen specificity redirected by a chimeric receptor

Chimeric antigen receptor

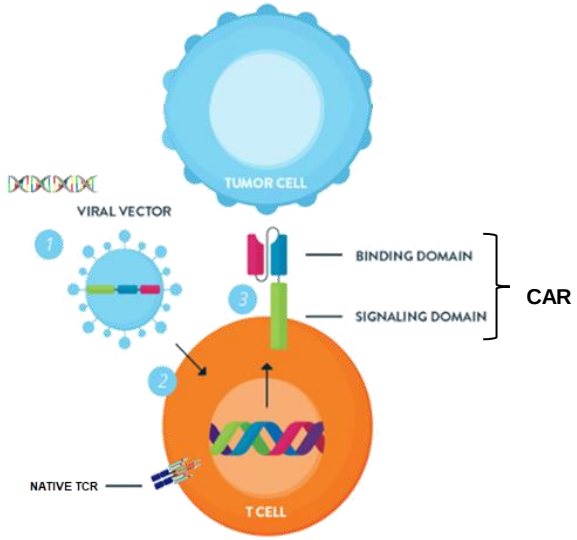
- composed of an extracellular antigen-binding domain (*e.g.*, CD19-binding) and tandem intracellular costimulatory and CD3-ζ signaling domains

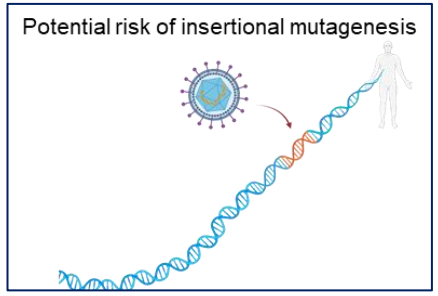
CD19 binding domain
- *e.g.*, FMC63

Fusion protein
- T cell costimulatory receptor signaling domain
- TCRζ activation domain

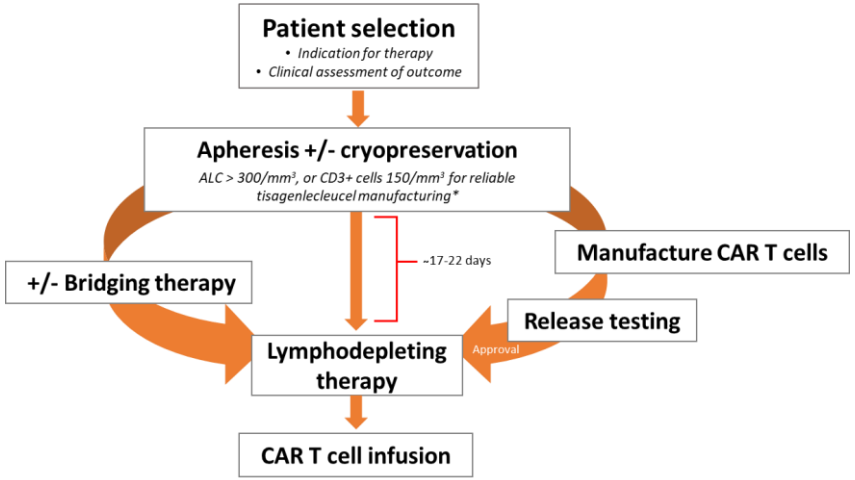


Transduction





Therapy

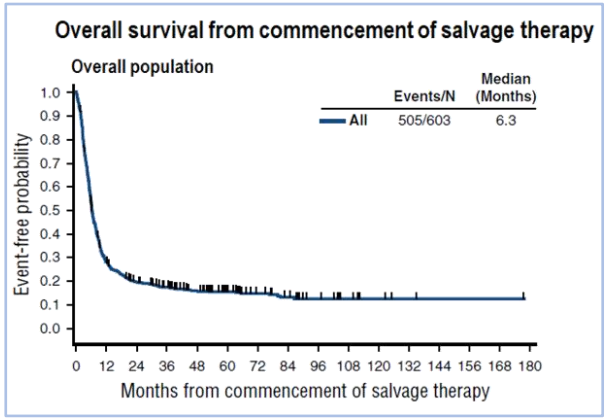


SJS 2021

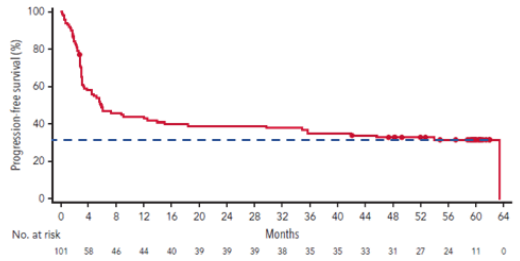
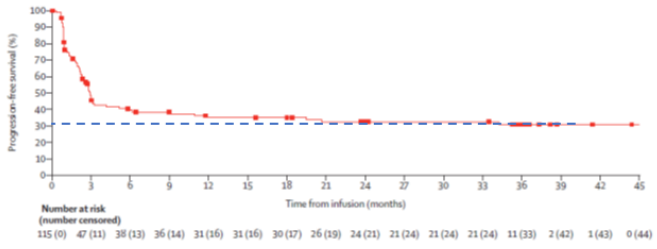
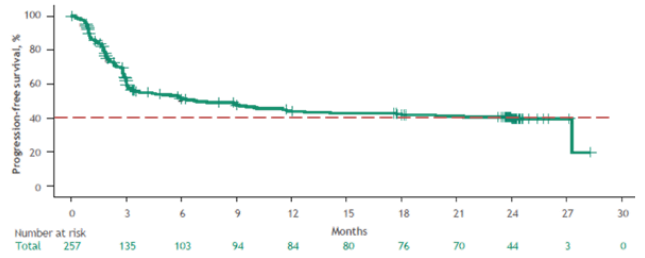
CD19-directed CAR-T saves the lives of many lymphoma patients

- **Relapsed/refractory large B-cell lymphoma outcomes *before* CAR-T**
  - **SCHOLAR-1 study[1]: an international multicenter, retrospective study**
  - **Data from 2001-2014 (N = 636)**
  - **Inclusion: progressive or stable disease (after ≥ 4 cycles of 1st-line and/or 2 cycles of later-line therapy) or relapse ≤ 12 months after ASCT**
    - **Response rates to next line of therapy: ORR = 26%; CR = 7% rate**
    - **Median overall survival = 6.3 months**

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



- **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***

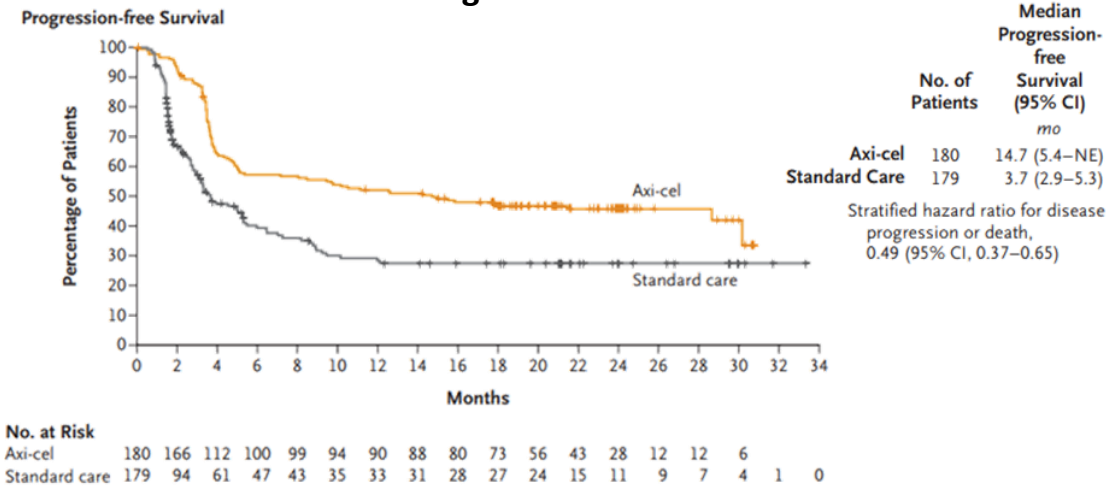
Axicabtagene ciloleucel[1]	Tisagenlecleucel[2]	Lisocabtagene maraleucel[3]
ZUMA-1[1]: axi-cel as ≥ 3rd-line therapy for LBCL N = 101 Median follow-up: 63.1 months **Estimated 5-year EFS: 30.3% (95% CI, 21.5-39.6)**	**JULIET**[2]: tisa-cel as > 3rd-line therapy for LBCL N = 115 Median follow-up: 40·3 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.

[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.

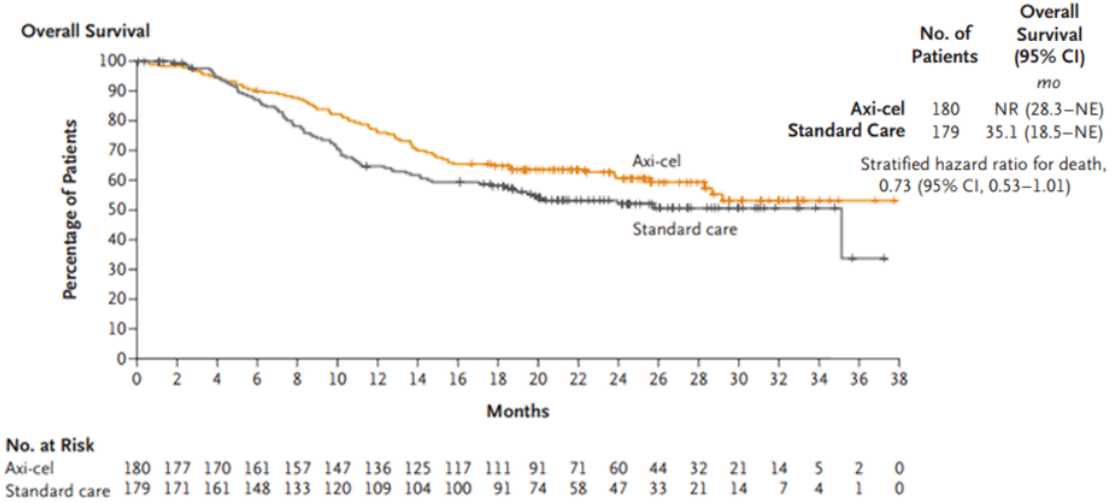
CAR-T is now the standard of care for 2nd-line therapy of refractory or relapsed large B-cell lymphomas

Axicabtagene ciloleucel vs SOC



	No. of Patients	Median Progression-free Survival (95% CI)
		mo
Axi-cel	180	14.7 (5.4–NE)
Standard Care	179	3.7 (2.9–5.3)

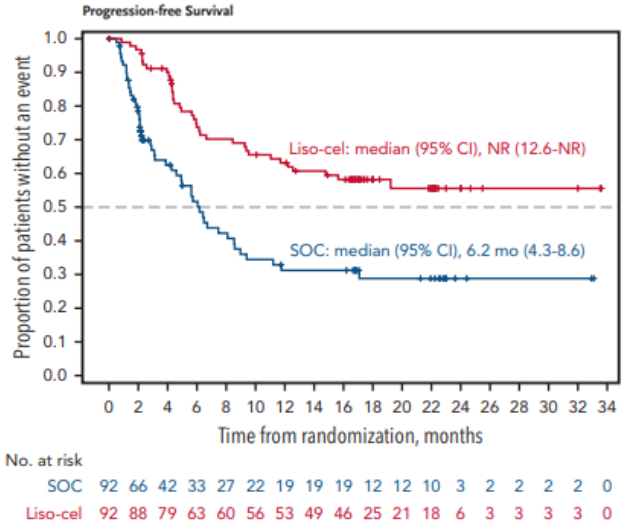
Stratified hazard ratio for disease progression or death, 0.49 (95% CI, 0.37–0.65)



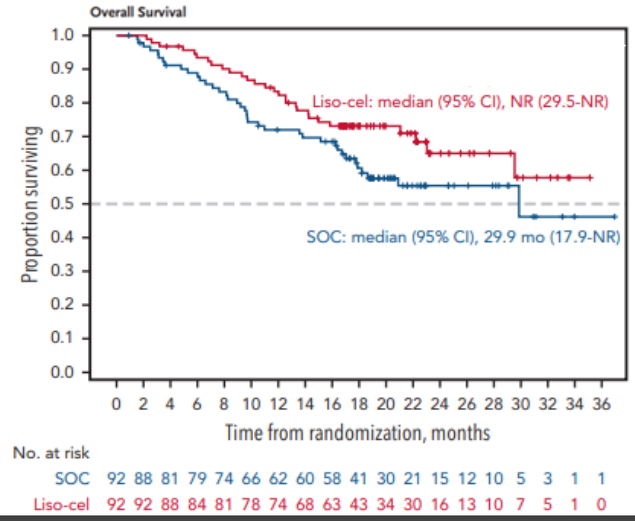
	No. of Patients	Median Overall Survival (95% CI)
		mo
Axi-cel	180	NR (28.3–NE)
Standard Care	179	35.1 (18.5–NE)

Stratified hazard ratio for death, 0.73 (95% CI, 0.53–1.01)

Lisocabtagene maraleucel vs SOC



Stratified HR, 0.400; 95% CI, 0.261-0.615; $P < .0001$



Stratified HR, 0.724; 95% CI, 0.443-1.183; $P = .0987$

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

On November 28, 2023, FDA announced investigation into T-cell lymphomas after CAR T therapies

FDA U.S. FOOD & DRUG ADMINISTRATION

FDA Investigating Serious Risk of T-cell Malignancy Following BCMA-Directed or CD19-Directed Autologous Chimeric Antigen Receptor (CAR) T cell Immunotherapies

22 cases of T-cell lymphoma occurring post-CART immunotherapies reported.
3/22 were CAR positive.

https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/fda-investigating-serious-risk-t-cell-malignancy-following-bcma-directed-or-cd19-directed-autologous

Non-relapse mortality after CAR-T for B-cell lymphoma & multiple myeloma

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

Clinical trial and real-world studies (46 studies; N = 7,604)

Large B-cell lymphoma

Axi-cel *n* = 3230, 16 studies	7.4%
Tisa-cel *n* = 729, 7 studies	4.1%
Liso-cel *n* = 422, 3 studies	3.8%

Multiple myeloma

Cilta-cel *n* = 328, 4 studies	15.2%
Ide-cel *n* = 699, 7 studies	6.3%

Indolent B-cell lymphomas

Axi-cel *n* = 168, 2 studies	5.7%
Tisa-cel *n* = 97, 1 study	

Mantle cell lymphoma

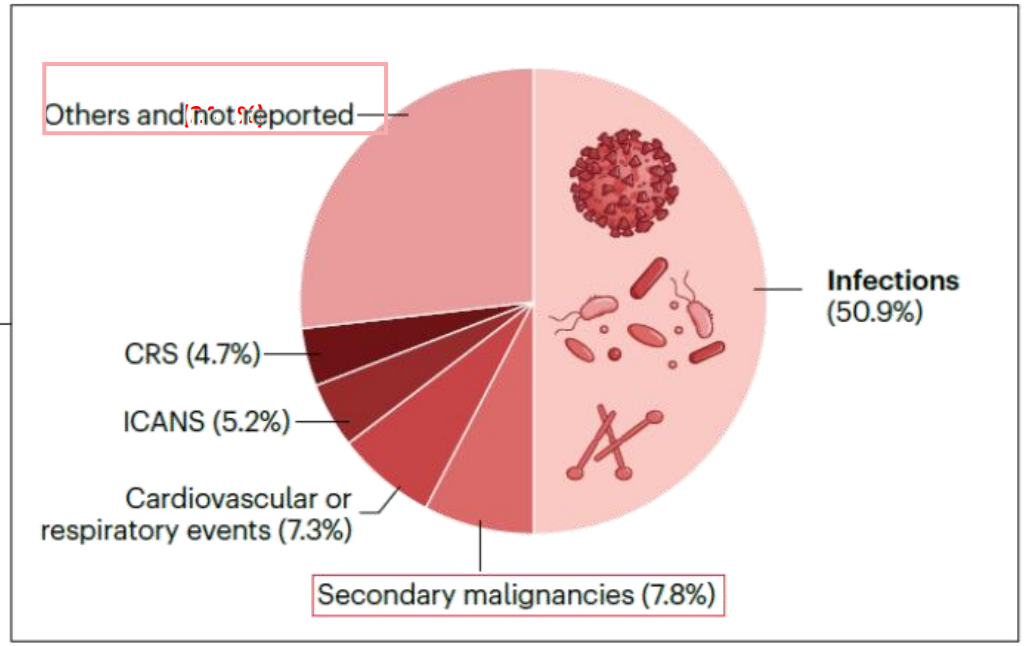
Brexu-cel *n* = 340, 5 studies	10.6%

Non-relapse mortality (6 CAR-T products)

NRM point estimates across diseases:

mantle cell lymphoma (10.6%) > multiple myeloma (8.0%) > large B-cell lymphoma (6.1%) > indolent lymphomas (5.7%)

Top five non-relapse-related causes of death



Cordas dos Santos et al, Nat Med 2024;30: 2667.
Blumenberg and Maus, Nat Med 2024;30: 2413.

UPenn CTL019 (*aka tisagenlecleucel)*: Secondary cancers at 5 years

The NEW ENGLAND JOURNAL of MEDICINE

CORRESPONDENCE | VOL. 384 NO. 7 | FEB 18, 2021

Five-Year Outcomes for Refractory B-Cell Lymphomas with CAR T-Cell Therapy

E.A. Chong, M. Ruella, and S.J. Schuster | N Engl J Med 2021;384:673-674

Figure S1: Patient allocation

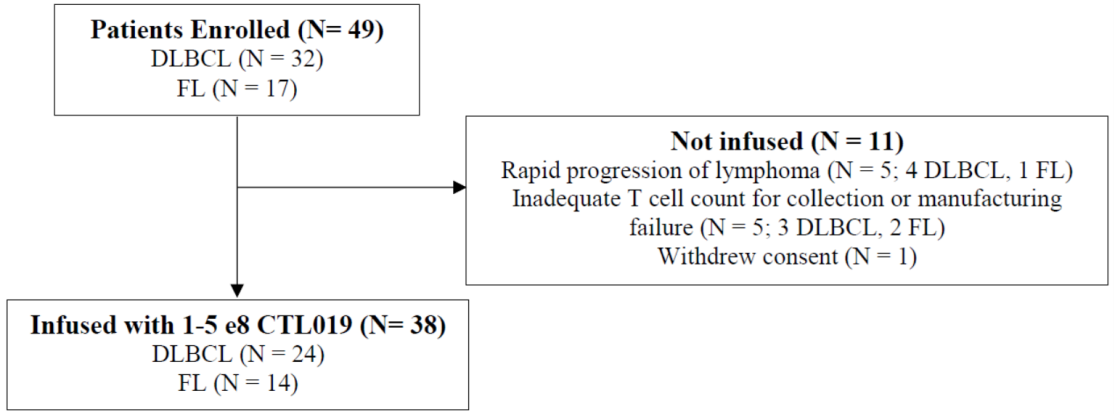


Table S6: Secondary cancers

All Infused Patients	
	Patients, N (%)
Secondary cancers	6/38 (16)
Non-small cell lung carcinoma[1]	2/38 (5)
Acute myeloid leukemia[2]	1/38 (3)
Myelodysplastic syndrome[3]	1/38 (3)
Prostate cancer	1/38 (3)
Melanoma	1/38 (3)

Clinical histories for selected patients:

[1] Both patients were former smokers with 22 pack-year and 40 pack-year smoking histories.
[2] 79-year-old man with past medical history of follicular lymphoma transformed to high grade B-cell lymphoma in remission after CTL019, who developed acute myeloid leukemia. He had previously received COPP + radiation (remission since 1977, relapsed 15 years later), CHOP, fludarabine, eight cycles of BR, clinical trial of heat shock protein (PU-H17), and three cycles of EPOCH.
[3] 68-year-old woman with past medical history of follicular lymphoma transformed to diffuse large B-cell lymphoma in remission after CTL019, who developed myelodysplastic syndrome. She initially received six cycles of CHOP (followed by a four-year remission), then rituximab monotherapy for several relapses. She developed transformed follicular lymphoma and received three cycles of R-ICE (stable disease), CUDC-907 on clinical trial (progressive disease), and lenalidomide/rituximab (mixed response) prior to CTL019.

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma

Secondary primary cancers after CAR-T: the UPenn experience

Study cohort: adult patients (**N = 449**) treated with commercial CAR T for NHL, MM and ALL between January 2018 and November 2023 at the University of Pennsylvania

Characteristics		All cases 449 (100%)	No second cancer 433 (96.4%)	Second cancer 16 (3.6%)	p
Sex	Female	162 (36.1%)	159 (36.7%)	3 (18.8%)	0.142
Age at CART infusion	>65	179 (39.9%)	170 (39.3%)	9 (56.2%)	0.173
Diagnosis	NHL MM ALL	317 (70.6%) 125 (27.8%) 7 (1.6%)	304 (70.2%) 122 (28.2%) 7 (1.6%)	13 (81.2%) 3 (18.8%) 0 (0.0%)	0.601
# of previous lines of therapies	>3	243 (54.1%)	232 (53.6%)	11 (68.8%)	0.232
Previous autologous SCT	Yes	158 (35.2%)	153 (35.3%)	5 (31.2%)	0.737
Product infused	Axi-cel	69 (15.4%)	65 (15.0%)	4 (25.0%)	0.412
	Tisa-cel	189 (42.1%)	182 (42.0%)	7 (43.7%)	
	Liso-cel	32 (7.1%)	32 (7.4%)	0 (0.0%)	
	Brexu-cel	34 (7.6%)	32 (7.4%)	2 (12.5%)	
	Ide-cel	67 (14.9%)	64 (14.8%)	3 (18.8%)	
	Cilta-cel	58 (12.9%)	58 (13.4%)	0 (0.0%)	
Previous neoplasm	Yes	75 (16.7%)	70 (16.2%)	5 (31.2%)	0.112

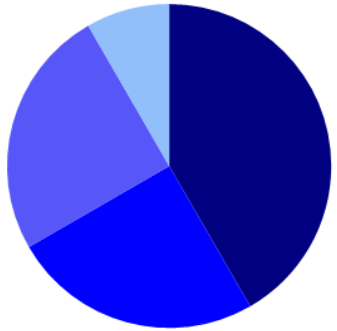
NHL, non-Hodgkin lymphoma; MM, multiple myeloma; ALL, acute lymphoblastic leukemia

Ghilardi et al. Nature Med. 2024;30:984–989.

Types and risk of secondary cancers after CAR-T: the UPenn experience

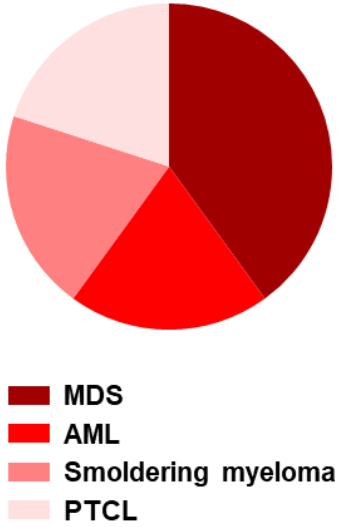
Solid neoplasms

N=12/449 (2.7%)

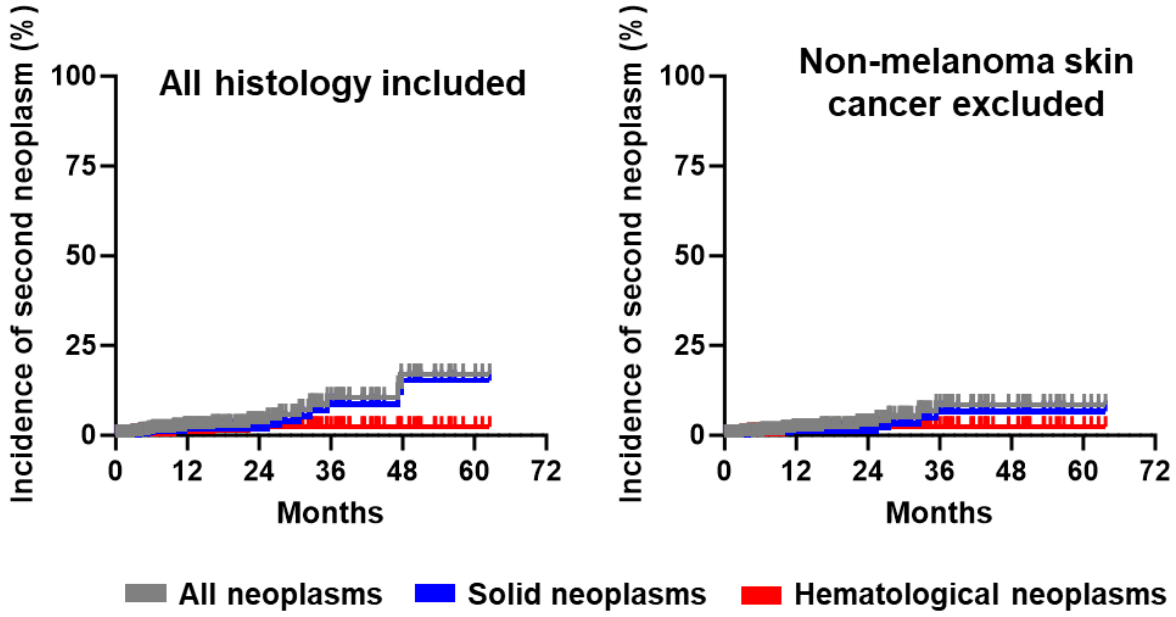


Hematological neoplasms

N=5/449 (1.1%)



Cumulative incidence of second cancers

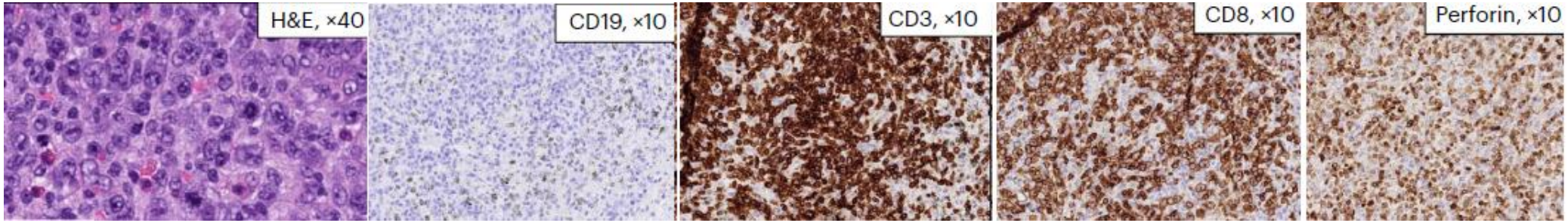


The projected 5-year cumulative incidence is 15.2% for solid and 2.3% for hematological malignancies

Secondary cancers after CAR-T: the UPenn experience

Among 16 second cancers observed after commercial CAR-T, a single case of T-cell lymphoma occurred 3 months after infusion of axicabtagene ciloleucel for B-cell 'grey zone' lymphoma

Pathologic diagnosis: CD8+ peripheral T-cell lymphoma NOS with cytotoxic phenotype



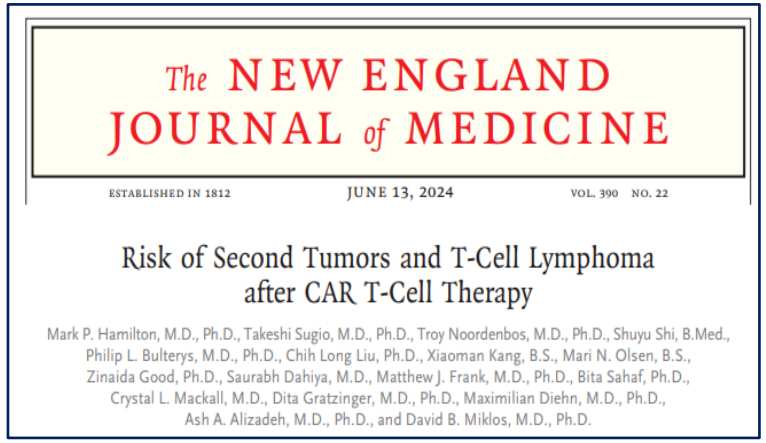
Molecular studies: JAK3 variant of uncertain significance, (p.D640Efs*30) with VAF 11%, not present in the B-cell lymphoma, was detected; a clonal TRG rearrangement was identified in the T-cell but not the original B-cell lymphoma.

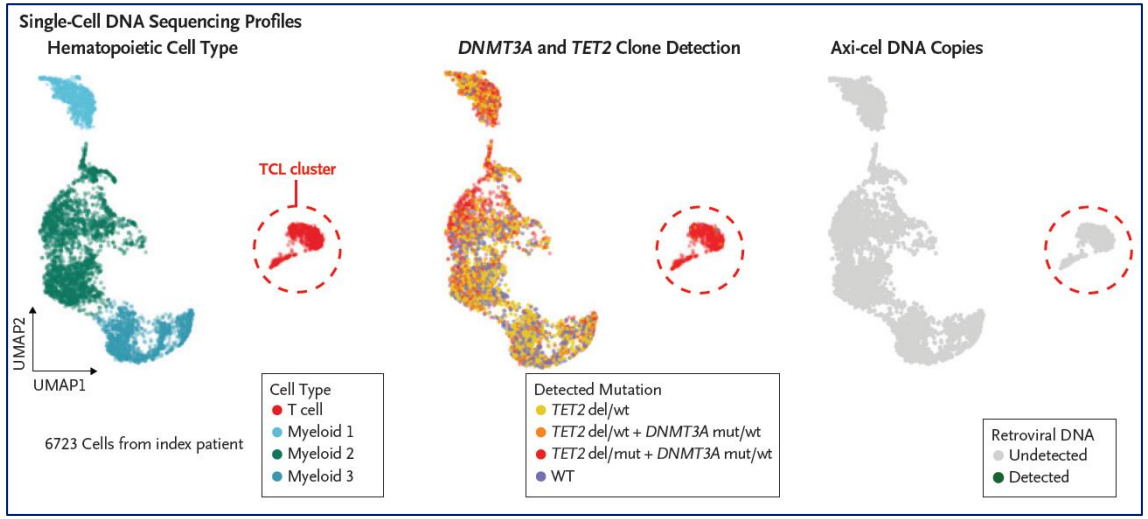
- qPCR revealed very low CAR transgene copies (8 copies/µg DNA or approximately 0.005% of cells), suggesting blood contamination or infiltrating CAR-T cells rather than CAR+ malignant T-cells.
- In diagnostic T-cell lymphoma tissue, a TRG clone represented approximately 20% of total TRG sequencing reads.
- This TRG clonotype was also detected reproducibly at very low copy number in pre-CAR T blood (less than 0.01% of total TRG sequencing reads; confidence >99% at 1 × 10−3)

Ghilardi et al. Nature Med. 2024;30:984–989.

Secondary cancers after CAR-T: the Stanford experience

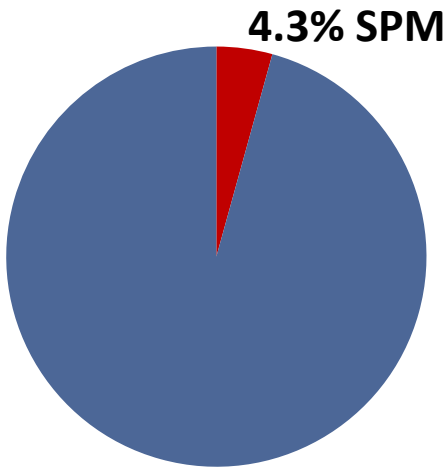
- 724 patients received T-cell therapies at Stanford between 2016-2023
- Second primary malignancies were observed in 25 cases (3.5%)
  - 11 solid tumors (44% of SPM; 1.5% of all pts)
  - 14 hematologic, 13 MDS/AML, 1 TCL (56% SPM; 1.9% all)
- Lethal T-cell lymphoma was identified in a single patient who received axi-cel for diffuse large B-cell lymphoma
- Both lymphomas were deeply profiled
  - each had molecularly distinct immunophenotypes and genomic profiles, but both were positive for EBV and associated with *DNMT3A* and *TET2* mutant clonal hematopoiesis
- **No evidence of oncogenic retroviral integration found using of multiple techniques.**



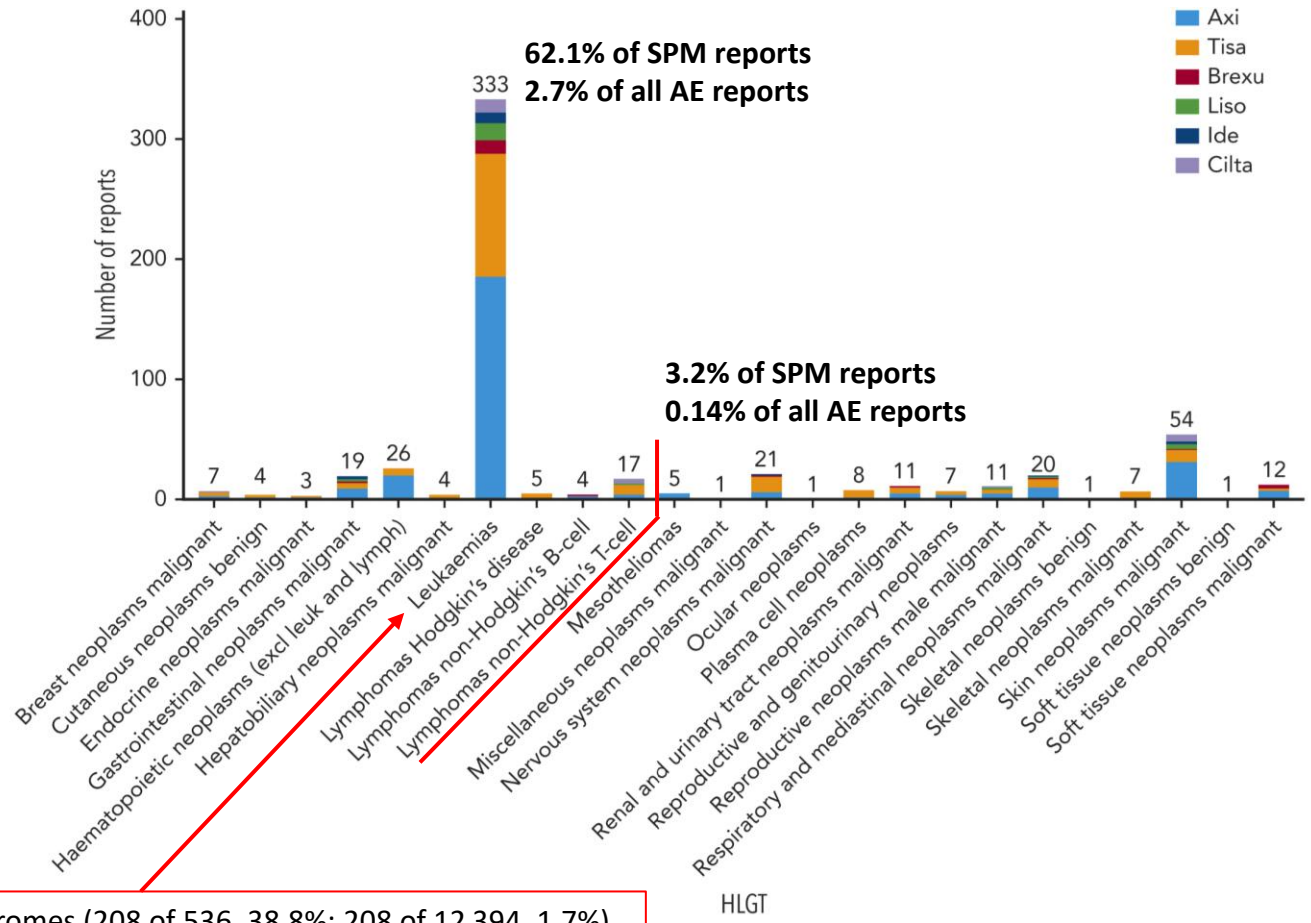


Secondary cancers after CAR-T: FDA Adverse Events Reporting System (FAERS) database

12,394 unique CAR-T AE reports



536 (4.3%) SPM reports



"Leukemias" include myelodysplastic syndromes (208 of 536, 38.8%; 208 of 12 394, 1.7%), acute myeloid leukemias (106 of 536, 19.8%; 106 of 12 394, 0.9%), and 2 cases of T-cell large granular lymphocytic leukemia

Secondary cancers after CAR-T: Systematic review and meta-analysis

326 SPMs in 5,517 (6.0%) patients from 18 clinical trials and 7 real-world studies

- hematologic malignancies (37%), solid tumors (27%), non-melanoma skin cancers (16%), T-cell malignancies (1.5%)

Overall SPM point estimate was 6.0% (95% CI, 4.8%-7.4%) at 21.7 months median follow-up.

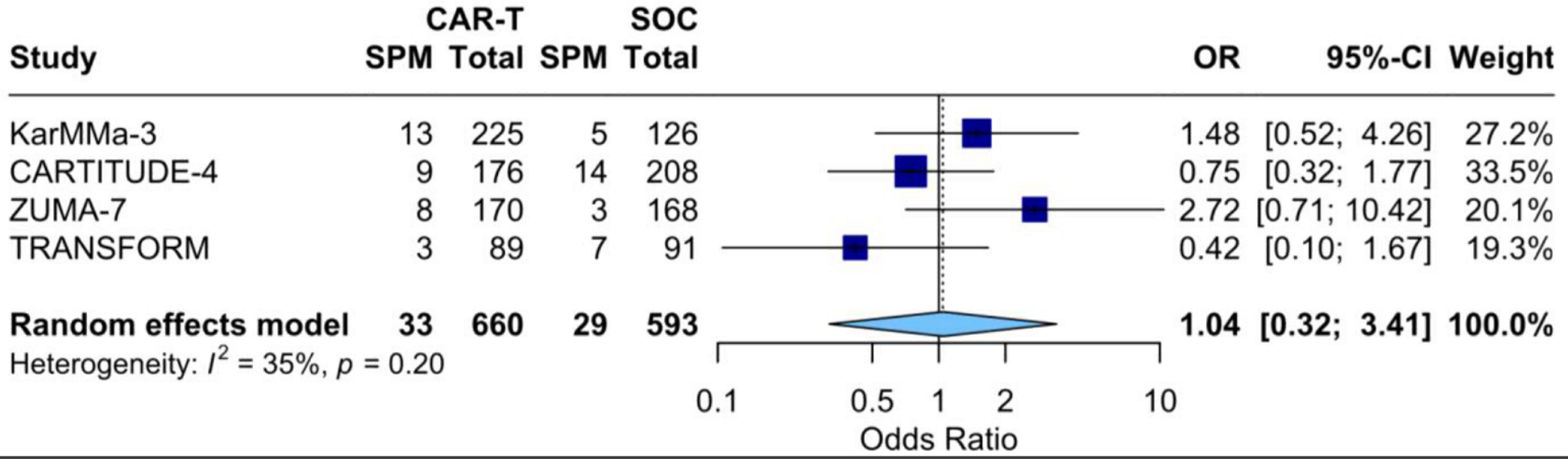
- point estimate for T-cell SPM across the entire population was 0.09% (95% CI, 0.04%–0.2%)

Data from 4 randomized trials evaluable for SPMs emergence after CAR-T

- BCMA-CART: KarMMa-3 (ide-cel) and CARTITUDE-4 (cilta-cel)
- CD19-CART: ZUMA-7 (axi-cel) and TRANSFORM (liso-cel)

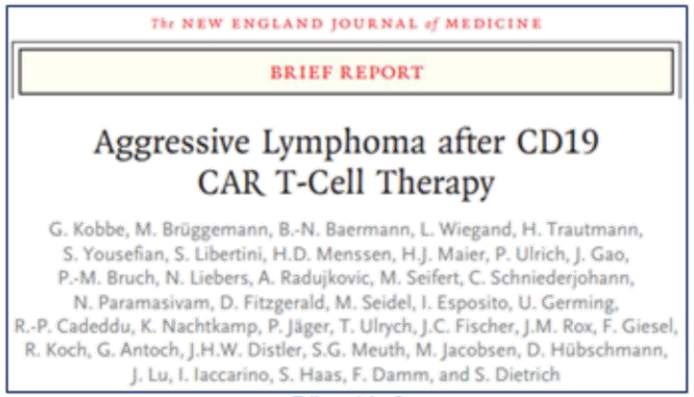
] 1,253 patients

- Pooled SPM frequency in randomized CAR-T arms was 5.0% (95% CI 3.6-6.9%).
- Pooled SPM frequency in randomized SOC arms was 4.9% (95% CI 3.4-6.9%).

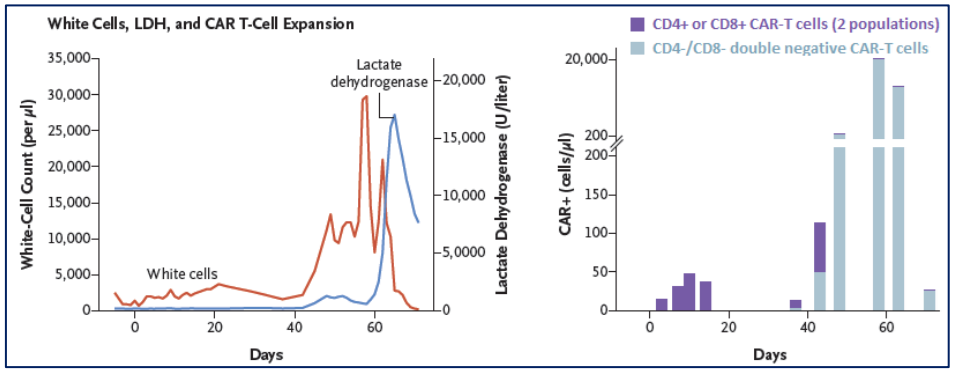


Aggressive CAR+ T-cell lymphoma after CAR-T: Contribution of clonal hematopoiesis

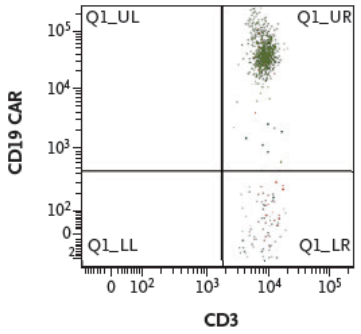
Case History: 60-year-old man developed a fatal, clonal, autonomously proliferating double CD4-/CD8- CAR+ peripheral T-cell lymphoma ~1 month after tisa-cel for relapsed primary CNS lymphoma (PCNSL). A PBSC product was collected ~ 7 months prior to CAR-T and stored.



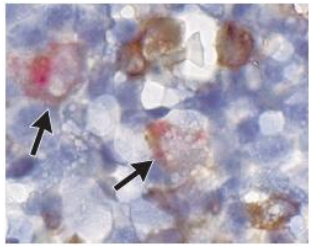
Clinical/laboratory course:



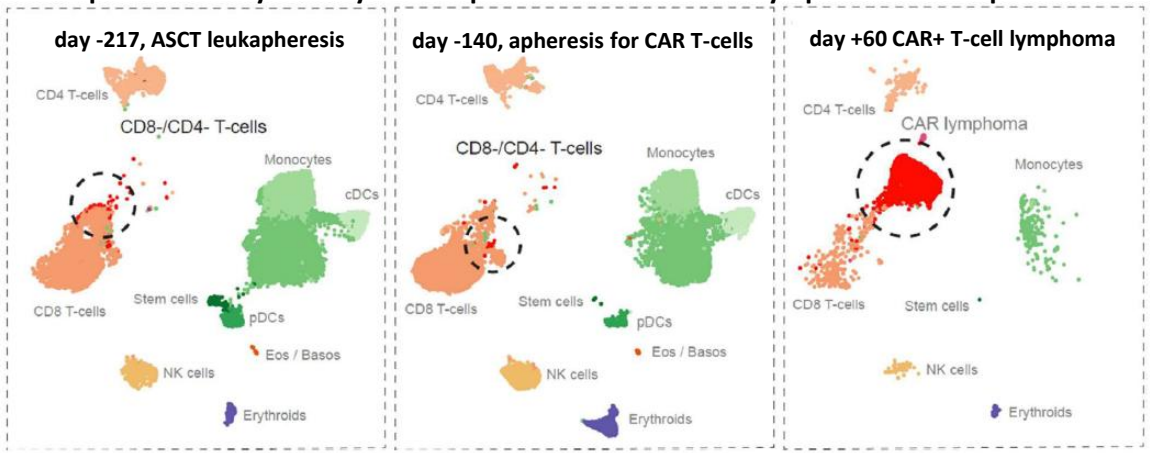
Analysis: Immunophenotype of CAR+ T-cell lymphoma: **CD19-CAR+ double negative (CD4- CD8-) peripheral T-cell lymphoma NOS with co-expression of CD2, CD3, CD5, CD7, CD38, granzyme B, CD26, CD28, HLA-DR, and TCR α/β**



Combination CD3 IHC and In Situ Hybridization of Anti-CD19 CAR (Day 52)



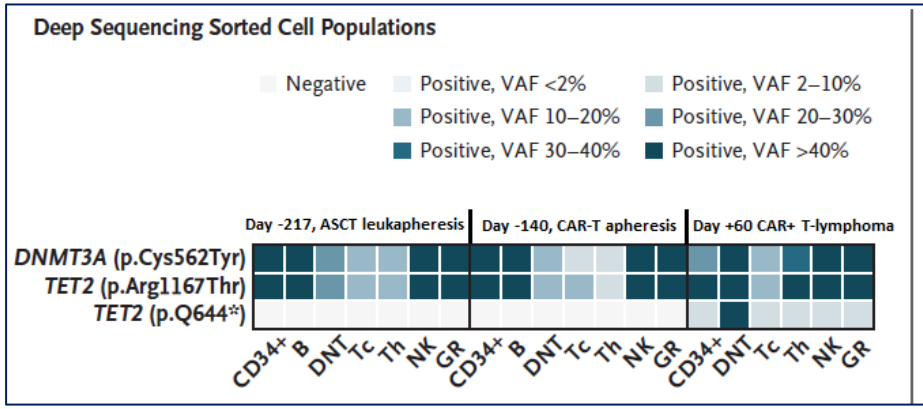
Spectral flow cytometry feature plots of the CAR+ T-cell lymphoma: 3 time points



Aggressive T-cell lymphoma after CAR-T

Analysis *(continued)*:

- Genomic characterization:



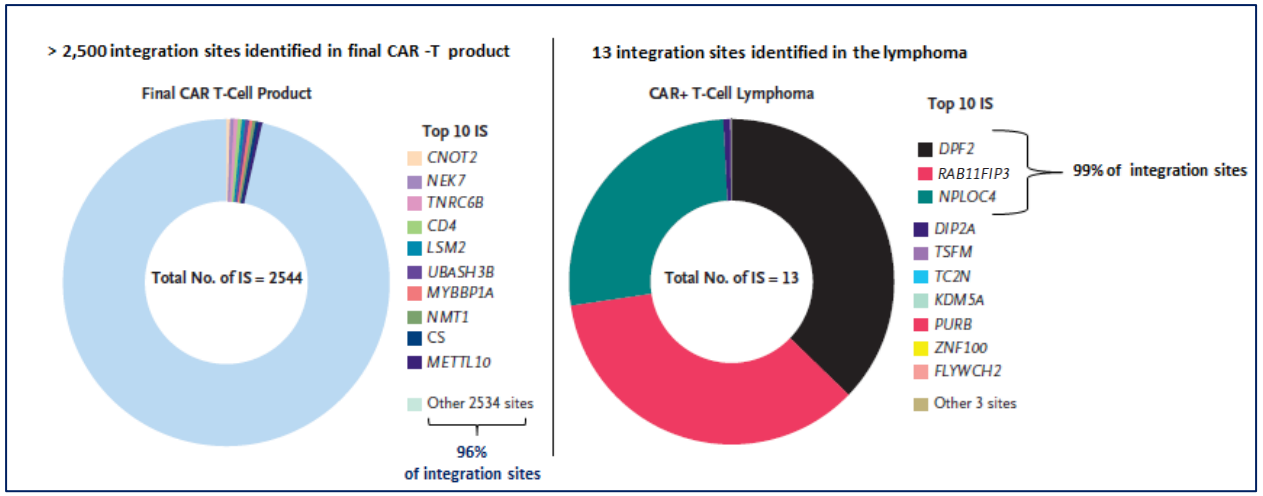
Duplex Sequencing for *TET2* Mutations

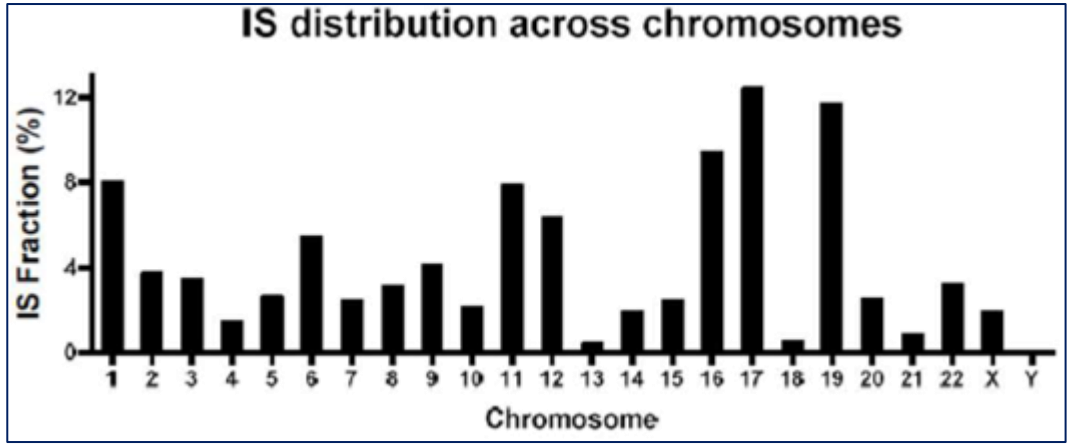
Sample	*TET2* (p.Q644*)	*TET2* (p.Arg1167Thr)
CAR T-cell apheresis (Day -140)	0.0012%	22.2%
Final CAR T-cell product	0.02%	21.3%
healthy donor	ND	ND
DNA control	ND	ND

- T-cell receptor sequencing and clonality analysis:

Target	Sequence	ASCT apheresis	CAR T-cell apheresis	Blood	Blood	Bone Marrow	Blood
	Day	-217	-140	7	14	51	56
TRB	V27 -2/17/-0 J2-7	0.00%	0.00%	0.00%	0.13%	80.56%	91.53%
TRB	V20-1 -0/15/-7 J2-2	0.58%	0.41%	5.54%	4.23%	2.04%	0.48%
TRB	V20-1 -6/11/-5 J2-1	0.49%	0.30%	6.40%	5.53%	0.50%	0.12%
TRB	V20-1 -3/11/-5 J1-3	1.73%	40.71%	3.99%	5.76%	0.21%	0.48%
TRB	V11-2 -5/11/-1 J1-1	0.81%	6.44%	0.21%	0.26%	0.00%	0.00%
TRG	V10 -7/3/-8 JP1	0.21%	0.20%	0.36%	0.54%	54.62%	54.85%
TRG	V2 -0/4/-6 JP2	0.00%	0.00%	0.00%	0.15%	32.63%	36.40%
TRG	V3 -3/9/-2 J1=J2	2.83%	4.14%	12.66%	15.48%	0.67%	0.33%
TRG	V4 -4/5/-3 J1=J2	1.48%	2.69%	8.31%	10.80%	0.44%	0.08%
TRG	V2 -2/6/-4 JP2	1.04%	8.56%	3.31%	2.63%	0.39%	0.04%
TRG	V8 -1/2/-2 JP2	1.10%	8.82%	4.43%	4.11%	0.28%	0.08%

- Vector integration site analysis:





Conclusion: Evidence suggests that clonal hematopoiesis contributed to lymphomagenesis

Bidirectional increased risk of B-cell lymphoma and T-cell lymphoma: SEER registry data (2000 – 2016)

Method

- Previously diagnosed BCL or TCL cohorts were followed over time to compare the observed incidence rate of subsequent lymphoma diagnoses to the number of lymphomas expected for the general population
- Expected new cases of lymphoma per year (both genders, age-adjusted): 18.6 per 100,000 (~ 0.02% per year)
- Standardized Incidence Ratio (SIR) = $\frac{\text{observed cases}}{\text{expected cases}}$

Sample size

First Lymphoma diagnosis (2000-2016)

- Total patients diagnosed (N = 312,225)
- B-cell lymphoma (n = 288,478)
- T-cell lymphoma (n = 23,747)

Results

TCL following BCL: SIR = 4.7 (95%CI 4.2-5.2)
BCL following TCL: SIR = 4.7 (95%CI 5 4.1-5.2)

PTCL-NOS following HL: SIR = 27.5
HL following PTCL-NOS: SIR = 31.6

AITL following DLBCL: SIR = 9.7
DLBCL following AITL: SIR = 15.3

SIRs were <5 for of TCL with CLL and FL

	Risk of TCL following BCL			
	No. of BCL patients	Observed no. of TCLs	SIR	(95% CI)
Total, N	288 478	354	4.7	(4.2, 5.2)
	Risk of BCL following TCL			
	No. of TCL patients	**Observed no. of BCLs**	**SIR**	**(95% CI)**
	23 747	300	4.7	(4.1, 5.2)

- increased risks were strongest within the first year following diagnosis but remained elevated even at ≥ 5 years
- ~5X higher risk than the general population is ~0.1% vs 0.02% per year

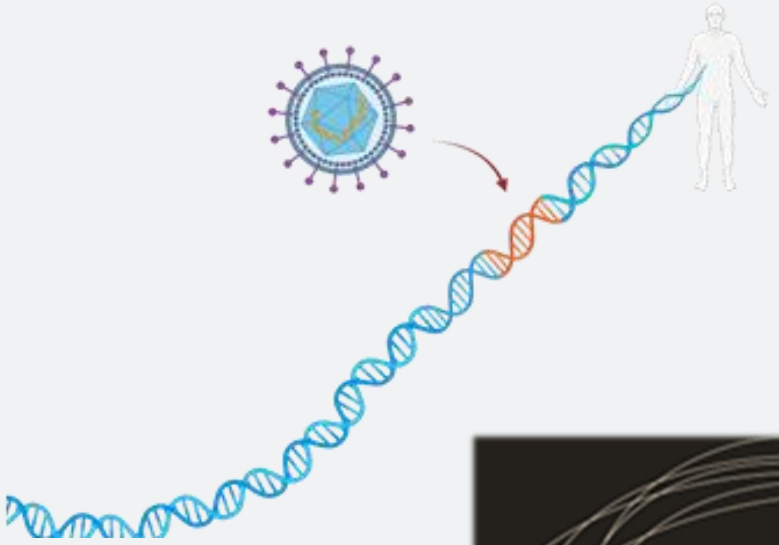
BCL, B-cell lymphoma; TCL, T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma not otherwise specified; HL, Hodgkin lymphoma; AILT, angioimmunoblastic T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma

Summary

- SPMs have been reported after CAR-T immunotherapy in ~5% of patients
- The incidence of SPMs after CAR-T does not appear higher than expected
  - At 30 years from primary cancer diagnosis, the cumulative incidence of all second cancers is 20.5% (95% CI 19.1%–21.8%)[1]
- T-cell lymphomas as SPMs are rare *after* CAR-T; reported cases of CAR+ TCLs are very limited
- Mechanisms of T-cell lymphoma development as a SPM may involve antecedent clonal hematopoiesis
- T-cell lymphoma with an integrated vector transgene was not observed in more than 1,500 CAR-T patients analyzed in depth at Penn and Stanford
- A baseline bidirectional risk between B-cell and T-cell lymphomas exists; risk of T-cell after B-cell lymphoma ~5X higher than the general population (0.1% *vs* 0.02%)
- Caution is warranted if underlying cancer susceptibility mutations, such as clonal hematopoiesis, or other drivers of lymphoproliferation, such as EBV, are present
- Remember the “Immortal Time Bias”, *i.e.*, patients can only develop SPM malignancies if they do not first die of their primary cancer

[1]https://doi.org/10.14694/EdBook_AM.2014.34.e57

Risk of Second Primary Tumors and T-cell lymphoma after CAR-T cell therapy



Grazie Molto!
Many Thanks!