Next Gen CAR-T

Stephen J. Schuster, M.D.

University of Pennsylvania, Philadelphia, PA, USA

Penn Medicine
Abramson Cancer Center

Disclosures of Prof. Stephen J. Schuster

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
AbbVie						X	
AstraZeneca						X	
BeiGene						X	
Caribou Biotech						X	Steering committee
Fate Therapeutics							Safety DSMB
Genentech/Roche	X					X	Steering committee
Genmab	X					X	Steering committee
Incyte/Morphosys						X	Honoraria for presentation
Kite Pharmaceuticals						X	
Legend Biotech						X	Steering committee
Novartis						X	Steering committee
Mustang Biotech						X	
Nordic Nanovector						X	Steering committee
Takeda							Honoraria for presentation

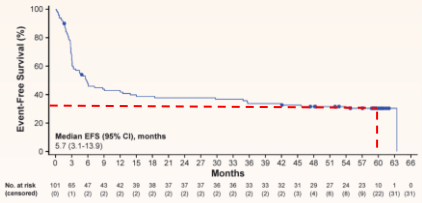
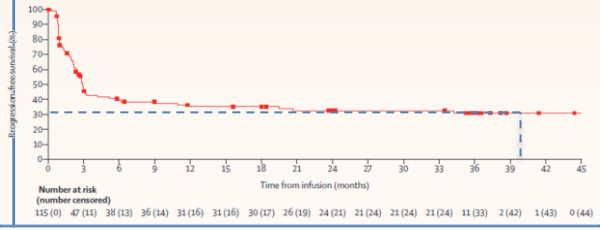
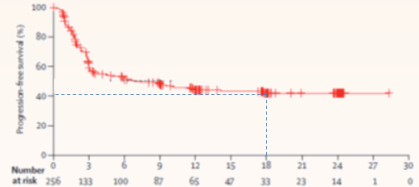
Who needs a new CAR?

Topics

- Large B-cell lymphomas (LBCL)
  - an unmet need remains
  - defining those LBCL patients with an unmet need
  - improving second generation anti-CD19 CAR-T products
  - overcoming tumor-specific mechanisms of resistance
- Other lymphomas require new targets
  - T-cell lymphomas

Large B-cell lymphomas: the remaining unmet need

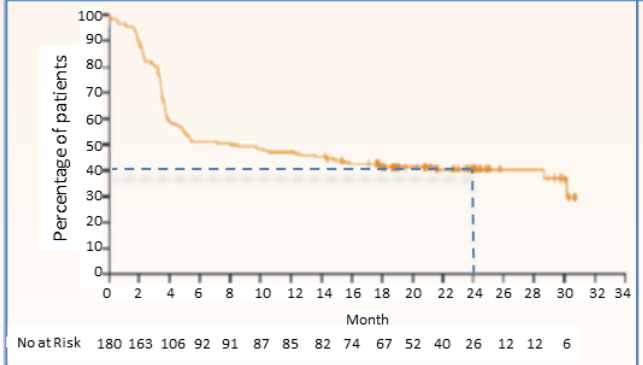
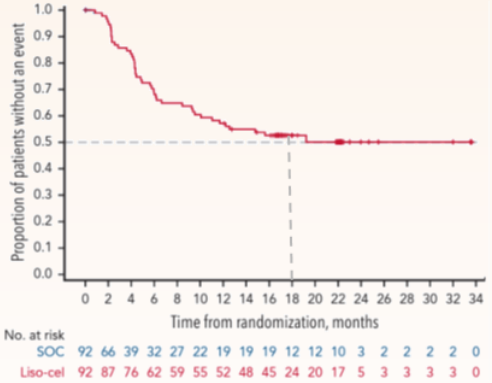
~ 2/3 of patients fail to achieve durable responses with commercially available CAR-T products as 3rd-line therapy

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%**	**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 = 256 Median follow-up: 12.3 months **Estimated 18-month PFS: 42.1%**
		

[1]Neelapu SS, et al. Blood. 2023;Epub ahead of print; [2]Schuster SJ, et al. Lancet Oncol 2021;22(10):1403-1415; [3]Abramson J, et al. Lancet. 2020;396(10254):839-852.

Large B-cell lymphomas: the remaining unmet need

As 2nd-line therapy, ~1/2 of patients will have disease progression or need new lymphoma treatment by 2 years after available CAR-T products

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.

The question is,

How can we improve these results?

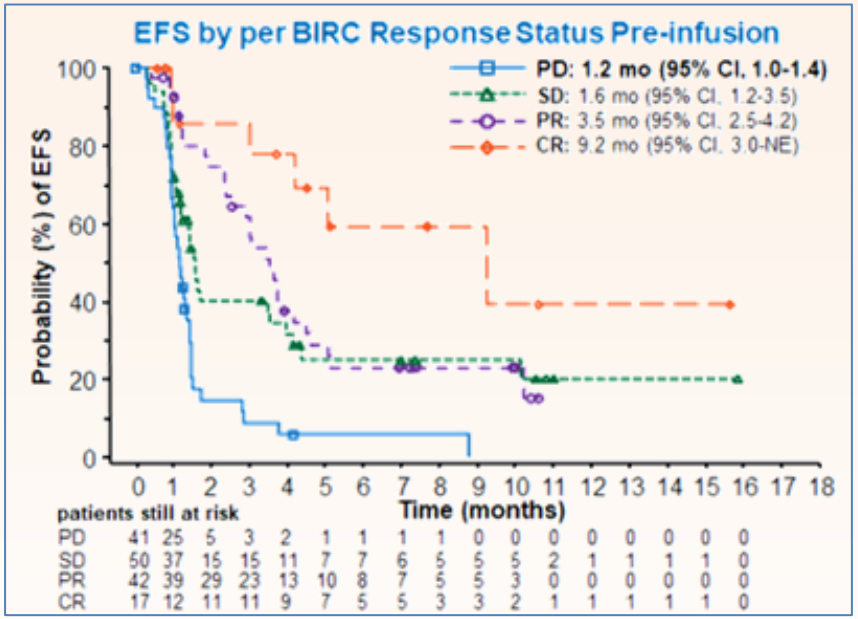
The easy answer is,

Treat patients who are likely to respond

and treat those destined to fail on clinical trials.

Patient characteristics impacting outcome

- **Disease status at the time of CAR-T infusion impacts best response post-infusion and EFS**
  - Data from the BELINDA trial: tisagenlecleucel vs SOC



Multivariate Logistic Regression Model for Post-Infusion Best Overall Response (CR/PR vs SD/PD/UNK) in Arm A (second-line CAR-T)

	Odds Ratio Estimates		
Variable	Point Estimate	95% Wald Confidence Limits	
CR/PR before infusion vs. SD/PD before infusion at mean cell dose	7.75	3.23	18.62

The odds ratio is the odds of having a best overall response of CR/PR vs. SD/PD/UNK; *i.e.*, an odds ratio >1 means patients are more likely to have a best overall response of CR/PR.

EFS time is relative to date of tisagenlecleucel infusion; median time from pre-infusion disease assessment to infusion was 10 days (range, 2-57; Q1-Q3, 8-15).
EFS events defined as PD/SD after day 71 from randomization or death at any time.

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

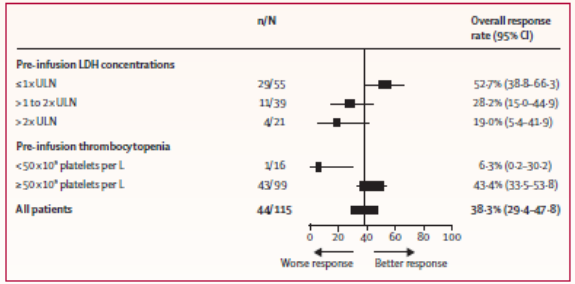
Patient characteristics impacting outcome

- **Pre-infusion LDH and platelet count impact CAR-T response and survival outcomes**
  - **Data from the JULIET trial: Phase 2 trial of tisagenlecleucel in r/r LBCL**

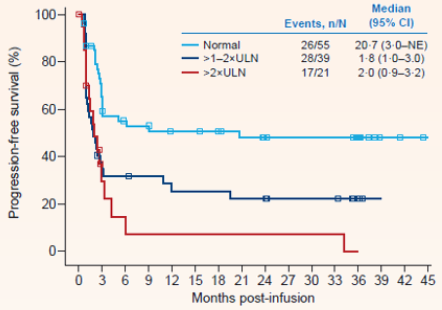
Multivariable analysis		
Predictive Factors from Univariable Analysis	**Responders/Patients**	**Odds Ratio (95% CI)**
LDH		
≤ x ULN	29/55	2.74 (0.71-10.56)
>2 x ULN	4/21	
>1 - 2 x ULN	11/39	0.97 (0.23-4.06)
>2 x ULN	4/21	
Thrombocytopenia		
CTCAE grades 0 - 2	43/99	7.23 (0.84-62.31)
CTCAE grades 3 - 4	1/16	

- Lab analytes are defined as the closest time before or on the day of infusion
  - 93% of values fell on the day of infusion
- Thrombocytopenia: grade 4, <25; grade 3, 25-50; grade 2, 50-75; grade 1, 75-LLN × 10^9/L

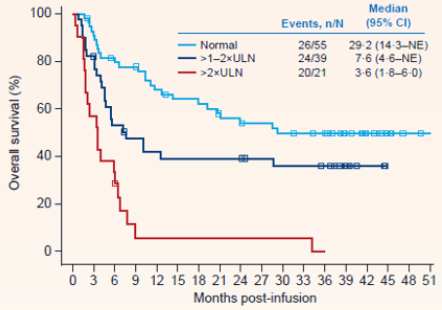
Overall response rates by LDH and platelet count



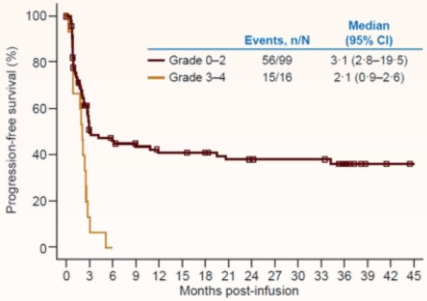
Progression-free survival by LDH



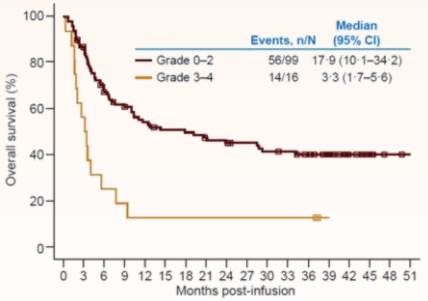
Overall survival by LDH



Progression-free survival by platelets



Overall survival by platelets

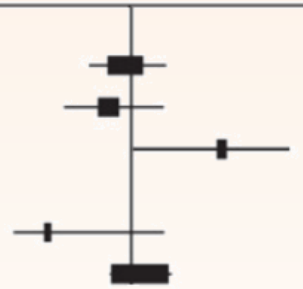


Schuster SJ, *et al*. Lancet Oncol. 2021;22(10):1403-1415.

Patient characteristics impacting outcome

- **Tumor bulk and its impact on response (“size matters”)**
  - **Data from the JULIET trial: Phase 2 trial of tisagenlecleucel in r/r LBCL**

	n/N	Overall response rate (95% CI)
Baseline tumour volume		
<100 mL	23/64	35·9% (24·3–48·9)
≥100 mL	11/36	30·6% (16·3–48·1)
Unknown	10/15	66·7% (38·4–88·2)
Bulky disease (> 10 cm)		
Yes	1/9	11·1% (0·3–48·2)
No	43/106	40·6% (31·1–50·5)

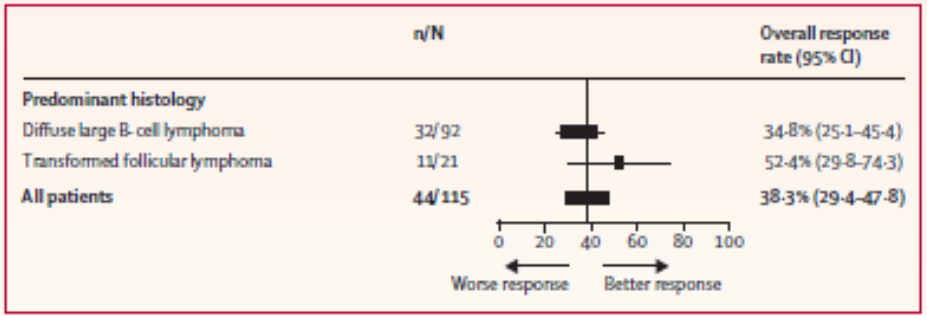


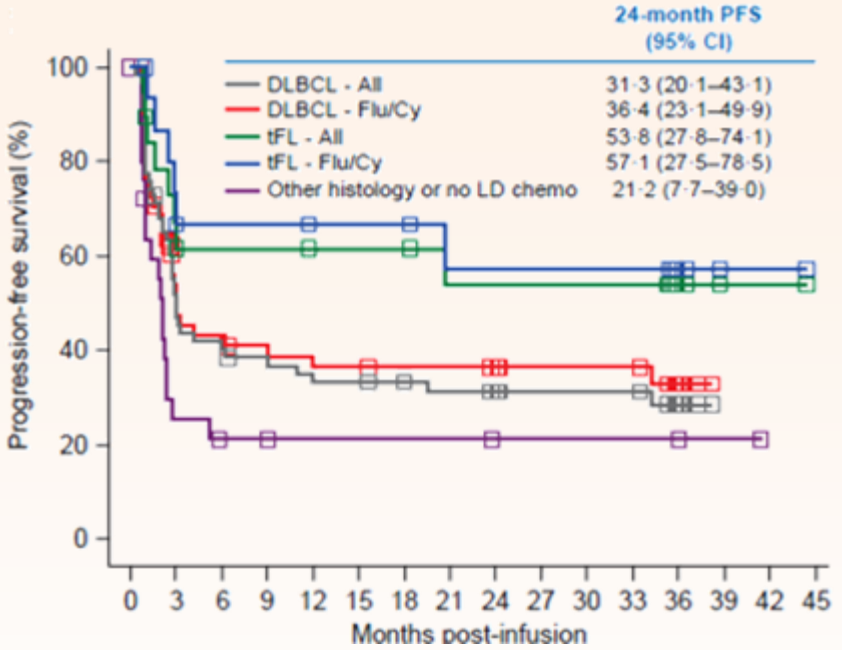
Schuster SJ, *et al*. Lancet Oncol. 2021;22(10):1403-1415.

Patient characteristics impacting outcome

- **Subtype of lymphoma impacts CAR-T response rates and progression-free survival**
  - **Data from the JULIET trial: Phase 2 trial of tisagenlecleucel in r/r LBCL**

Overall response rates by lymphoma subtype



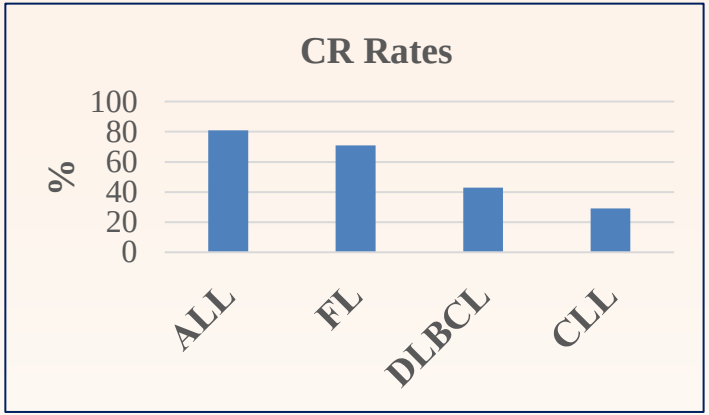


Schuster SJ, *et al*. Lancet Oncol. 2021;22(10):1403-1415.

Patient characteristics impacting outcome

- **Determinants of CAR-T success or failure are probably *disease-specific***
  - **Early CTL019 efficacy data from Penn and CHOP**

Disease	N	CR rate	Median DOR	Median Follow-Up
r/r ALL[1]	75	81%	Not Reached	13.1 mo (2.1-23.5)
r/r FL[2]	14	71%	Not Reached	28.6 mo (3.5-37.9)
r/r DLBCL[2]	14	43%	Not Reached	46.8 mo (6.0-54.6)*
r/r CLL[3]	14	29%	40.0 mo (21.0-53.0)	19.0 mo (6.0-53.0)

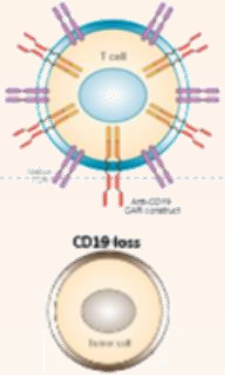


*Data updated December 2018

[1]Maude S, *et al*. NEJM. 2018;378(5): 439-448; [2]Schuster SJ, *et al*. N Engl J Med. 2017;377(26):2545-2554; [3]Porter DL, *et al.* Sci Transl Med. 2015; 7(303): 1-12.

Mechanisms of resistance to CAR-T

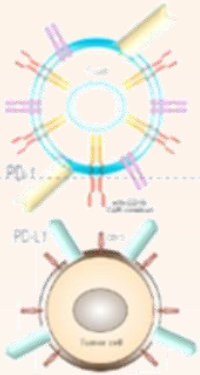
- **Putative mechanisms of tumor resistance to CAR T cells in DLBCL**



CD19 antigen loss

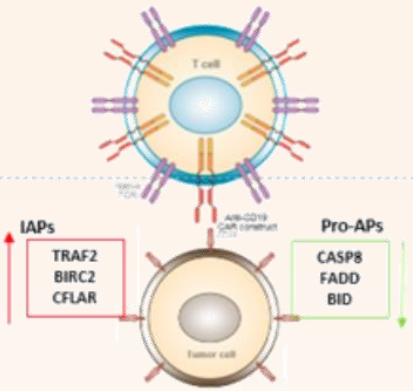
- acquired mutations and alternative splicing of CD19

(Sotillo et al. Cancer Disc. 2015)



T-cell exhaustion/hypofunction

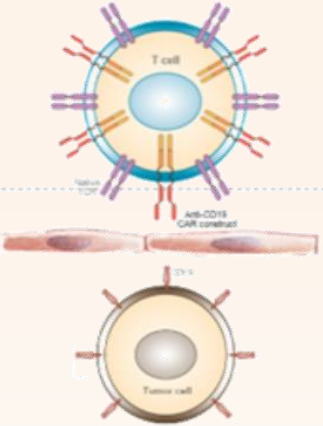
- mediated by inhibitory ligands on tumor cells and cells in the TME
- peripheral self-tolerance (B cell recovery? late relaspses?)
- TME-induced T cell hypofunction (reversible)



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

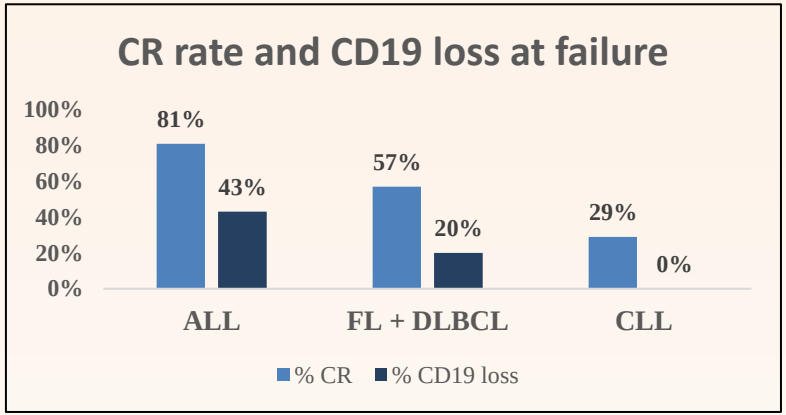
- T cells paralysis
- physiologic factors (high interstitial fluid pressure, hypoxia, pH)

Overcoming mechanisms of resistance to CAR-T

- **CD19 loss or downregulation: early CTL019 efficacy data from Penn and CHOP**

- **Penn and CHOP Data**

Disease	N	CD19 loss at PD
ALL[1]	30	3/7
FL + DLBCL[2]	28	1/5
CLL[3]	14	0/10



- **More responsive diseases seem more likely to fail due to CD19 loss**
- **Less responsive diseases, like CLL, require alternative explanations**

[1]Maude S, *et al*. NEJM. 2014; 371(16): 1507-1517; [2]Schuster SJ, *et al*. N Engl J Med. 2017;377(26):2545-2554; [3]Porter DL, *personal communication 2018 Mar 12.*

Overcoming mechanisms of resistance to CAR-T

- **Active and upcoming clinical trials at UPenn addressing tumor-specific mechanisms of resistance**

CD19 antigen loss

Phase II study of dual targeting of CD19 and CD20 antigens using CD19-CAR T cells and CD20-BsAb

PI: E. Chong

NCT04889716

- Recruiting

T-cell exhaustion/hypofunction

Interleukin-18 secreting anti-CD19 CAR T cells [huCART19-IL18 cells]

PI: J. Svoboda

NCT04684563

- Recruiting

KIR-CAR/Dap12–modified T cells

- Pre-clinical completed*

*Wang, et al. Cancer Imm Res 2015; 3; 815–26.

- Clinical trial planned

PI: S. Schuster

CD5 knockout CAR T cells

- Pre-clinical completed*

*Patel RP, ASH, 2022 #662

- Clinical trial planned

PI: S. Barta

Intrinsic tumor resistance

Venetoclax-resistant CAR T overexpressing mutated BCL-2(F104L) [BCL-2(F104L)-CART19]

- Pre-clinical completed*

*Lee, et al. Cancer Discov 2022;12:2372–91.

- Clinical trial planned

PI: M. Ruella

Insufficient T-cell infiltration

Under non-disclosure agreement

Overcoming mechanisms of resistance to CAR-T

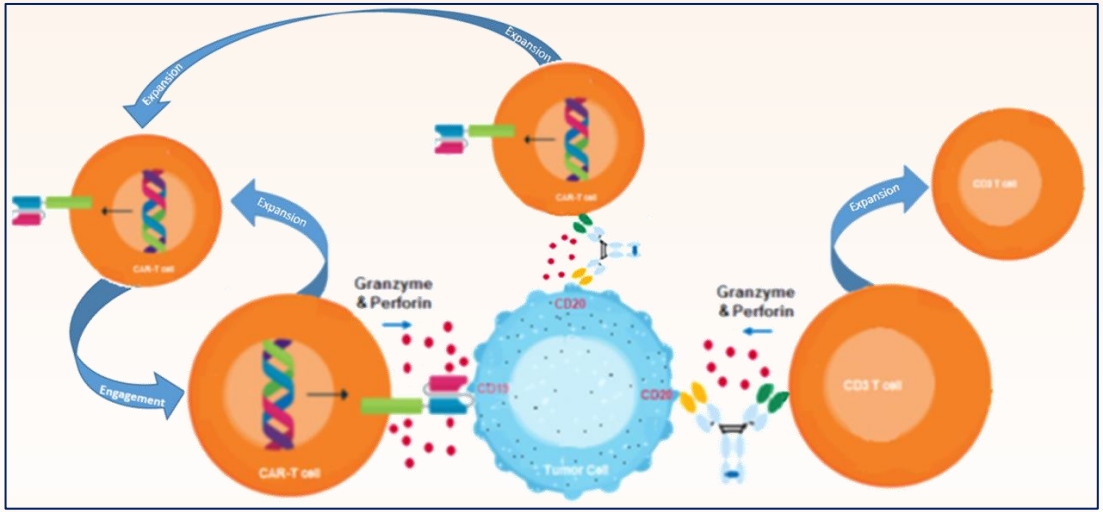
- **Active UPenn clinical trial addressing CD19 antigen loss or downregulation**

Phase II Study of Dual Targeting of CD19 and CD20 Antigens Using Sequential CD19-directed 4-1BB-CD3ζ CAR-T Cells Followed by Mosunetuzumab or Glofitamab in Relapsed or Refractory DLBCL or Transformed FL

Rationale:

Early administration of CD20:CD3 bispecific antibodies (mosunetuzumab or glofitamab) after CD19-directed CAR-T cell therapy may enhance tumor cytotoxicity by:

- synergistic or additive B cell cytotoxicity via simultaneously targeting two different B cell (tumor) antigens, *i.e.*, CD19 and CD20
- reducing CD19-negative tumor cell escape by targeting a second B cell antigen
- enhancing *in vivo* expansion of CAR T cells, as observed for T cells in general, after bispecific T cell engaging antibody exposure



ClinicalTrials.gov Identifier: NCT04889716

Recruitment Status : Recruiting
First Posted : May 17, 2021

Study Type :	Interventional (Clinical Trial)
Estimated Enrollment :	42 participants
Allocation:	Non-Randomized
Intervention Model:	Sequential Assignment
Actual Study Start Date :	November 5, 2021
Estimated Primary Completion Date :	December 31, 2023
Estimated Study Completion Date :	December 31, 2025

Overcoming mechanisms of resistance to CAR-T

- **Active UPenn clinical trial addressing T cell exhaustion**

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

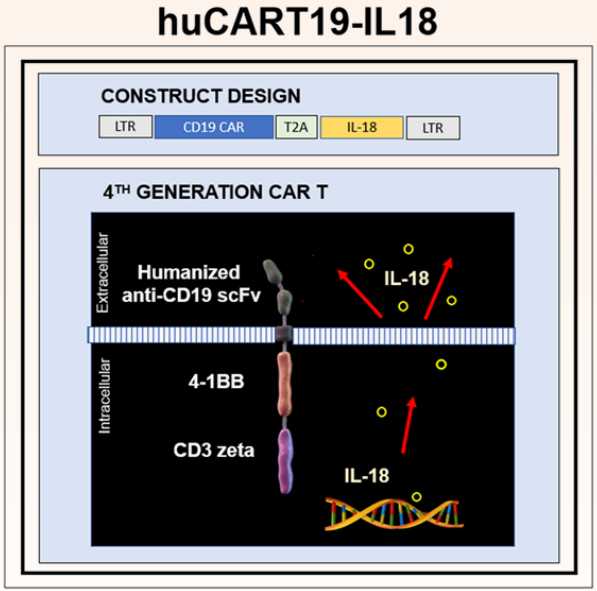
Rationale: to utilize IL-18 as a pro-inflammatory cytokine to:

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

ClinicalTrials.gov ID NCT04684563 **Sponsor** University of Pennsylvania

Brief Summary		The purpose of this study is to find the maximum dose of huCART19-IL18 cells that is safe for use in humans with CD19+ cancers.
Detailed Description		• Cohort A: Non-Hodgkin Lymphoma (NHL) • Cohort B: Chronic Lymphocytic Leukemia (CLL) • Cohort C: Acute Lymphoblastic Leukemia (ALL)
Study Type	ICMJE	Interventional
Study Phase	ICMJE	Phase 1
Study Design	ICMJE	Allocation: Non-Randomized Interventional Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Treatment
Condition	ICMJE	• Chronic Lymphocytic Leukemia • Non-hodgkin Lymphoma • Acute Lymphoblastic Leukemia
Recruitment Status	ICMJE	Recruiting
Enrollment (Estimated) (Submitted: 2023-03-30)	ICMJE	72
Original Enrollment (Estimated) (Submitted: 2020-12-21)	ICMJE	30
Study Start Date (Actual)	ICMJE	2021-05-06

Treated, so far:
NHL, n = 21
CLL, n = 1
ALL, n = 2



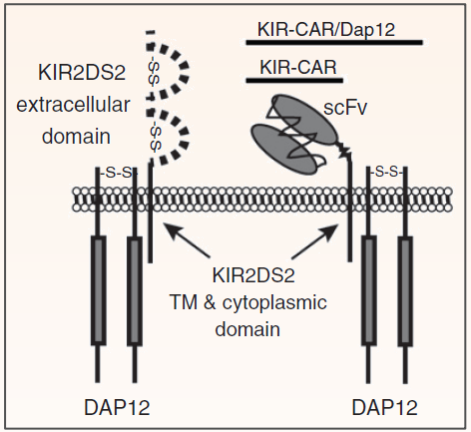
PI: Jakub Svoboda

Overcoming mechanisms of resistance to CAR-T

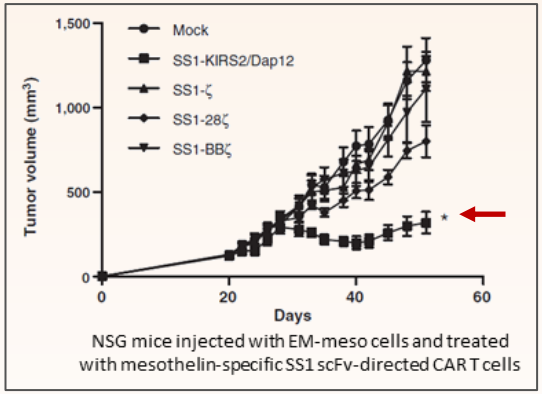
- **Planned UPenn clinical trial addressing T cell exhaustion or hypofunction**

CD19-directed KIR-CAR/DAP12-modified cells for CD19+ lymphomas

Rationale: KIR-CAR/Dap12 expressing CAR T cells have potent *in vivo* antitumor activity that is resistant to the tumor- and/or TME-induced T-cell hypofunction observed with CD3ζ-based CAR T cells[1]. This potent activity *may* be of benefit in large B-cell lymphomas with bulky disease.

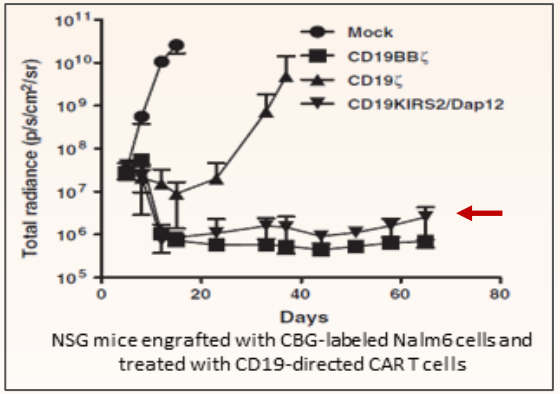


Solid tumor model



NSG mice injected with EM-meso cells and treated with mesothelin-specific SS1 scFv-directed CAR T cells

B-cell tumor model



NSG mice engrafted with CBG-labeled Nalm6 cells and treated with CD19-directed CAR T cells

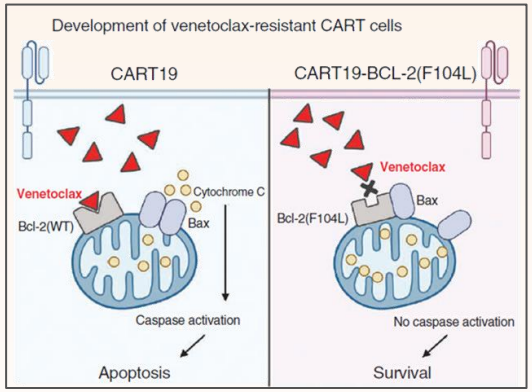
[1]Moon, et al. Clin Cancer Res 2014;20:4262–73.
[2]Wang, et al. Cancer Imm Res 2015;3:815-826. (*data show on the right*)

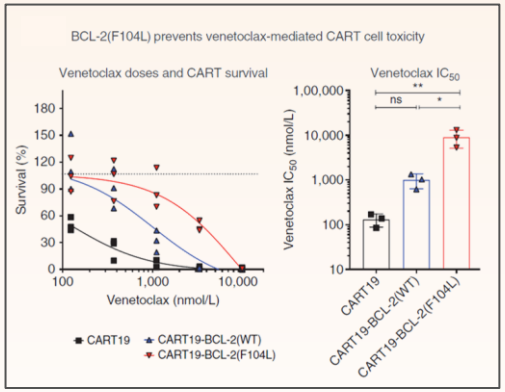
Overcoming mechanisms of resistance to CAR-T

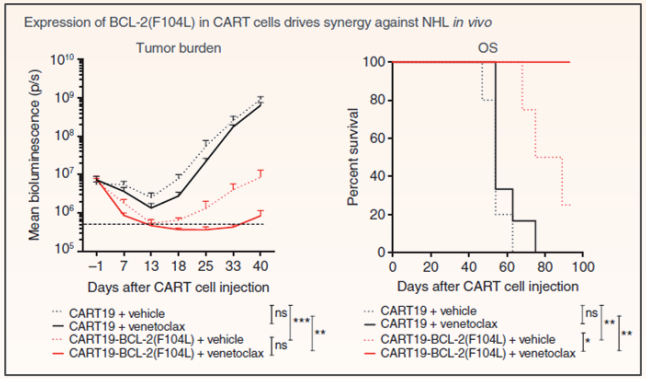
- **Planned UPenn clinical trial addressing intrinsic tumor resistance to CAR-T**

Venetoclax-resistant CAR-T cells engineered to express mutated BCL-2(F104L) for combination therapy

Rationale: BCL-2 *overexpression in CAR T cells* and *inhibition in tumor cells* enhances CAR T cell efficacy in pre-clinical models by reducing apoptosis in CAR T cells and enhancing apoptosis in cancer cells. Thus, combination venetoclax and CAR T cell therapy is a compelling approach for B-cell lymphomas failing standard CAR T therapy.







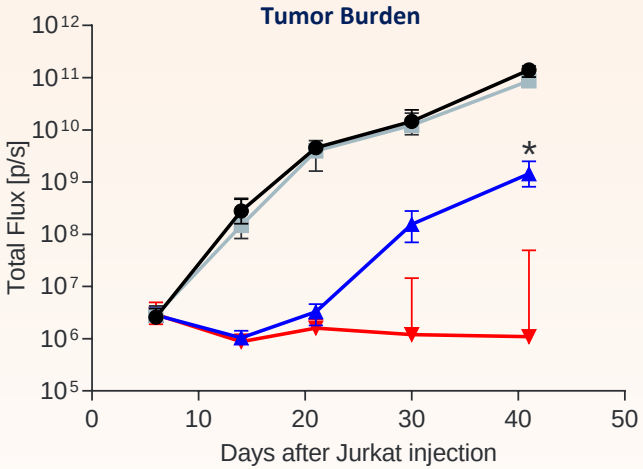
MINO xenografted mice treated with CART19 or CART19-BCL-2(F104L)

[1]Lee, et al. Cancer Disc 2022;12:2372–91.

Overcoming mechanisms of resistance to CAR-T

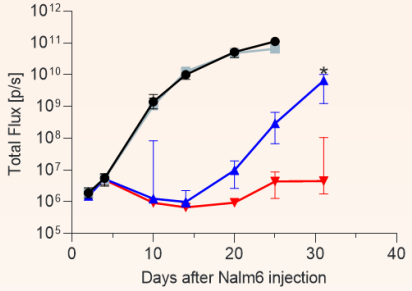
CD5 KO CAR T cells enhance efficacy in multiple liquid + solid tumor models

T-cell leukemia or lymphoma
CD5 KO vs Traditional CD5 CAR-T

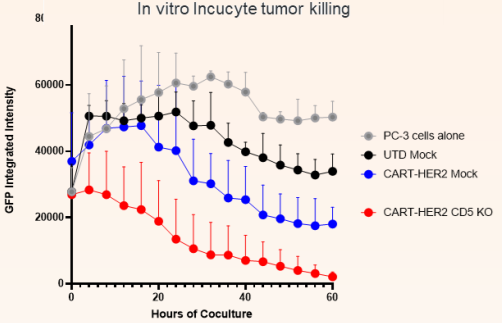


Ruella lab data
Patel RP, ASH, 2022 #662

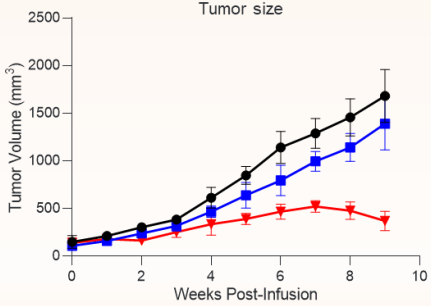
B-CELL LEUKEMIA AND LYMPHOMA
CD5 KO vs Traditional CD19 CAR-T
Tumor burden



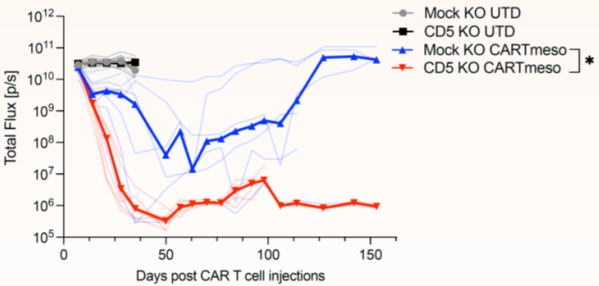
OVARIAN CANCER
CD5 KO vs Traditional HER2 CAR-T
In vitro Incucyte tumor killing



HODGKIN LYMPHOMA
CD5 KO vs Traditional CD30 CAR-T
Tumor size



PANCREATIC CANCER
CD5 KO vs Traditional Mesothelin CAR-T
Tumor size

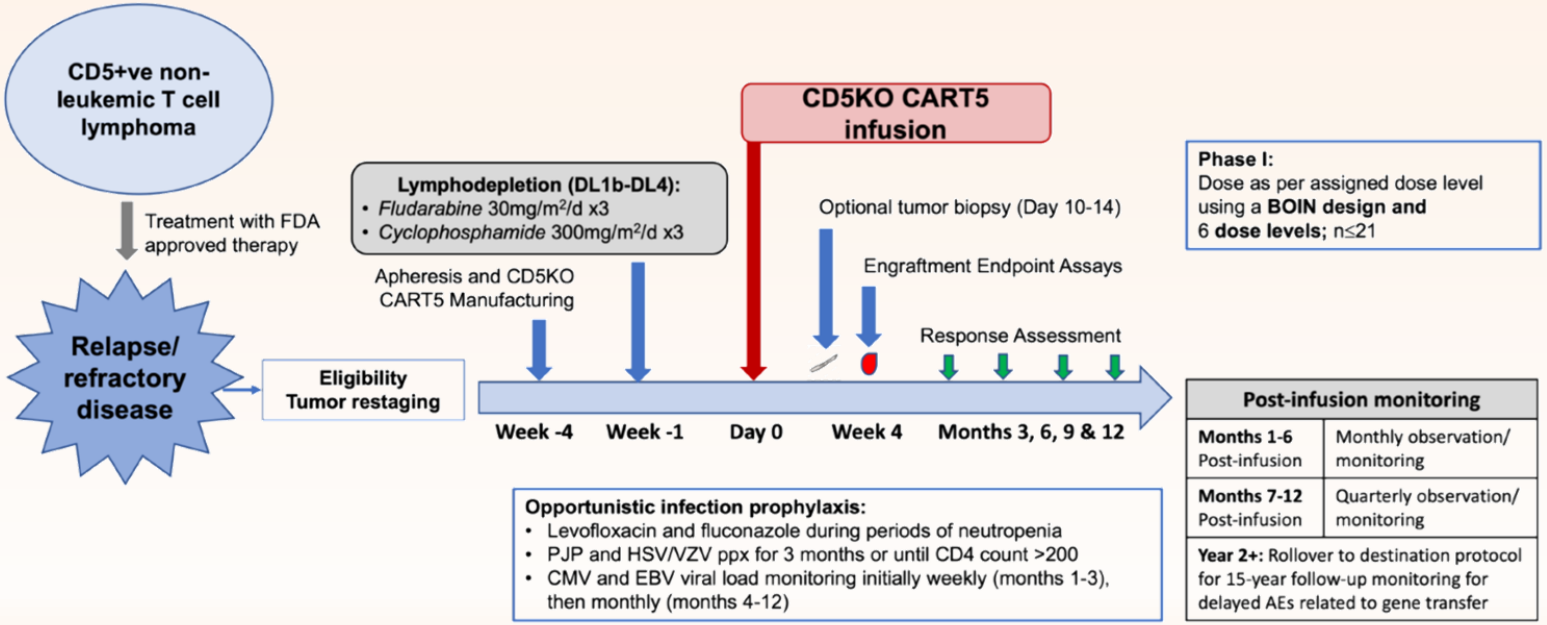


Phase I clinical trial of CD5 KO CART5 for T-cell lymphomas

- **Upcoming UPenn clinical trial for 2024**

PI: Stefan Barta

- Patients with relapsed or refractory CD5+ nodal non-leukemic T cell lymphoma
- Bayesian optimal interval design for dose level assignment



Grazie molte / Many Thanks!