Intro to CAR-T:
Driving Shiny New Cars

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Intro to CAR-T:
Driving Shiny New Cars

- **What are CAR-T?**

- **How do CAR-T kill work?**

    Heme malignancies

    "Solid" malignancies

- **How do CAR-T fail?**

    CAR-T intrinsic causes   → **Action Plan:**

    Tumor intrinsic causes:

        Antigen Escape   → **Action Plan:**

        Other Resistance   → **Action Plan:**

    TME intrinsic causes   → **Action Plan:**

- **The Future**
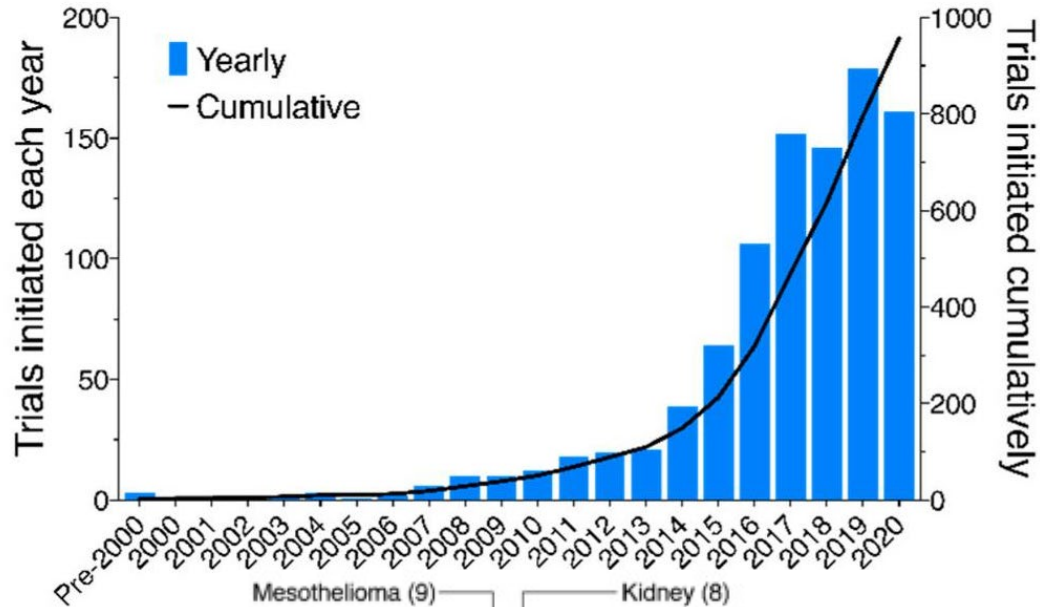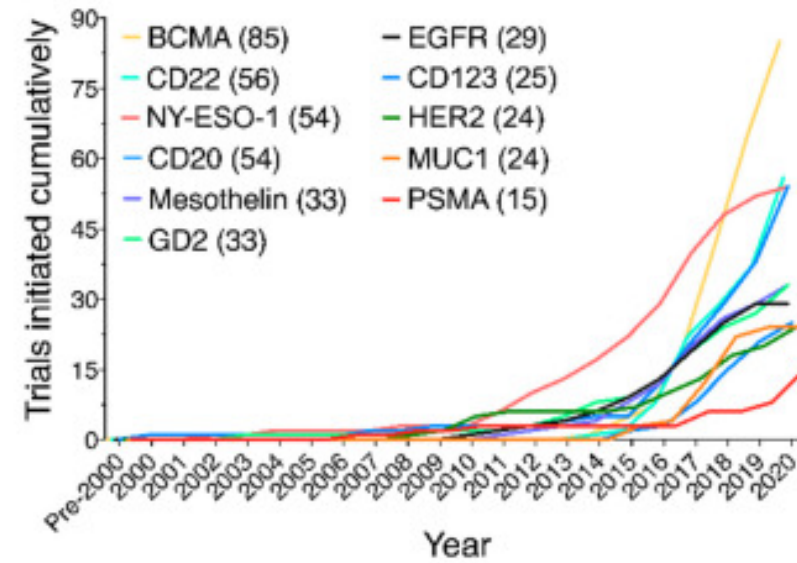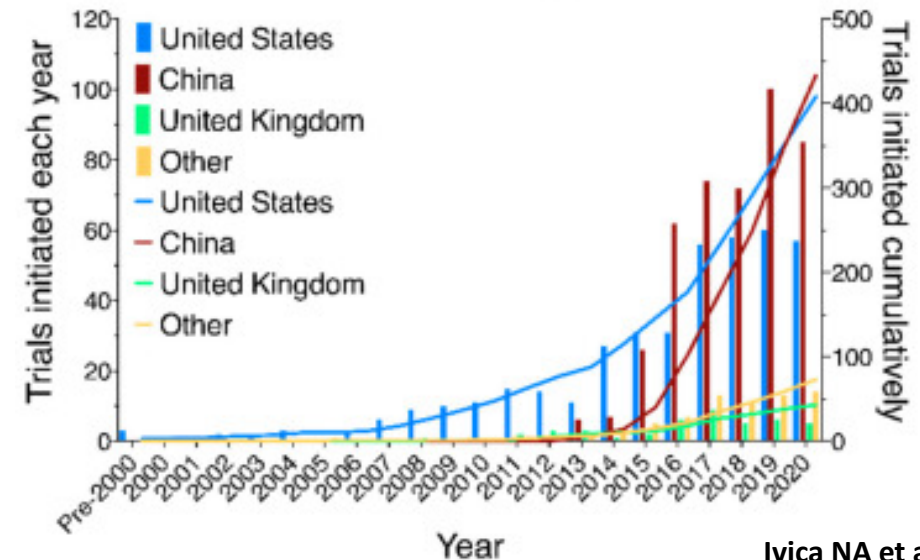
How can we measure CAR-T optimism?

CTgov Trials Initiations for CAR-T, TCR-T and Related Therapies
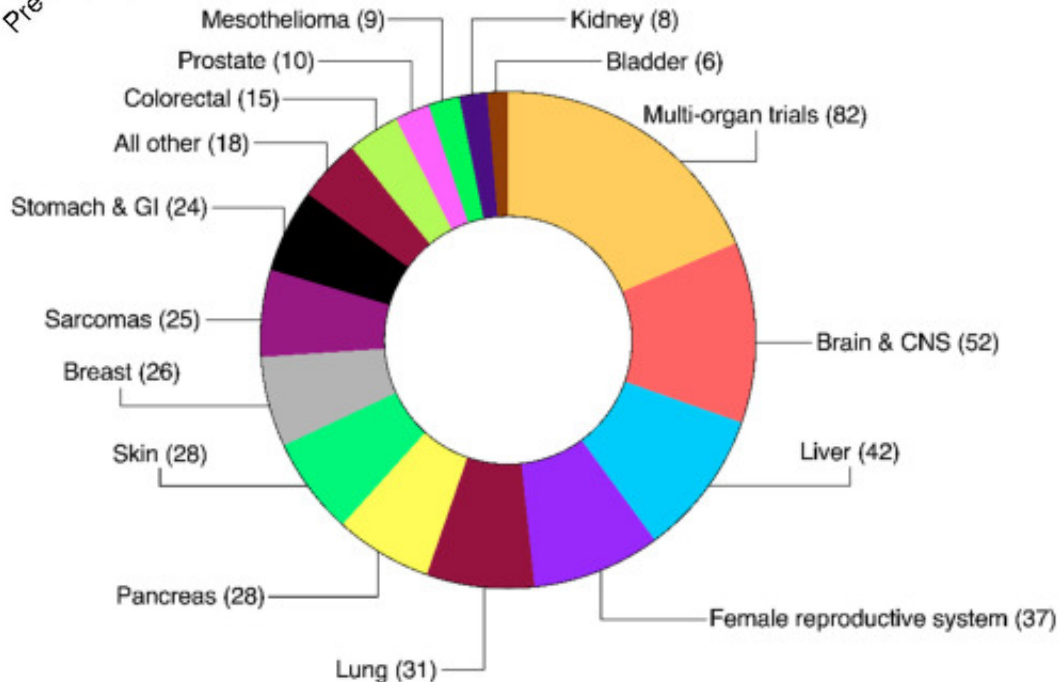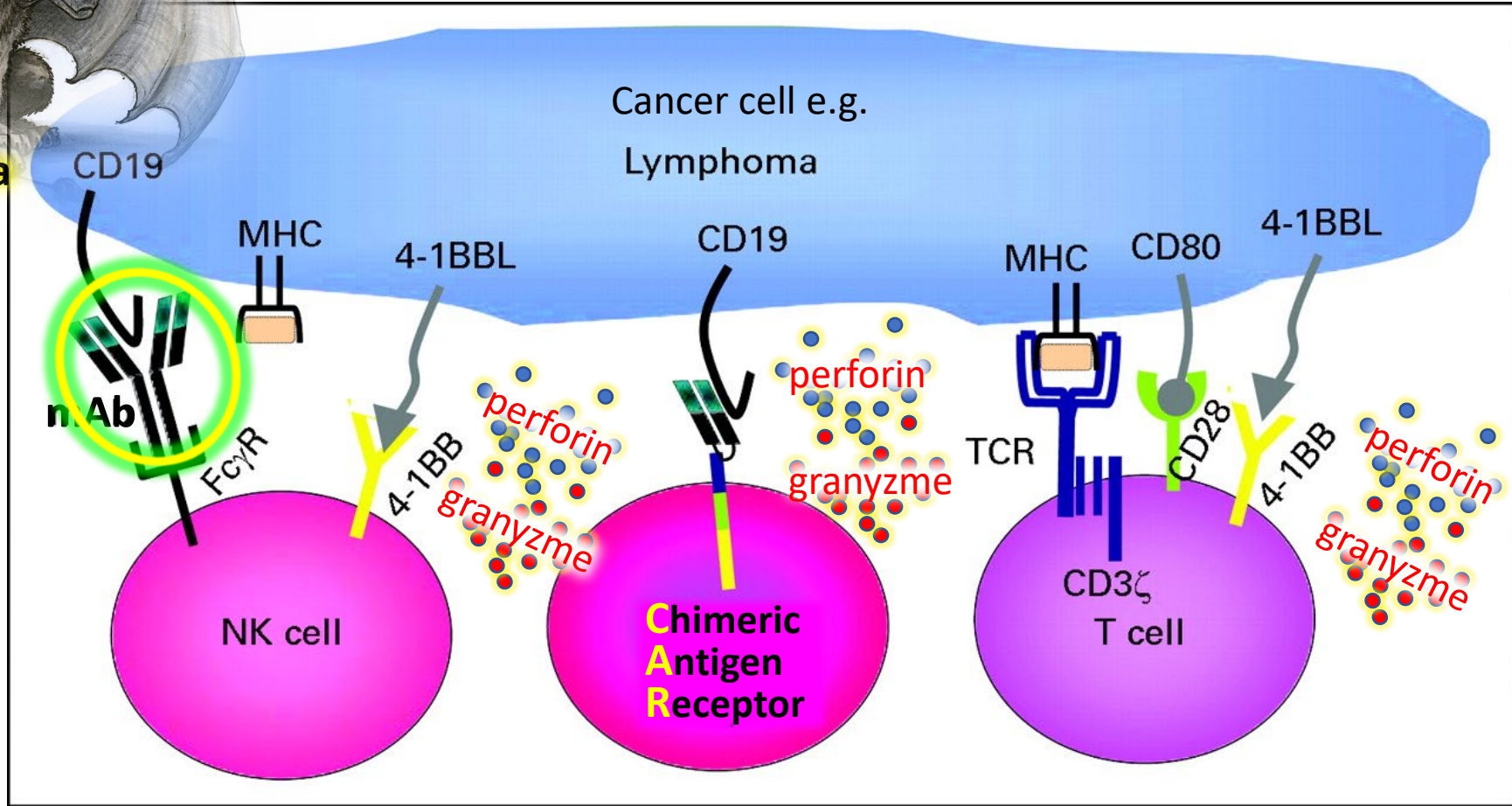

Most Common Target Antigens (CD19 excluded)

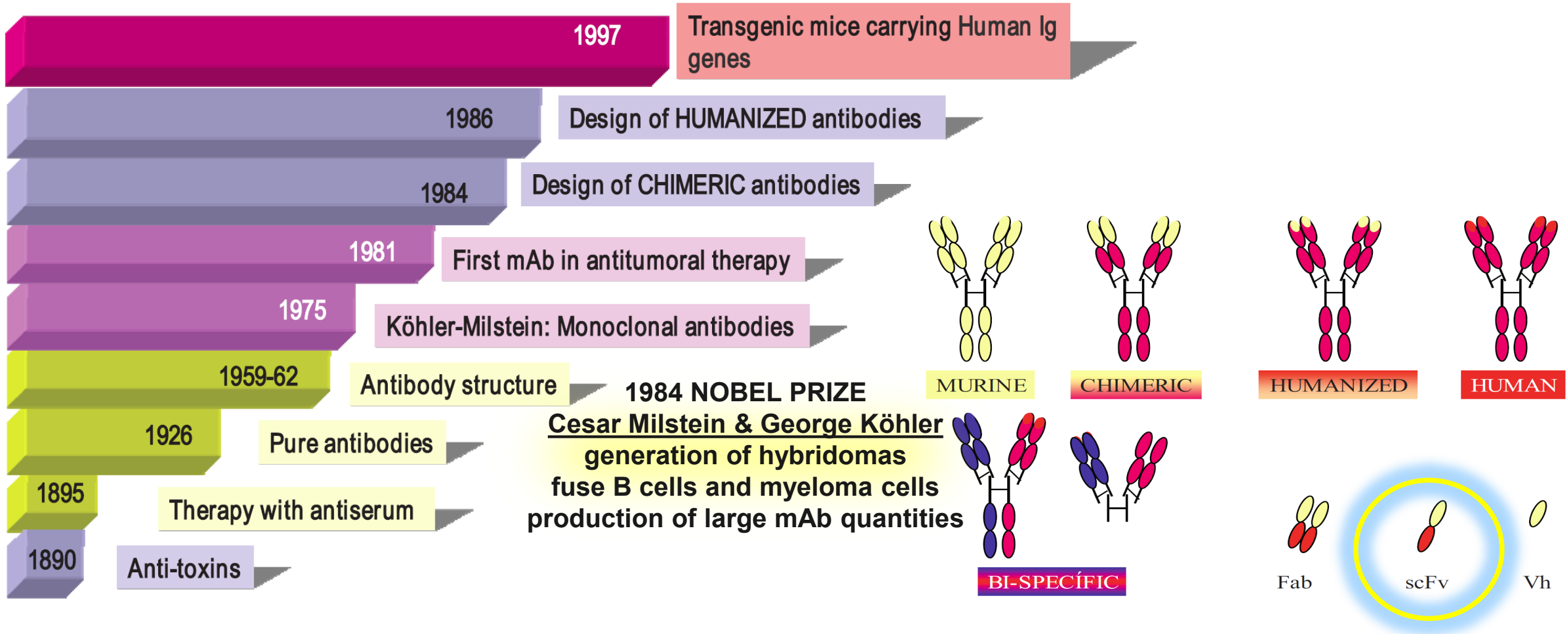

Countries of Trial Originators

Ivica NA et al., Healthcare 2021

What are CAR-T?
recipe: monoclonal antibodies (mAbs) + T cells

Brody J, et al., *Active and passive immunotherapy for lymphoma: proving principles and improving results*. J Clin Oncol. 2011

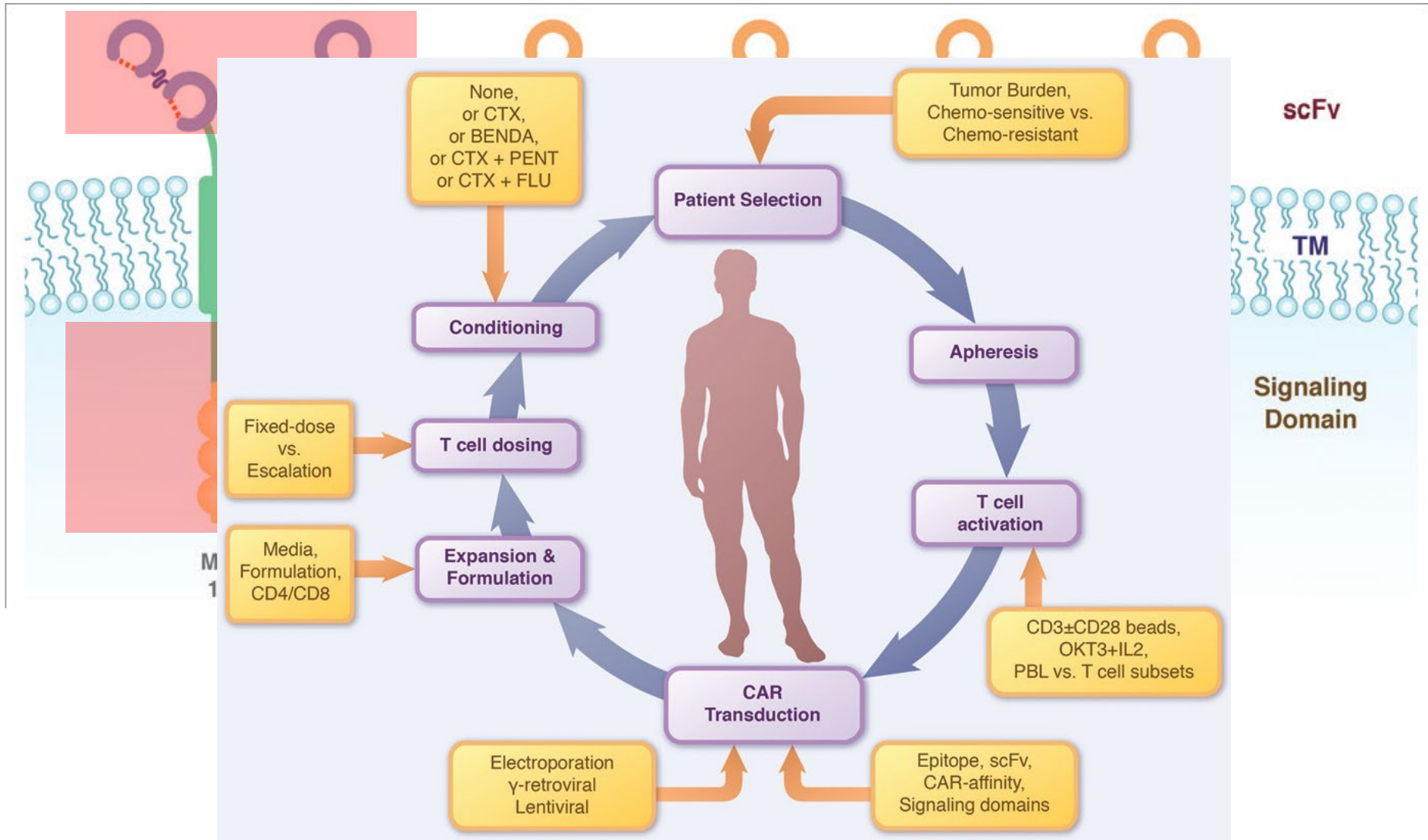# What are CAR-T?

recipe: monoclonal antibodies (mAbs) + T cells

Year	Event
1997	Transgenic mice carrying Human Ig genes
1986	Design of HUMANIZED antibodies
1984	Design of CHIMERIC antibodies
1981	First mAb in antitumoral therapy
1975	Köhler-Milstein: Monoclonal antibodies
1959-62	Antibody structure
1926	Pure antibodies
1895	Therapy with antiserum
1890	Anti-toxins

1984 NOBEL PRIZE
<u>Cesar Milstein & George Köhler</u>
generation of hybridomas
fuse B cells and myeloma cells
production of large mAb quantities

MURINE   CHIMERIC   HUMANIZED   HUMAN
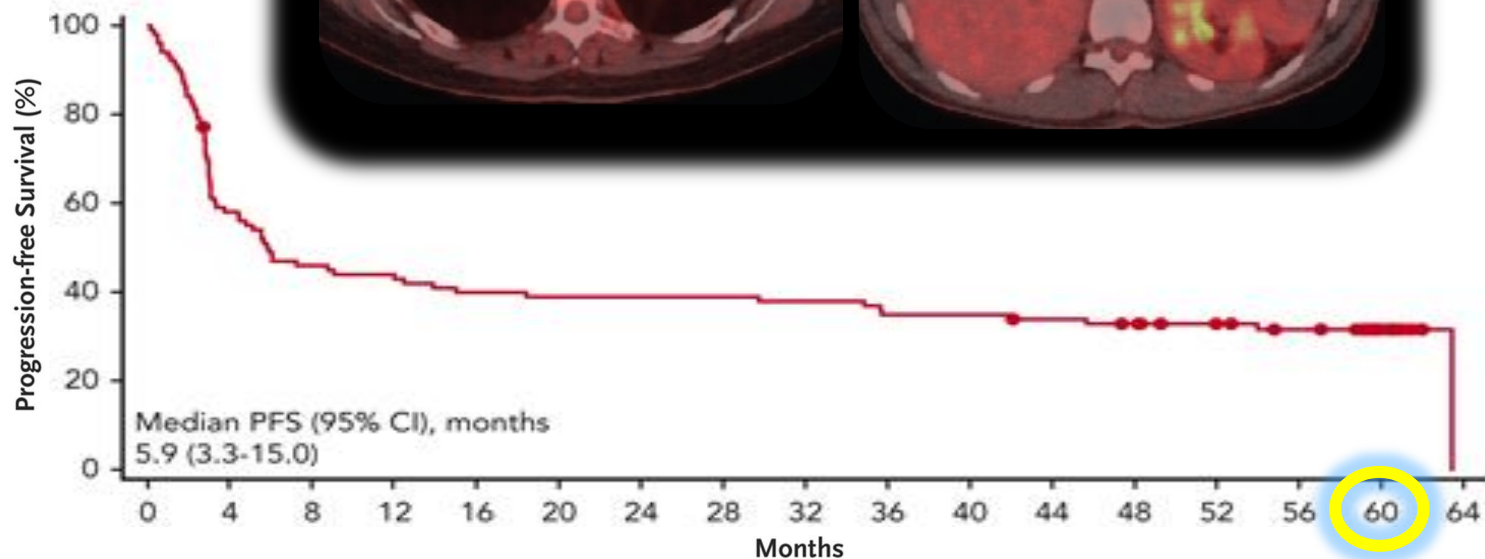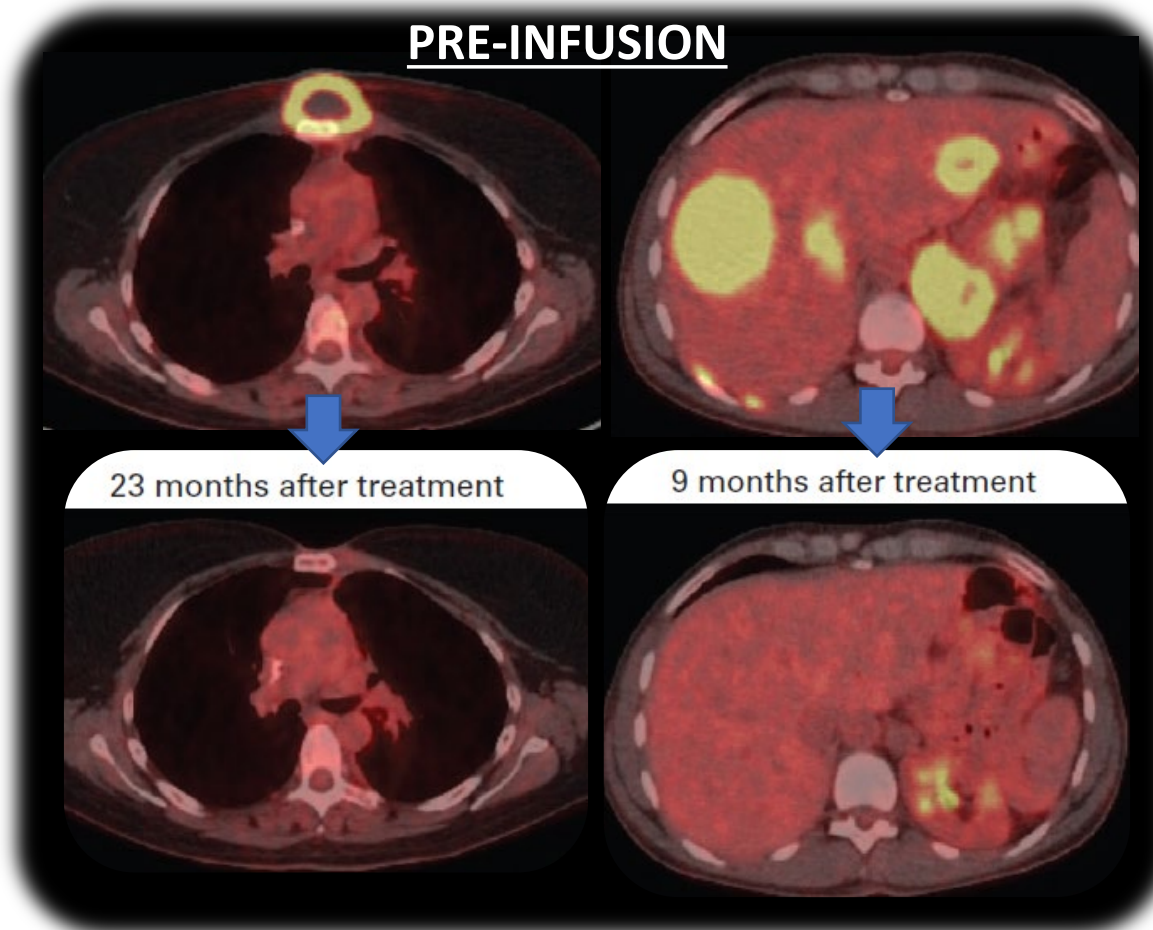
BI-SPECÍFIC

Fab   scFv   Vh

What are CAR-T?
putting the ingredients together
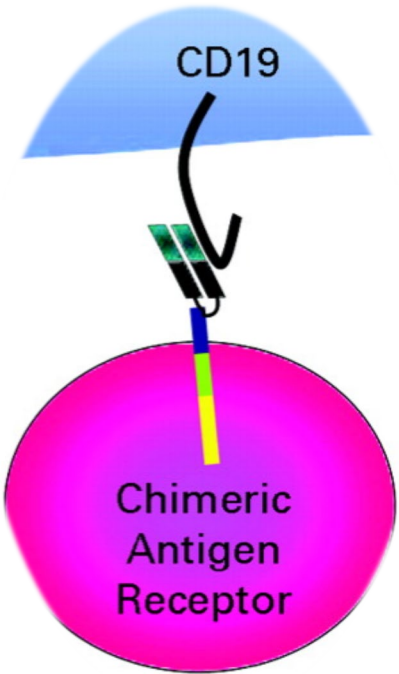
How do CAR-T kill cancer?

**axi-cel
anti-CD19**

PRE-INFUSION

23 months after treatment
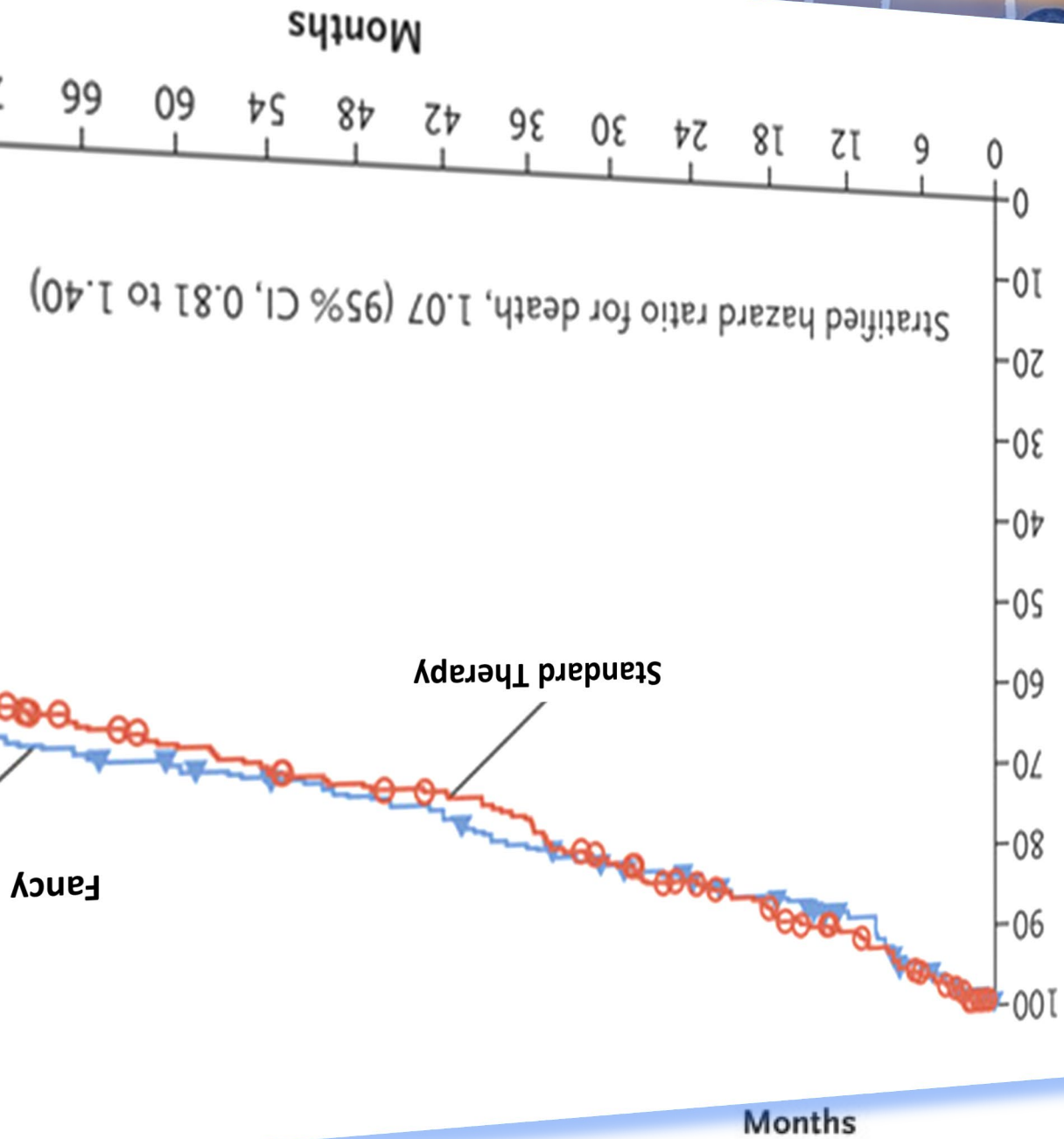
9 months after treatment

Answer:
better than other therapies for relapsed aggressive lymphoma

Median PFS (95% CI), months
5.9 (3.3-15.0)

Kochenderfer JN et al., J Clin Oncol. 2014
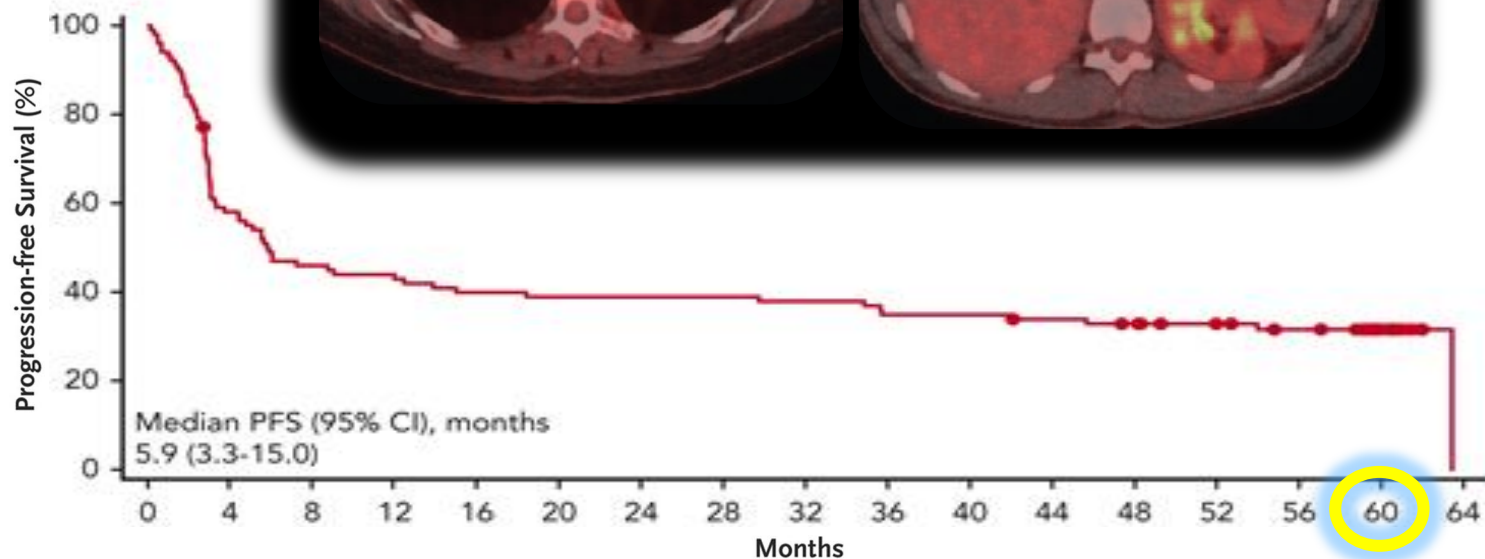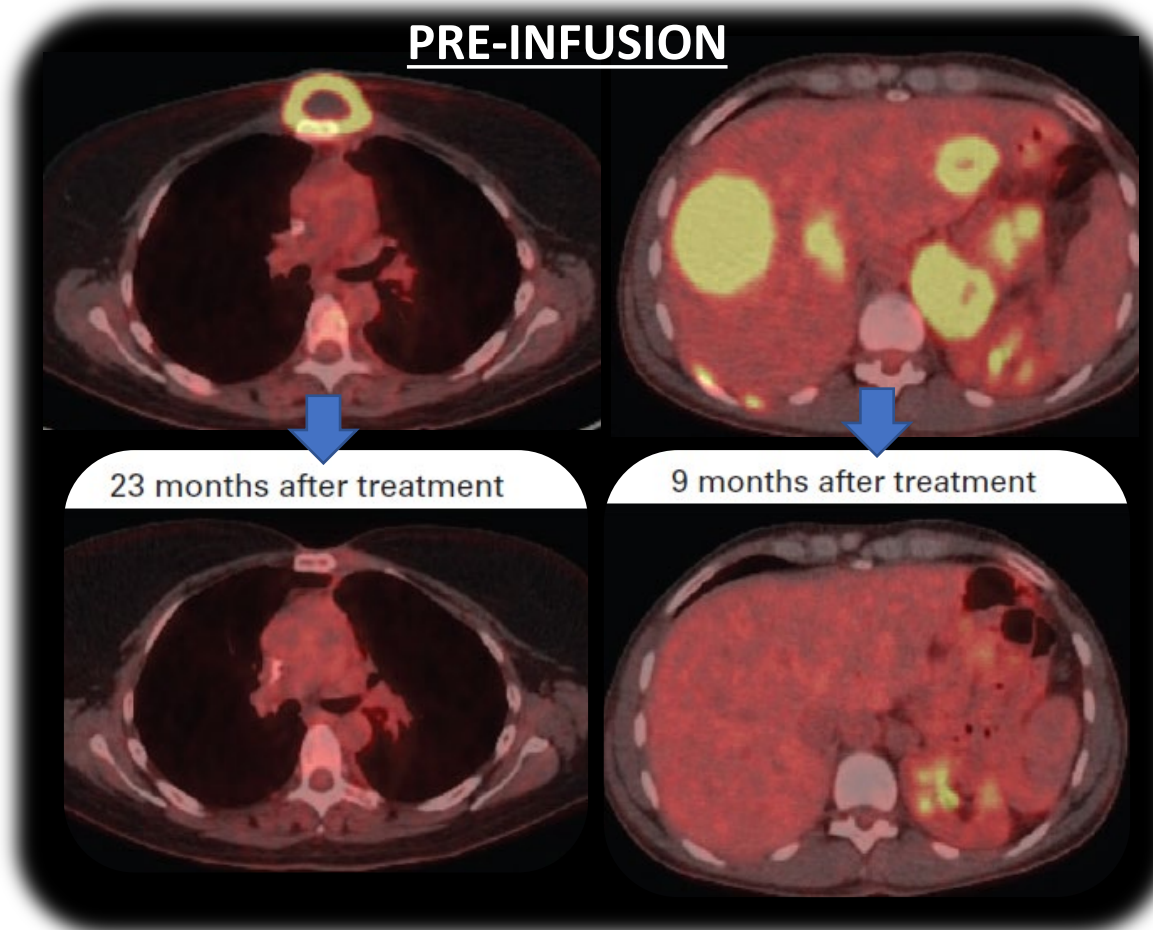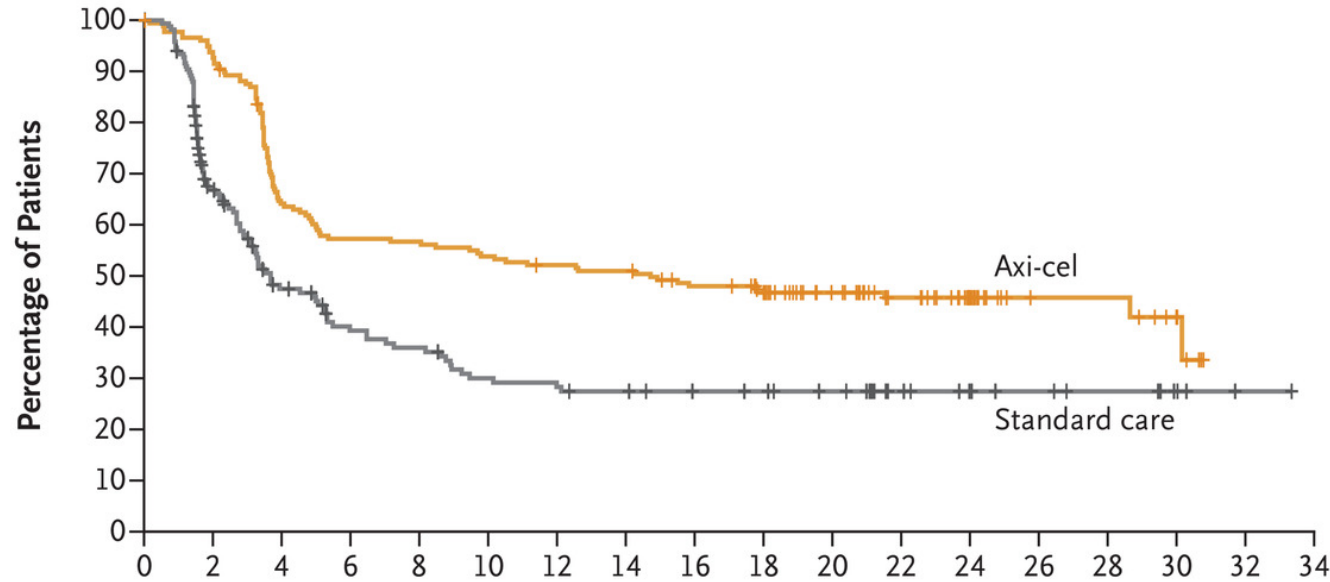Neelapu SS, et al., N Engl J Med. 2017
Neelapu SS, et al., Blood 2023

Quick aside - what *most* Kaplan Meier curves look like:
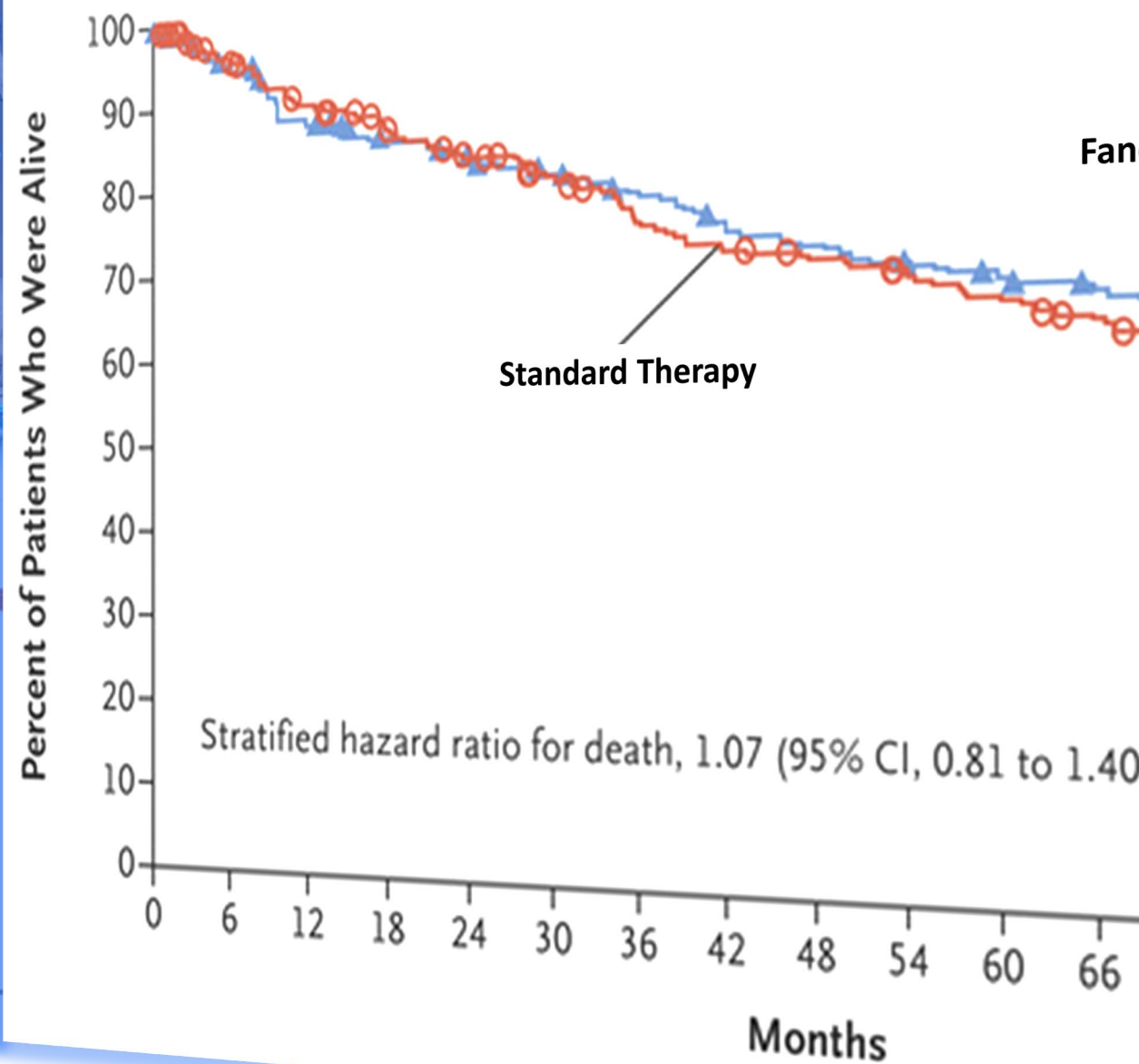
How do CAR-T kill cancer?

**Axi-Cel
anti-CD19**

PRE-INFUSION

23 months after treatment

9 months after treatment

Answer:
better than other
therapies for relapsed
aggressive lymphoma

Median PFS (95% CI), months
5.9 (3.3-15.0)

Progression-free Survival (%) vs Months

**Kochenderfer JN et al., J Clin Oncol. 2014
Neelapu SS, et al., N Engl J Med. 2017
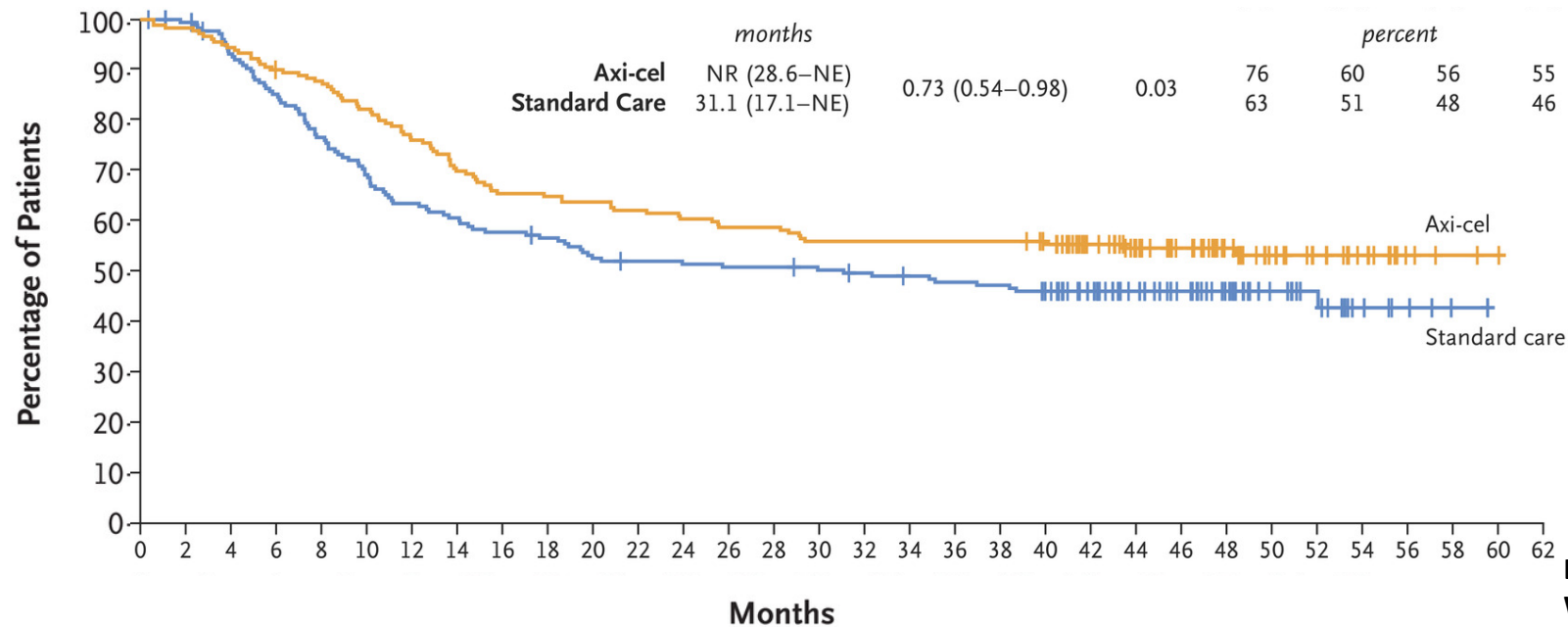Neelapu SS, et al., Blood 2023**

How do CAR-T kill cancer?

Axi-Cel anti-CD19

Progression-free Survival

Axi-cel

Standard care

Overall Survival

	months			percent			
Axi-cel	NR (28.6–NE)			76	60	56	55
Standard Care	31.1 (17.1–NE)	0.73 (0.54–0.98)	0.03	63	51	48	46

Axi-cel

Standard care

Months

Answer:
better than other therapies for relapsed aggressive lymphoma

Locke FL et al., N Engl J Med. 2022
Westin JR et al., N Engl J Med. 2023

How do CAR-T kill cancer?

Answer: better than other therapies for relapsed aggressive lymphoma

Liso-Cel anti-CD19

liso-cel
standard rx
one-sided p = 0.0001

liso-cel
standard rx
one-sided p = 0.026

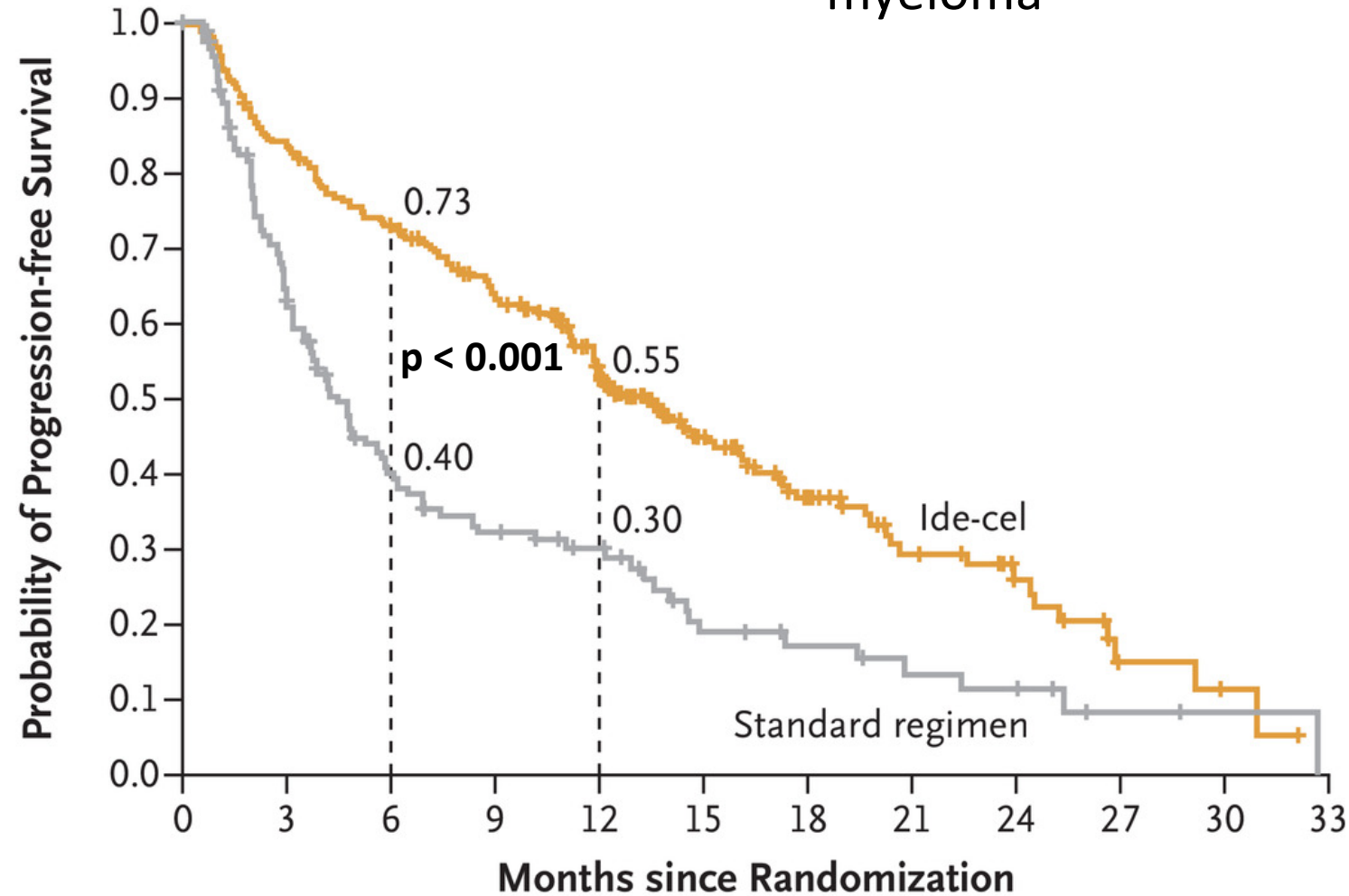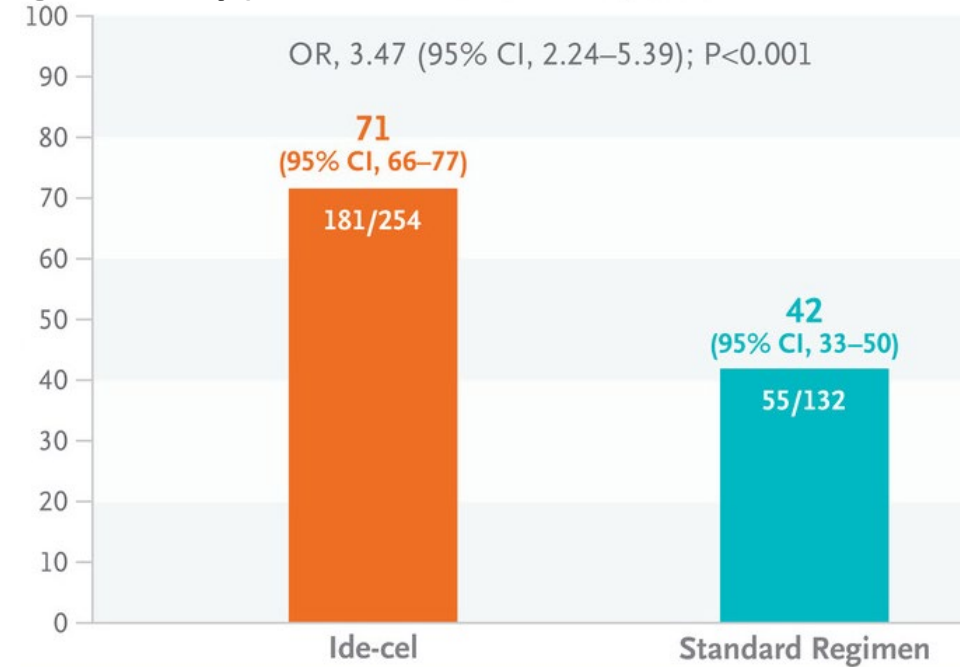Kamdar M et al., Lancet 2022
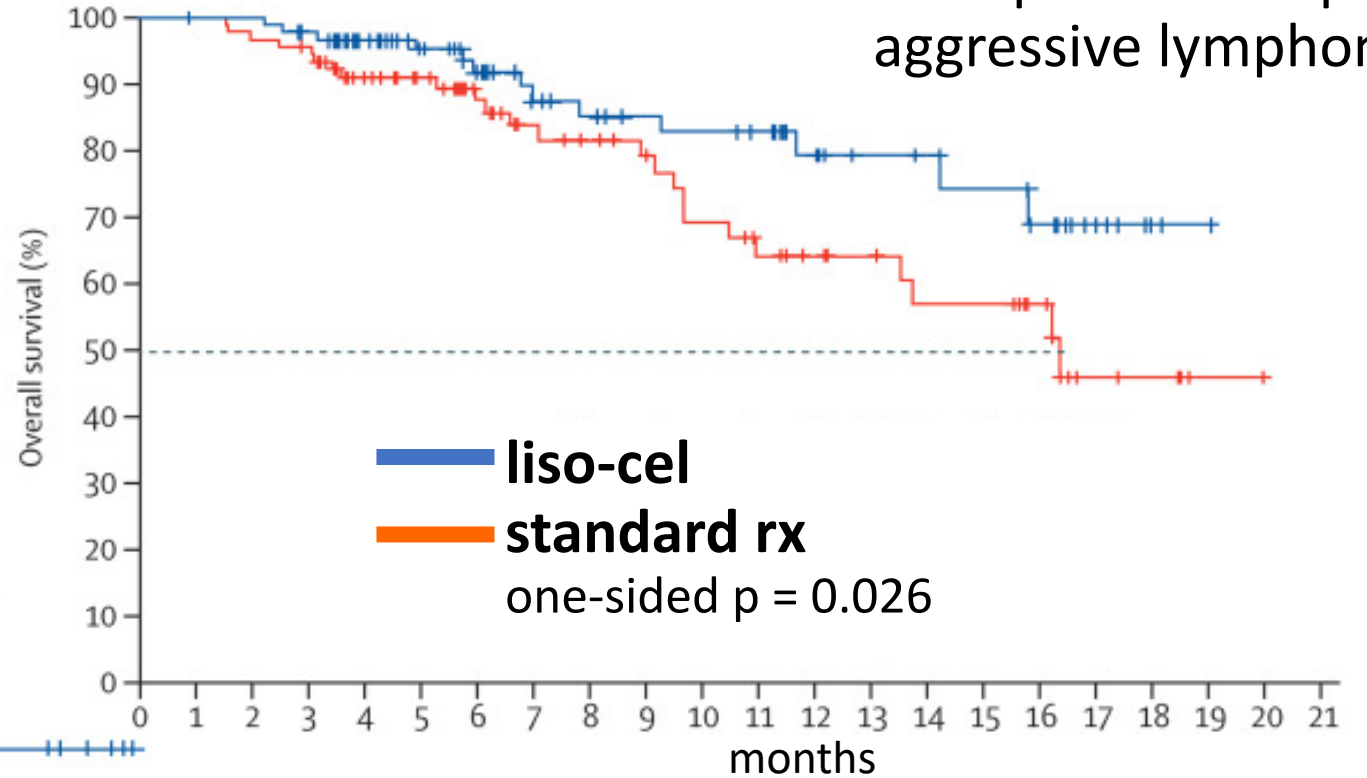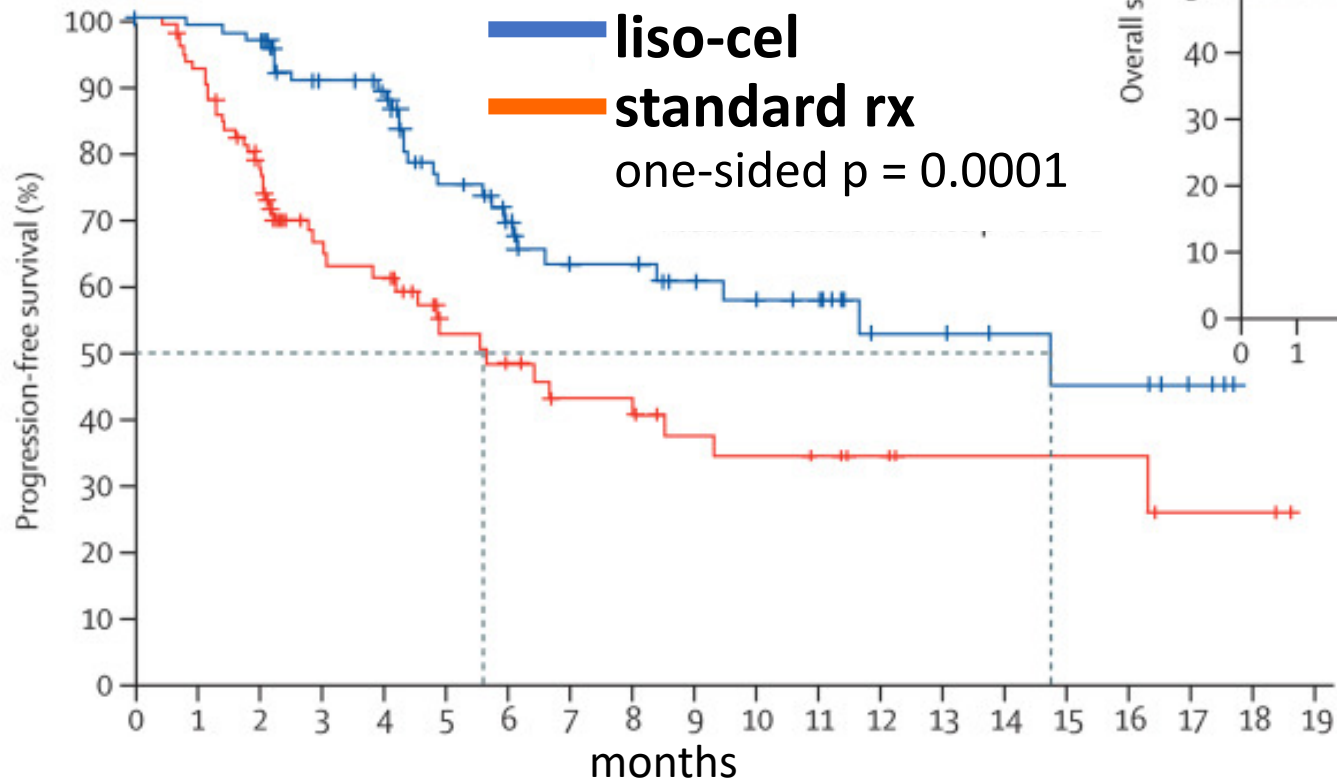
How do CAR-T kill cancer?

Answer: better than other therapies for relapsed myeloma

Ide-Cel anti-BCMA ORR 71%

Overall Response

OR, 3.47 (95% CI, 2.24–5.39); P<0.001

71 (95% CI, 66–77) 181/254 — Ide-cel

42 (95% CI, 33–50) 55/132 — Standard Regimen

Probability of Progression-free Survival vs. Months since Randomization

0.73

p < 0.001

0.55

0.40

0.30

Ide-cel

Standard regimen

Rodriguez-Otero P et al., N Engl J Med. 2023

How do CAR-T kill cancer?

Answer: better than other therapies for relapsed myeloma

Cilta-Cel anti-BCMA ORR 96.9%

Berdeja JG Lancet 2021
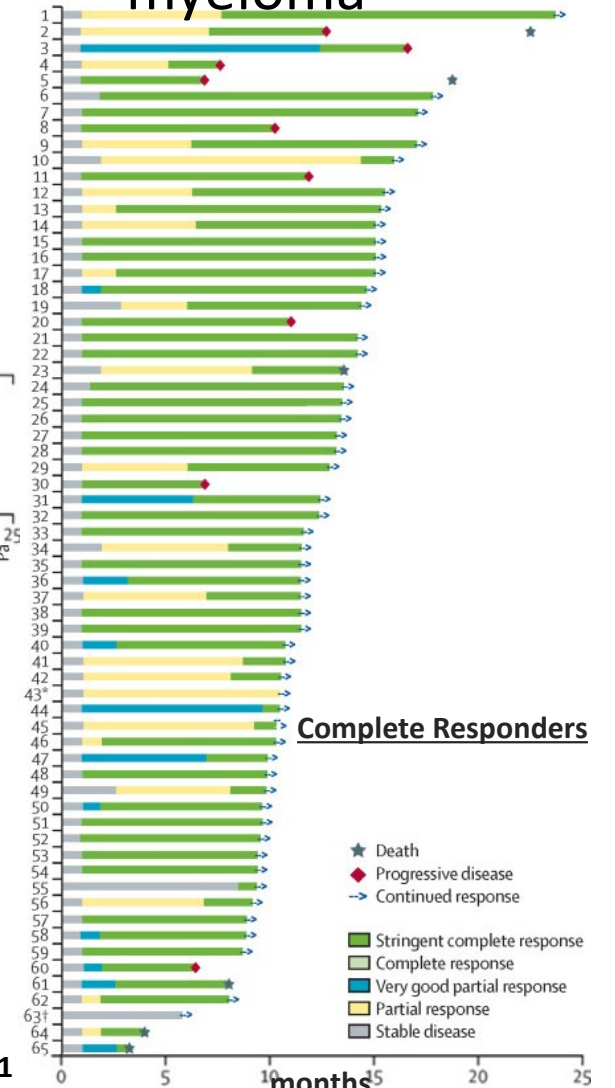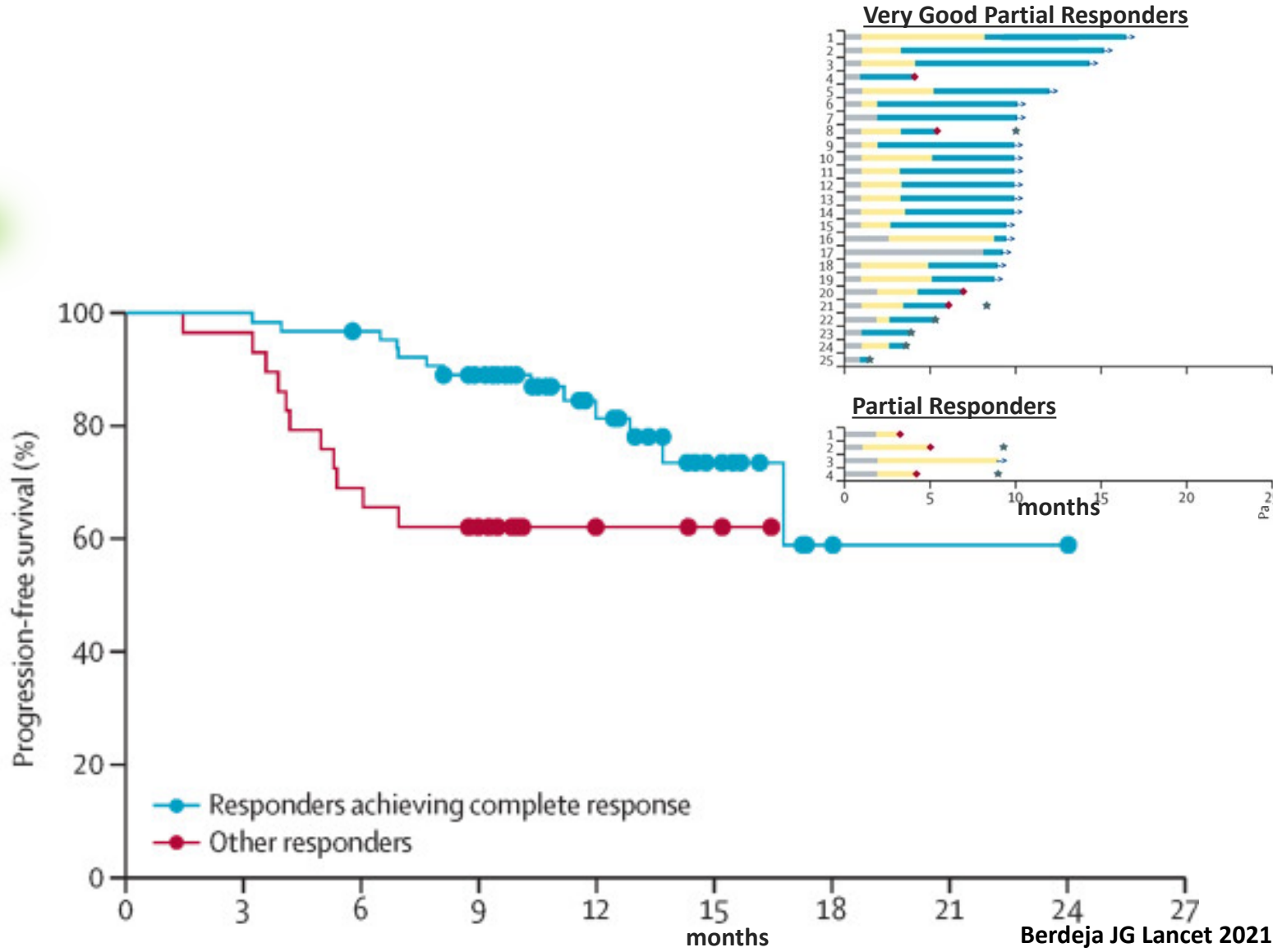
How do CAR-T kill cancer?

Answer:
better than other therapies for relapsed myeloma

**MCARH109
anti-GPRC5D**
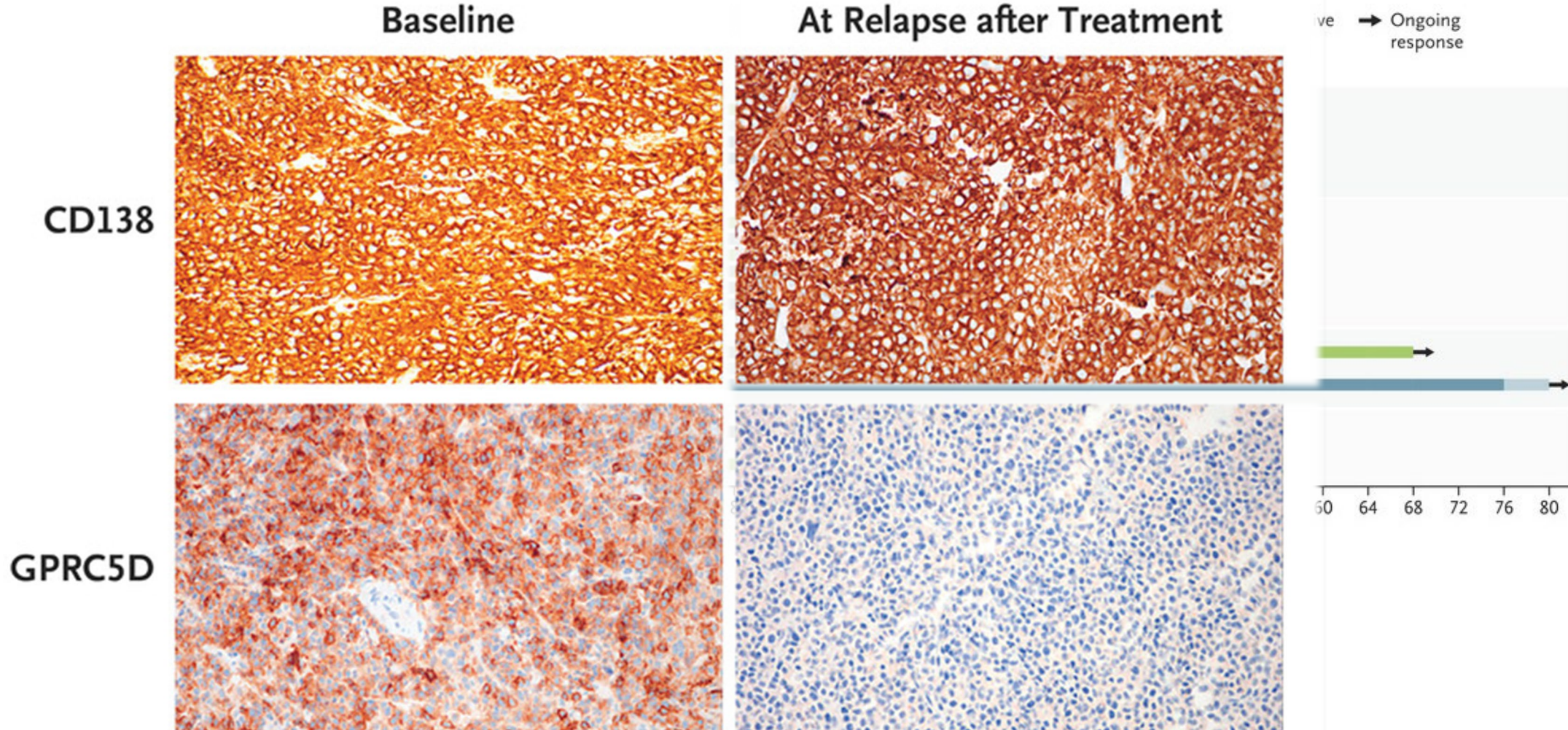
Mailankody S et al., NEJM 2022

How do CAR-T kill cancer?
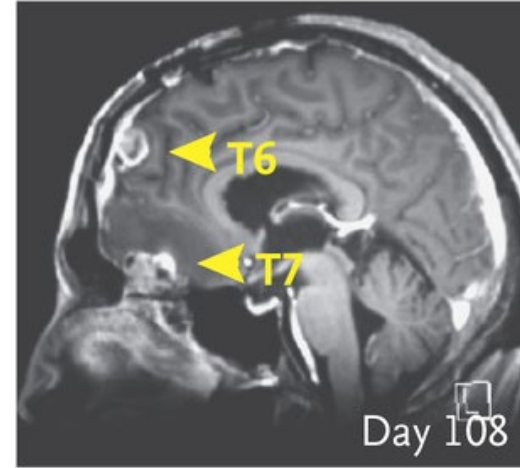
anti-IL13Rα2
"zetakine"
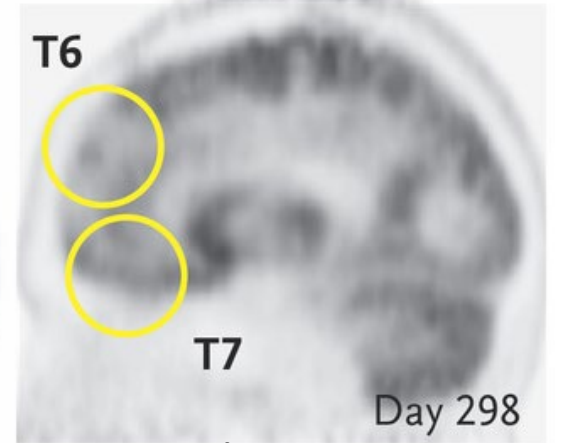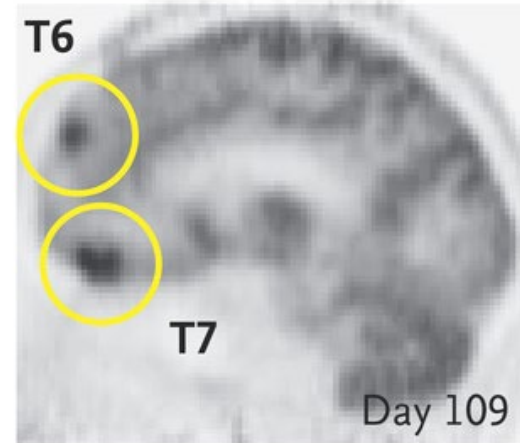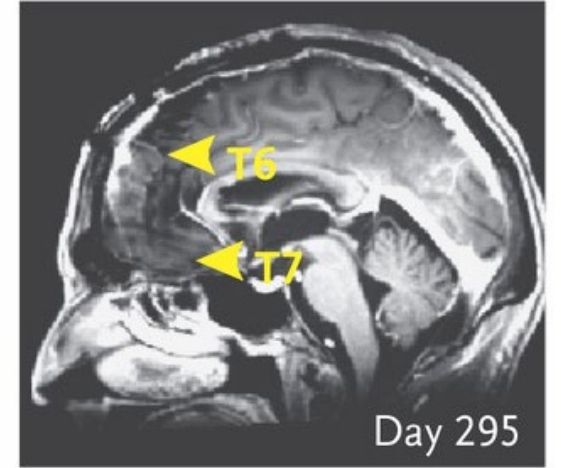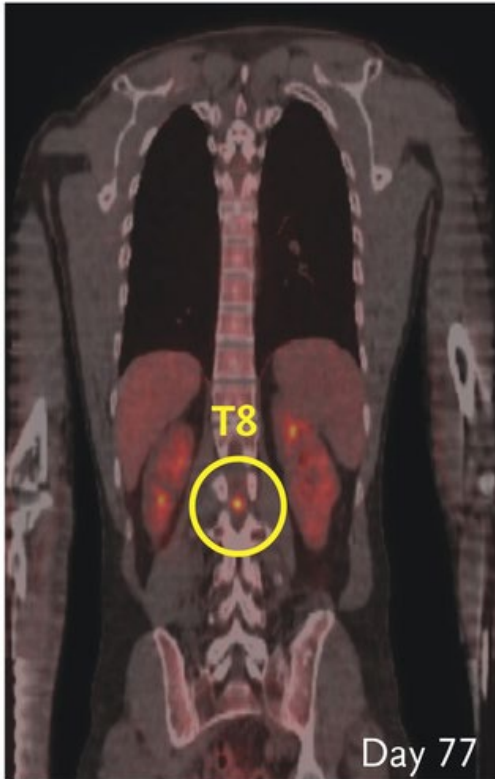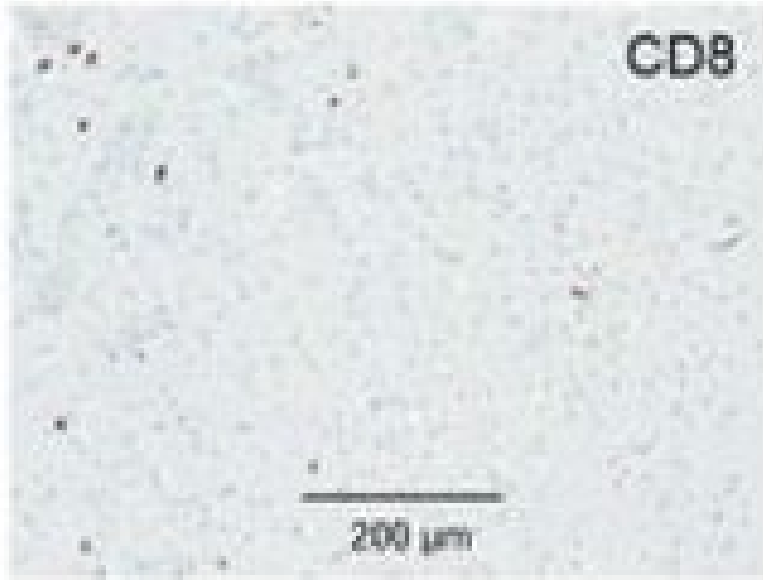
Answer: ?better than other therapies for relapsed Glioblastoma?

Brown CE et al., NEJM 2016

How do CAR-T kill cancer?

O'Rourke DM et al., Sci Trans Med 2017
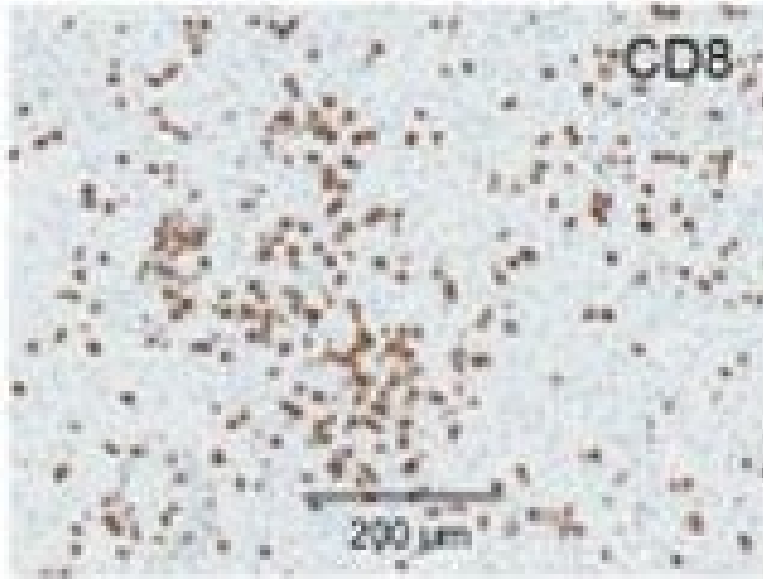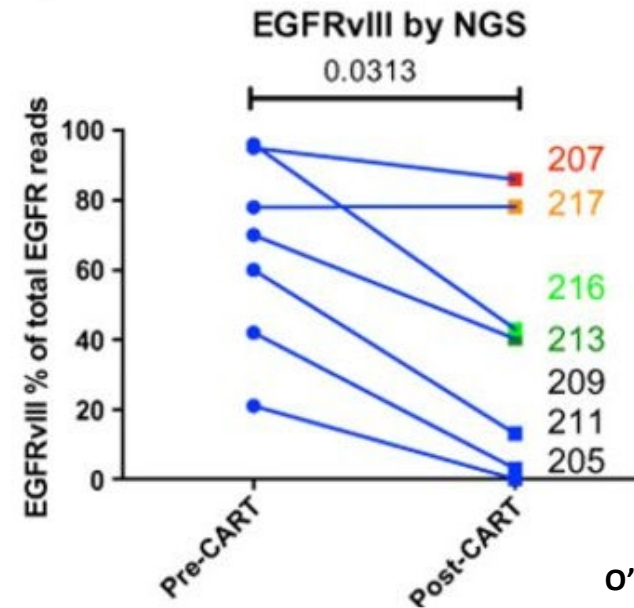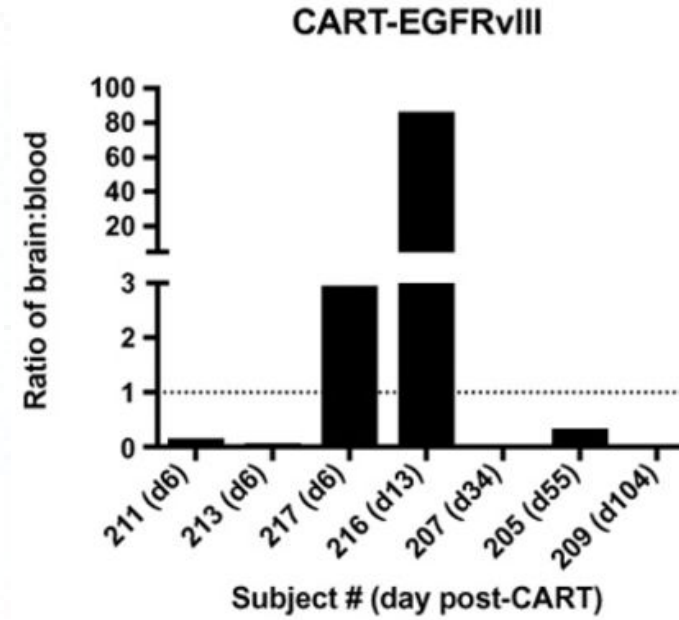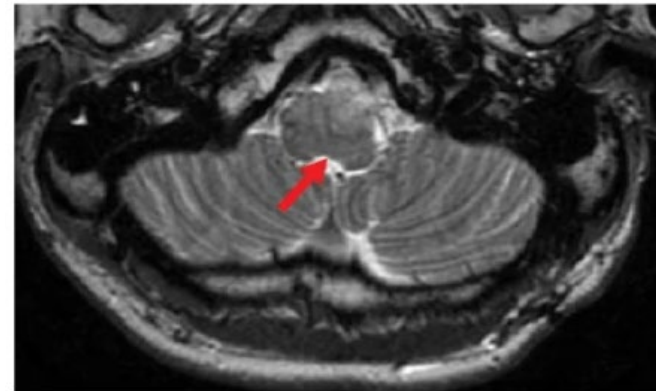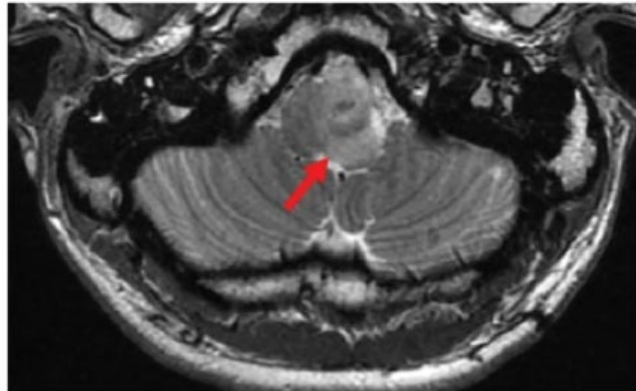
How do CAR-T kill cancer?

anti-GD2 CAR-T

Pre-treatment · Post-treatment · i.v. · i.c.v. · Pre-i.c.v. · Post-i.c.v.
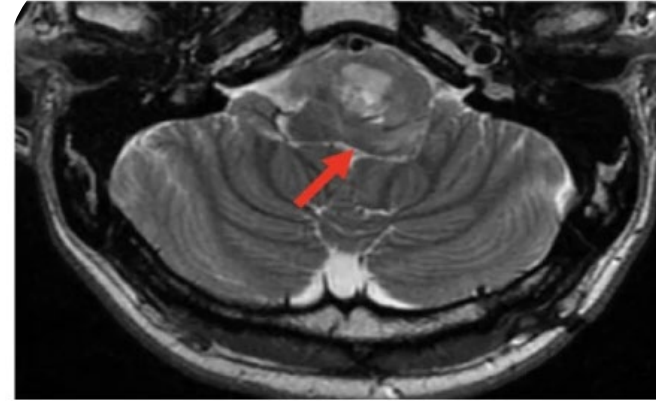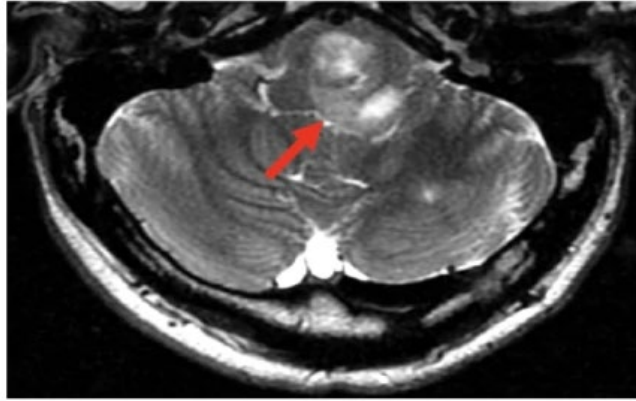
Answer:
?better than other therapies for midline gliomas?

How do CAR-T kill cancer?

anti-Claudin 18.2 CAR-T

Answer:
?better than other therapies for relapsed gastric cancer?
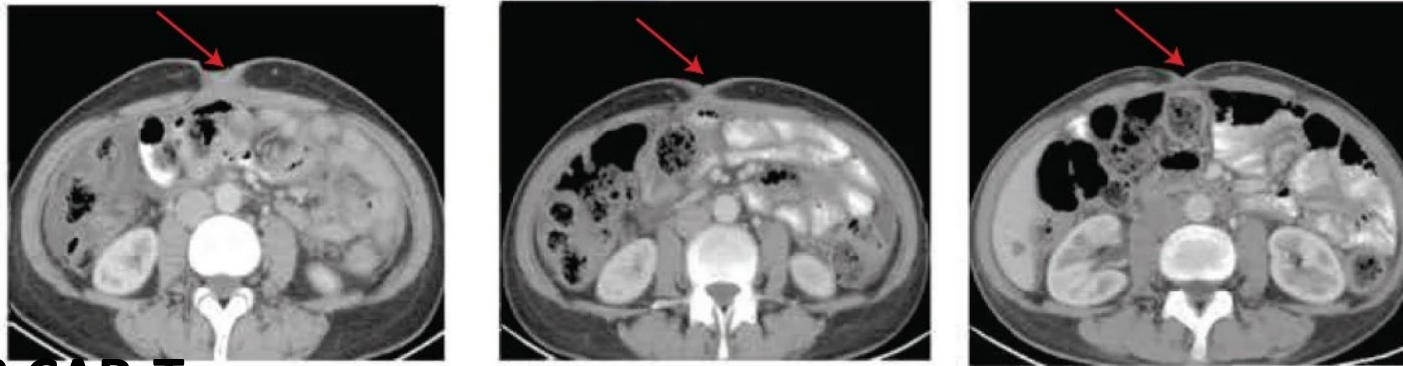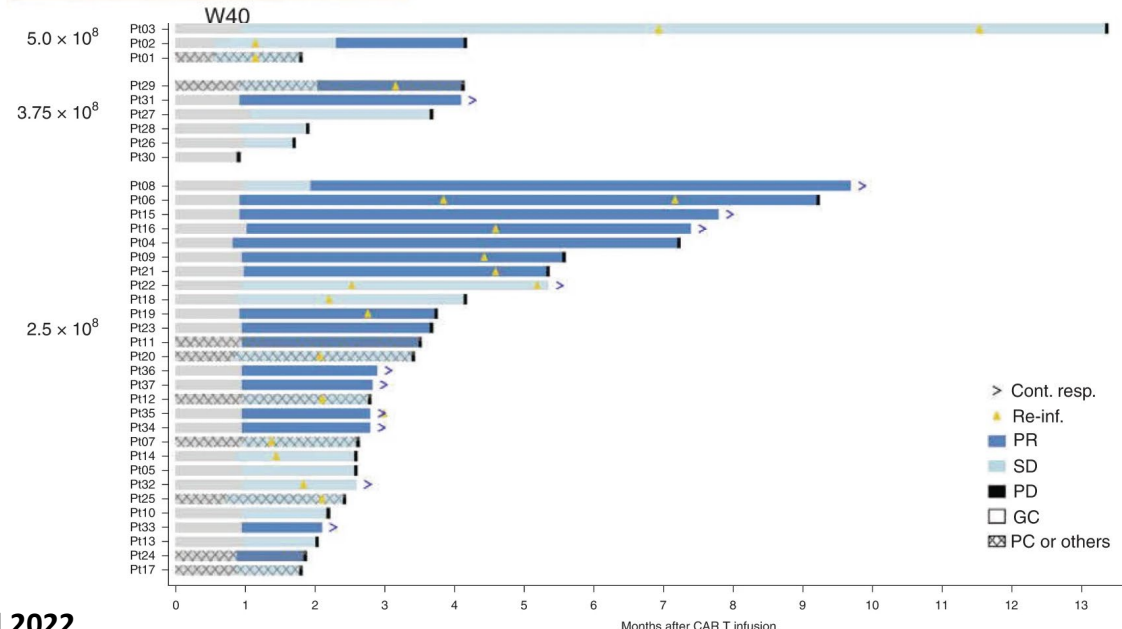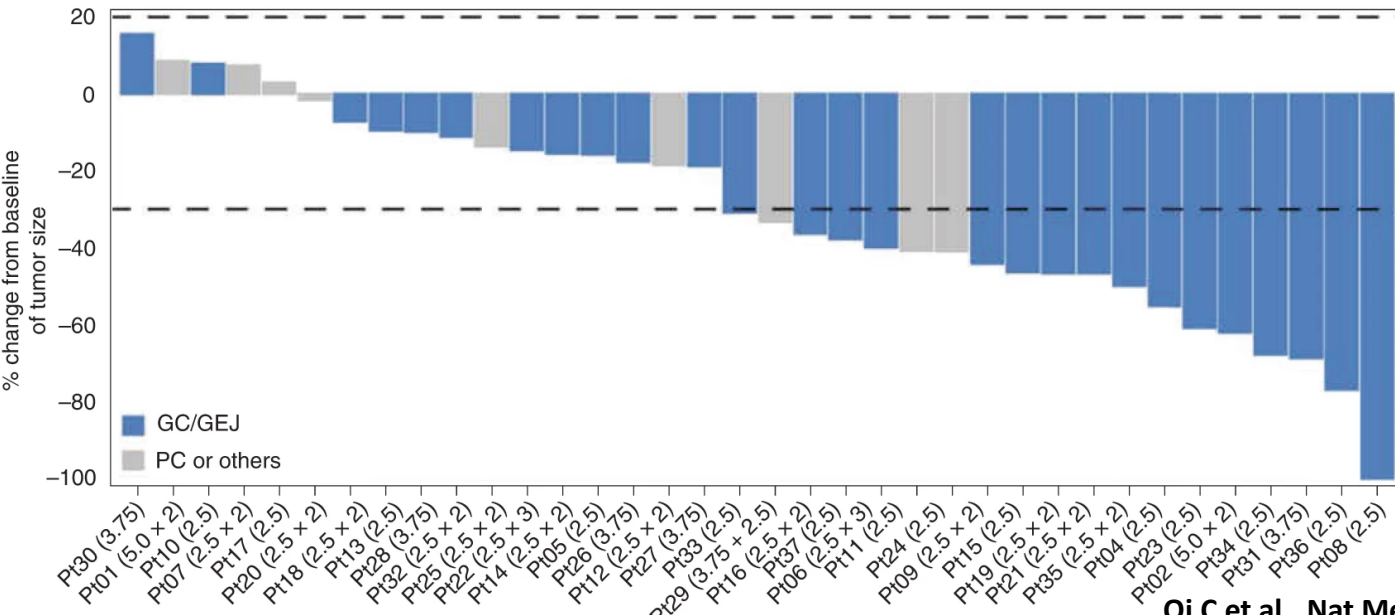
Qi C et al., Nat Med 2022

How do CAR-T fail?

CAR-T intrinsic causes   →   Action Plan:

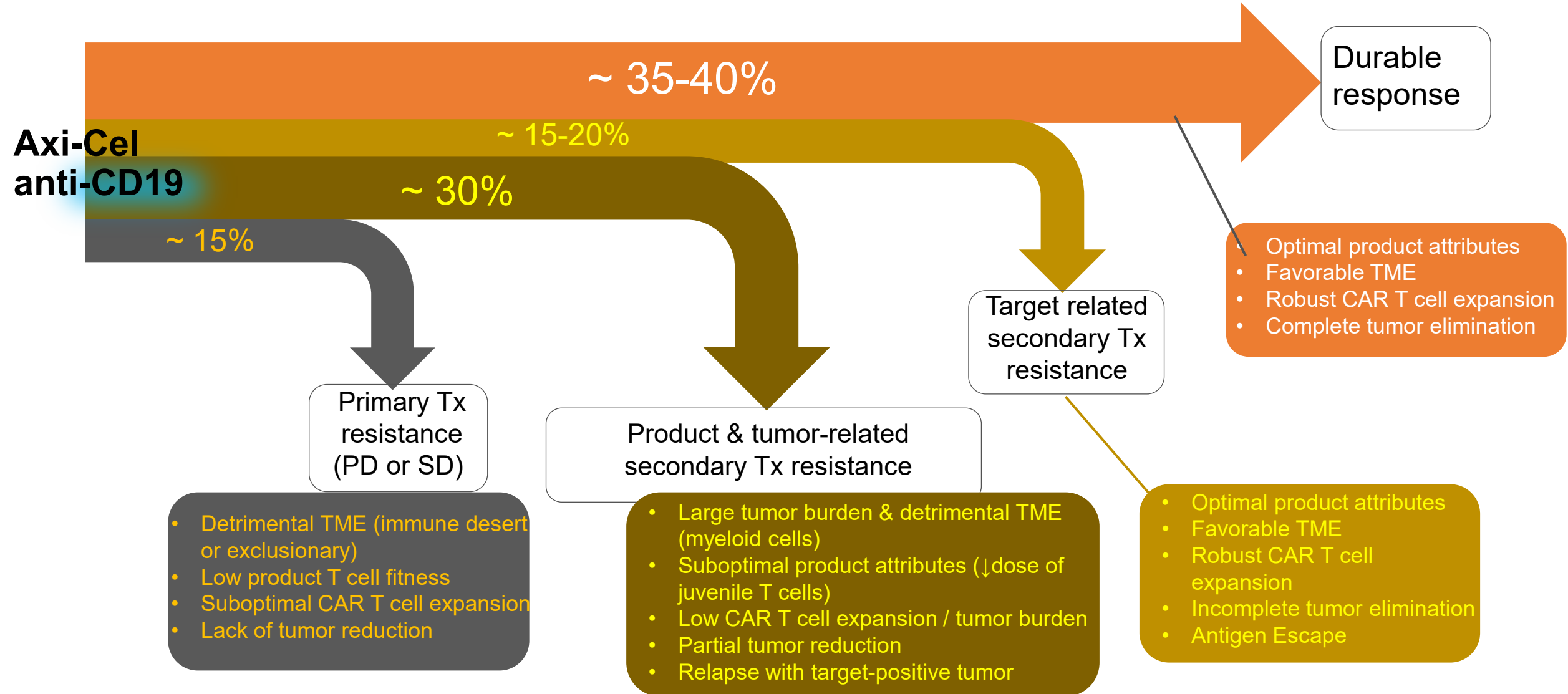Tumor intrinsic causes:

     Antigen Escape   →   Action Plan:

     Other Intrinsic   →   Action Plan:

TME intrinsic causes   →   Action Plan:

CAR-T Resistance: When and How

Axi-Cel anti-CD19

~ 35-40% → Durable response

~ 15-20%

~ 30%

~ 15%

Durable response:
- Optimal product attributes
- Favorable TME
- Robust CAR T cell expansion
- Complete tumor elimination

Primary Tx resistance (PD or SD):
- Detrimental TME (immune desert or exclusionary)
- Low product T cell fitness
- Suboptimal CAR T cell expansion
- Lack of tumor reduction

Product & tumor-related secondary Tx resistance:
- Large tumor burden & detrimental TME (myeloid cells)
- Suboptimal product attributes (↓dose of juvenile T cells)
- Low CAR T cell expansion / tumor burden
- Partial tumor reduction
- Relapse with target-positive tumor

Target related secondary Tx resistance:
- Optimal product attributes
- Favorable TME
- Robust CAR T cell expansion
- Incomplete tumor elimination
- Antigen Escape

CAR-T Resistance: Overall Action Plans

Primary Tx resistance

Secondary Tx resistance

Low product T cell fitness "Cold" TME	Large tumor burden & detrimental TME Low CAR T cell expansion / tumor burden	Antigen (e.g. CD19) escape

Manufacturing and product attributes optimization; off the shelf products

Combinations or sequencing with checkpoints; CAR armoring

Re-dosing with CAR T cells (consolidative)

Combination / consolidation with anti-CD20/CD22 agents (R^2, Bites, ADC)

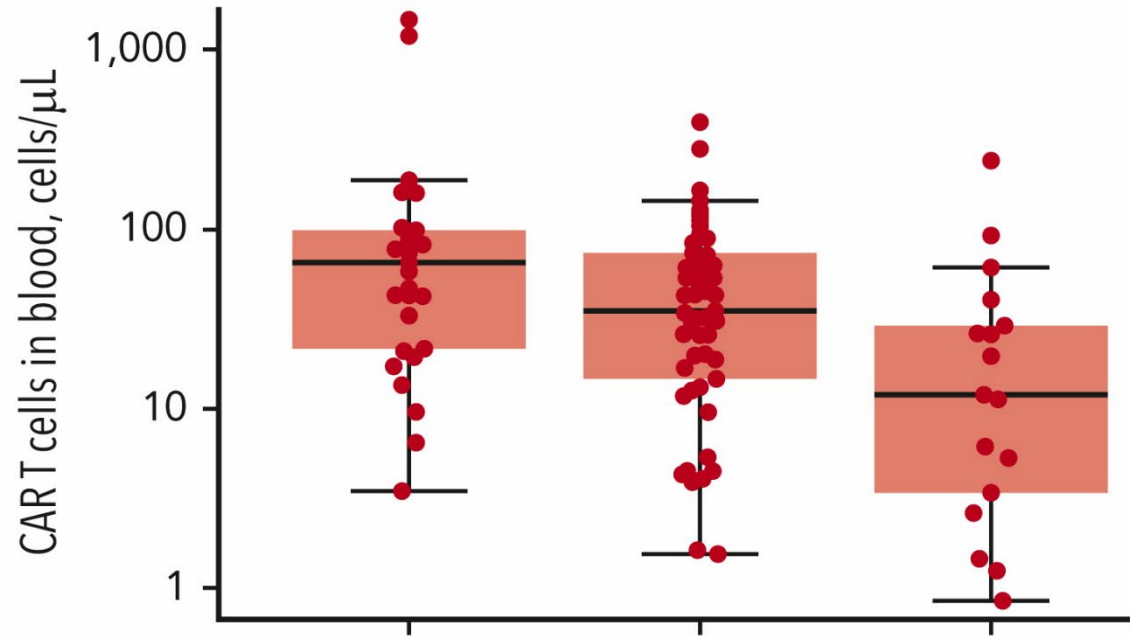Dual targeted CAR T cell products

Potentiate 'Bystander' Killing

How do CAR-T fail?

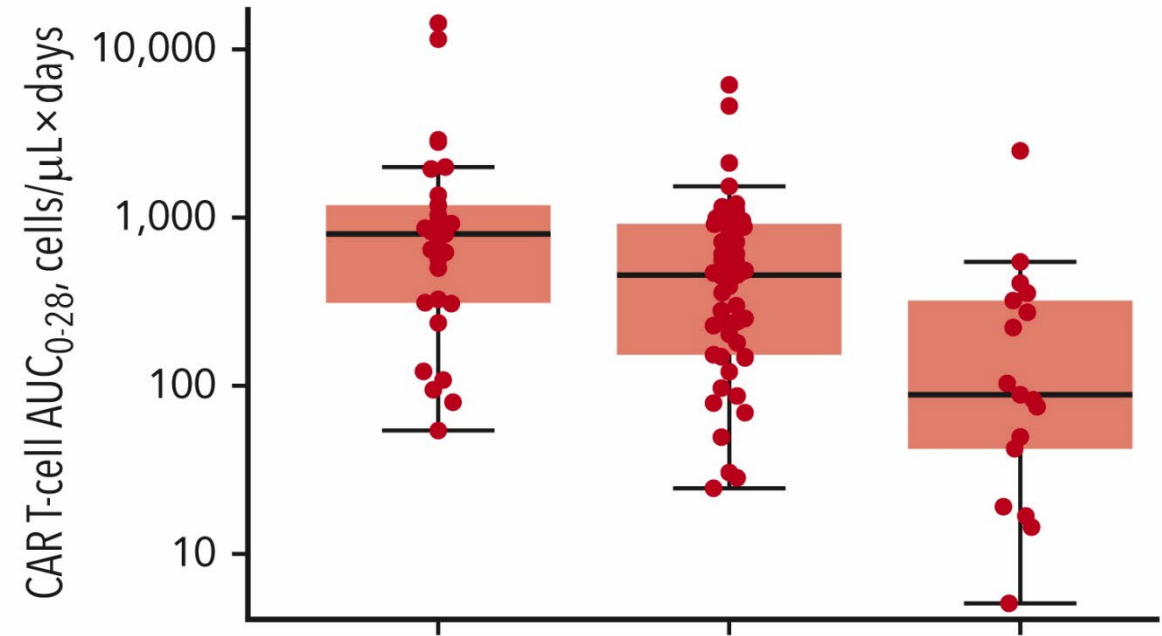**CAR-T intrinsic causes → Insufficient expansion**

CAR T-Cell Peak

Median peak, cells/μL	Ongoing Response (n = 29)	Relapse (n = 51)	Nonresponder (n = 17)
	65.76	35.27	12.08

CAR T-Cell AUC_{0-28}

Median AUC_{0-28}, cells/μL×days	Ongoing Response (n = 29)	Relapse (n = 51)	Nonresponder (n = 17)
	799.69	455.32	88.47

Neelapu, SS et al., Blood (2023) 141 (19): 2307–2315

How do CAR-T fail?

CAR-T intrinsic causes → Action Plan: IL-18 producing CAR-T

huCART19-IL18 anti-CD19

7/8 pts relapsed after prior CAR-T (mostly refractory)
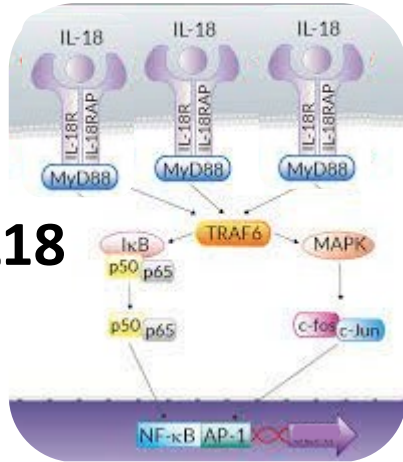
Svoboda J et al., Blood (2022) 140 (Supplement 1): 4612–4614.
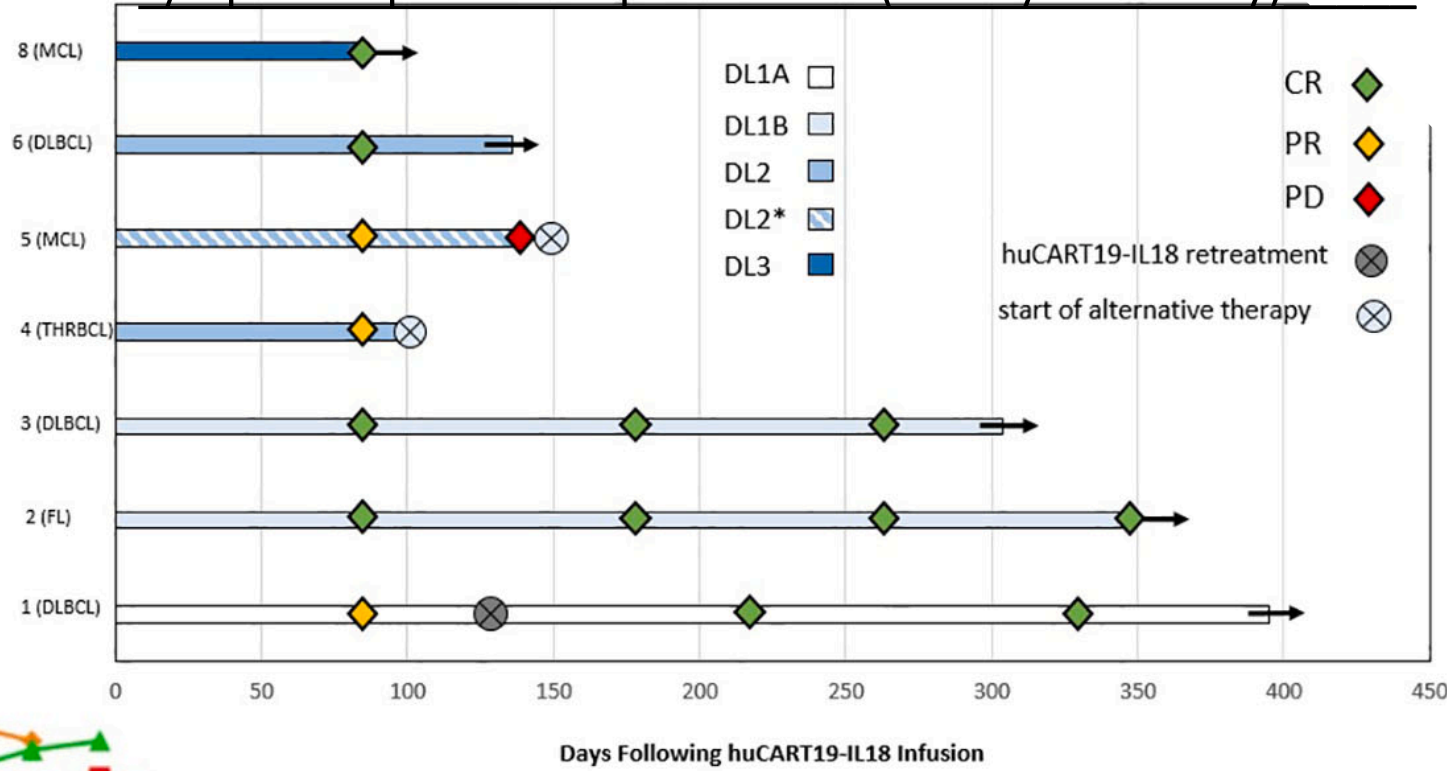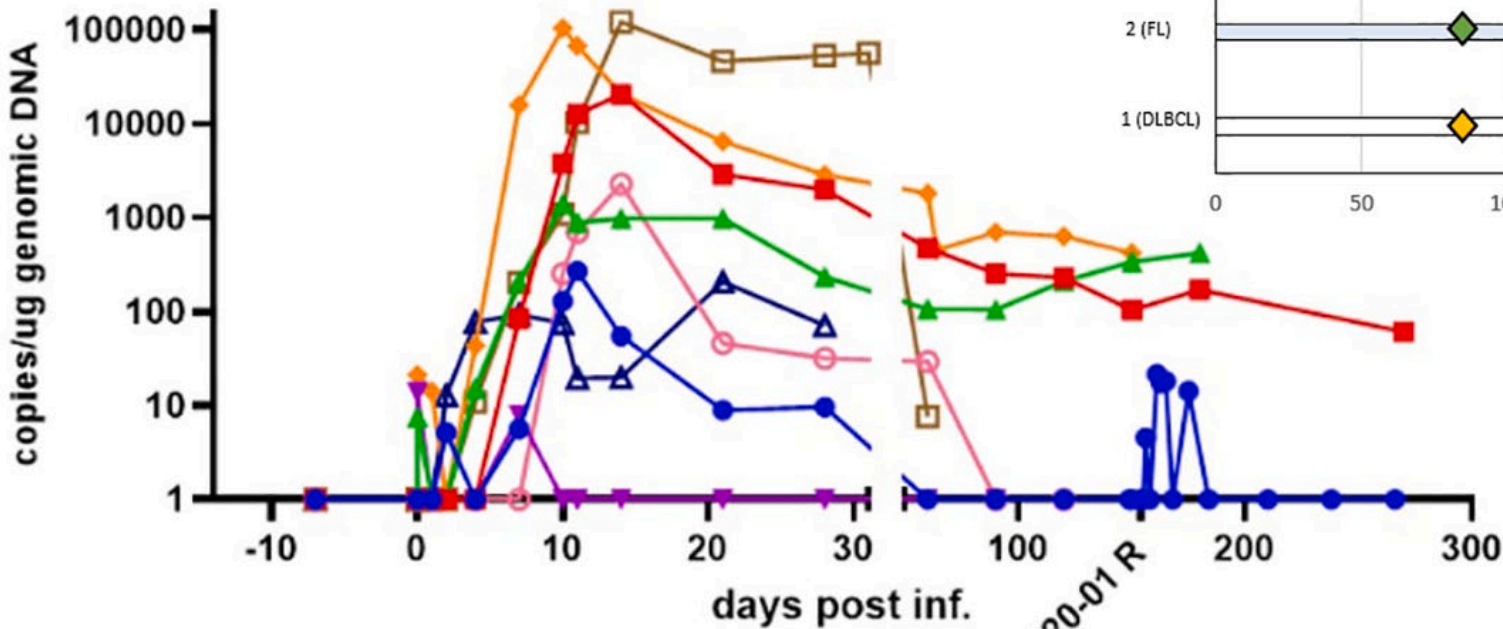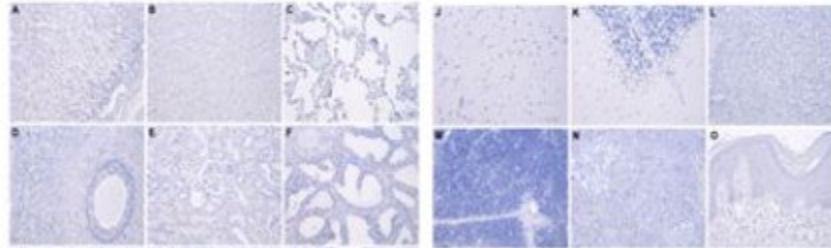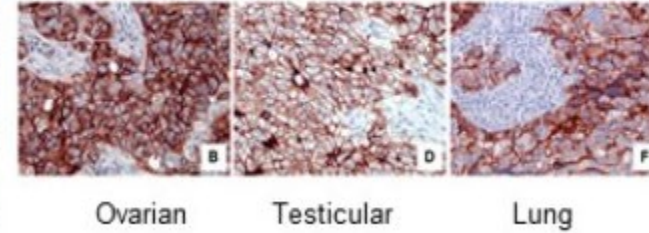
How do CAR-T fail?

CAR-T intrinsic causes → Action Plan: CARVac re-prime CAR-T *in vivo*

CLDN6 not present in healthy tissues

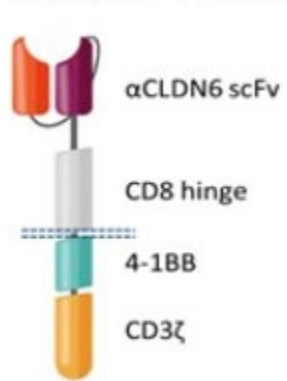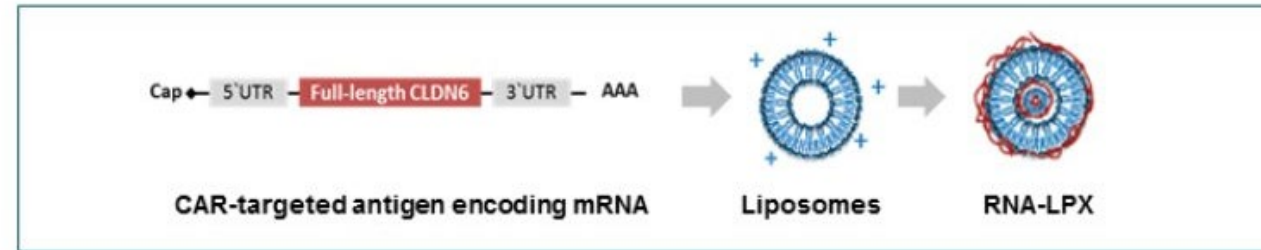CLDN6 expressed in multiple cancers
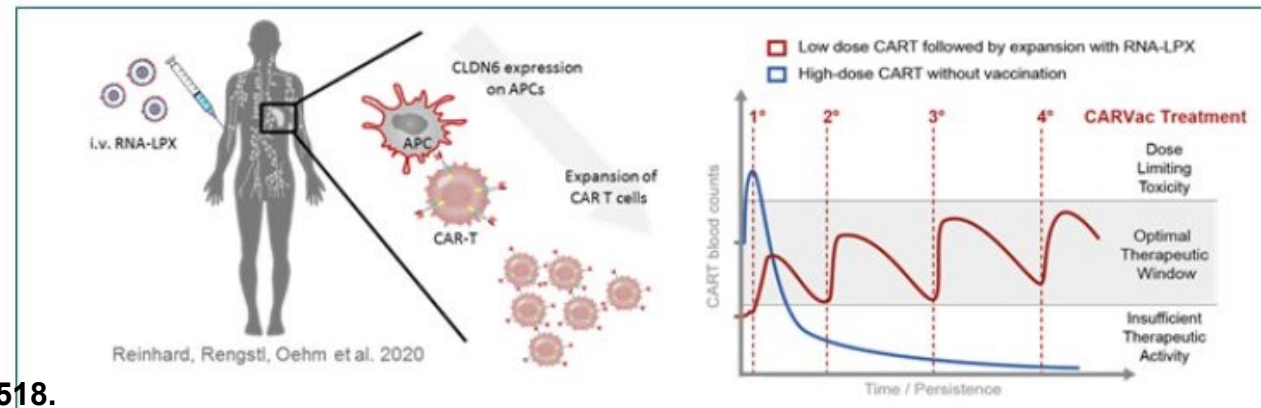
Ovarian   Testicular   Lung

BNT211
anti-CLDN6 CAR-T

αCLDN6 scFv

CD8 hinge

4-1BB

CD3ζ

CAR-T cell Amplifying RNA Vaccine (CARVac) drives in vivo expansion and efficac

CARVac production

Cap — 5'UTR — Full-length CLDN6 — 3'UTR — AAA

CAR-targeted antigen encoding mRNA   Liposomes   RNA-LPX

CARVac based CAR-T expansion

i.v. RNA-LPX

CLDN6 expression on APCs

APC

Expansion of CAR T cells

CAR-T

□ Low dose CART followed by expansion with RNA-LPX
□ High-dose CART without vaccination

CARVac Treatment

Dose Limiting Toxicity

Optimal Therapeutic Window

Insufficient Therapeutic Activity

Time / Persistence

CART blood counts

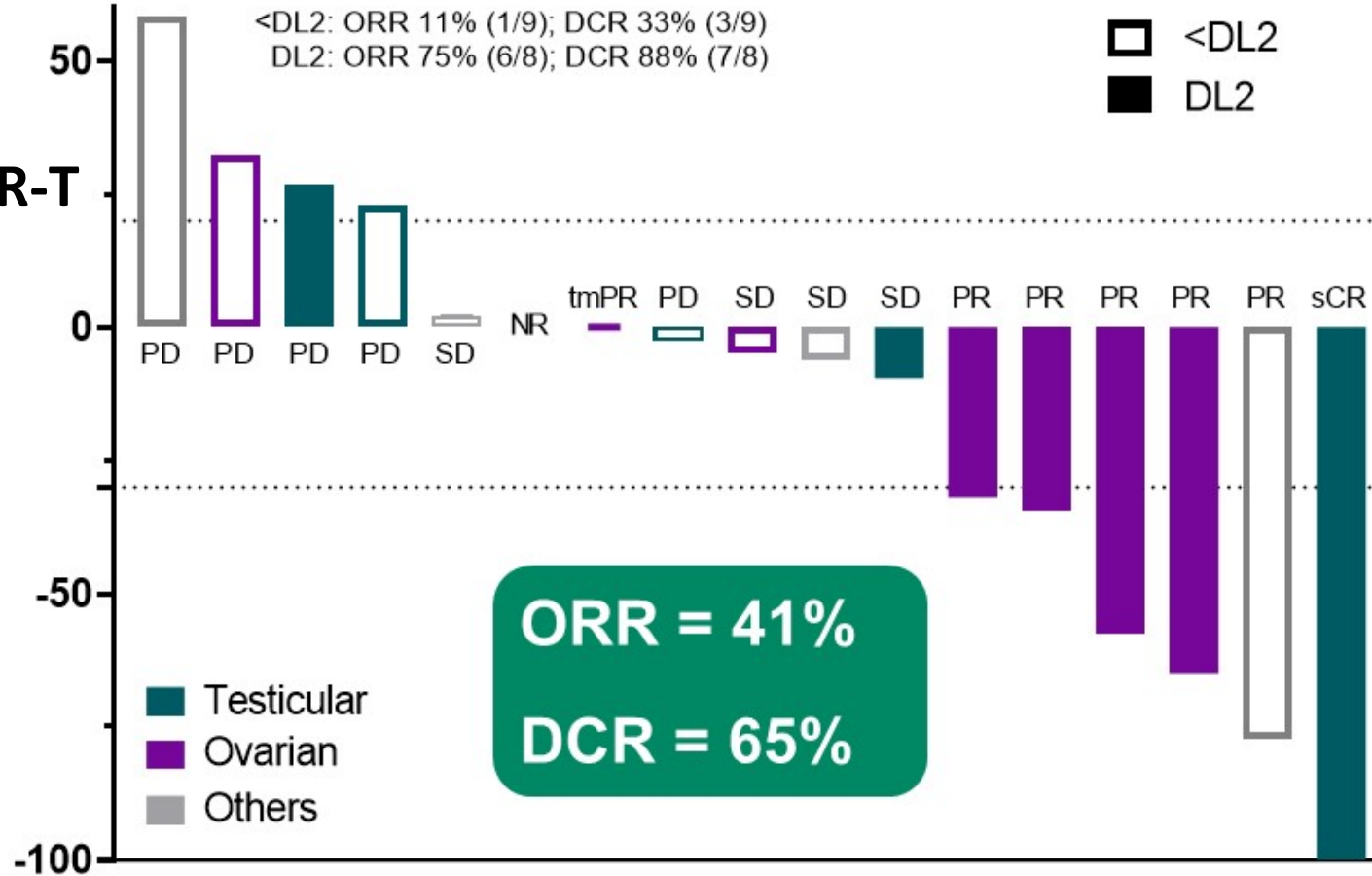Reinhard, Rengstl, Oehm et al. 2020

Mackensen A et al. J Clin Oncol. 2023;41(suppl 16):2518.

How do CAR-T fail?

CAR-T intrinsic causes → Action Plan: CARVac re-prime CAR-T *in vivo*

BNT211 anti-CLDN6 CAR-T

<DL2: ORR 11% (1/9); DCR 33% (3/9)
DL2: ORR 75% (6/8); DCR 88% (7/8)

<DL2
DL2

ORR = 41%
DCR = 65%

Testicular
Ovarian
Others

PD PD PD PD SD NR tmPR PD SD SD SD PR PR PR PR PR sCR

Tumor marker response (tmPR)

55% reduction   56 days
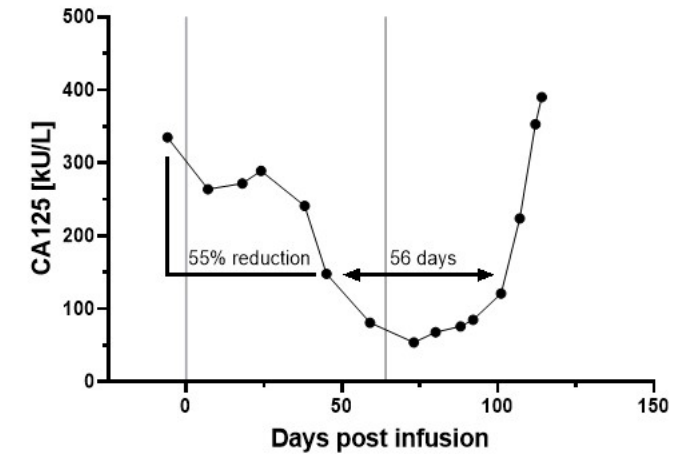
Surgical complete response (sCR)

Surgery

Mackensen A et al. J Clin Oncol. 2023;41(suppl 16):2518.
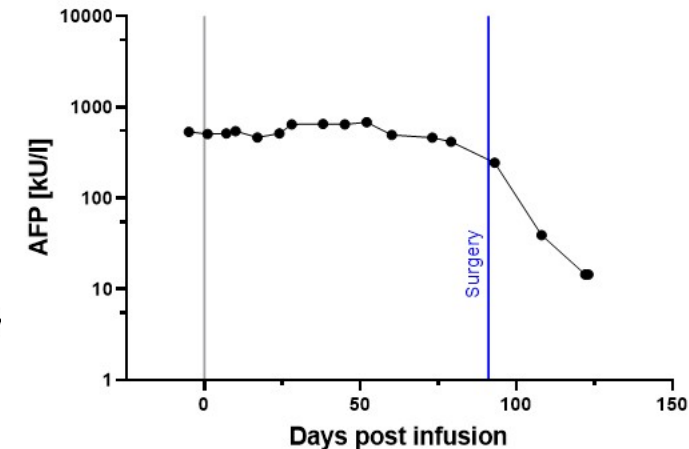
How do CAR-T fail?

CAR-T intrinsic causes → Action Plan: start with *fresh* (allogeneic) T cells
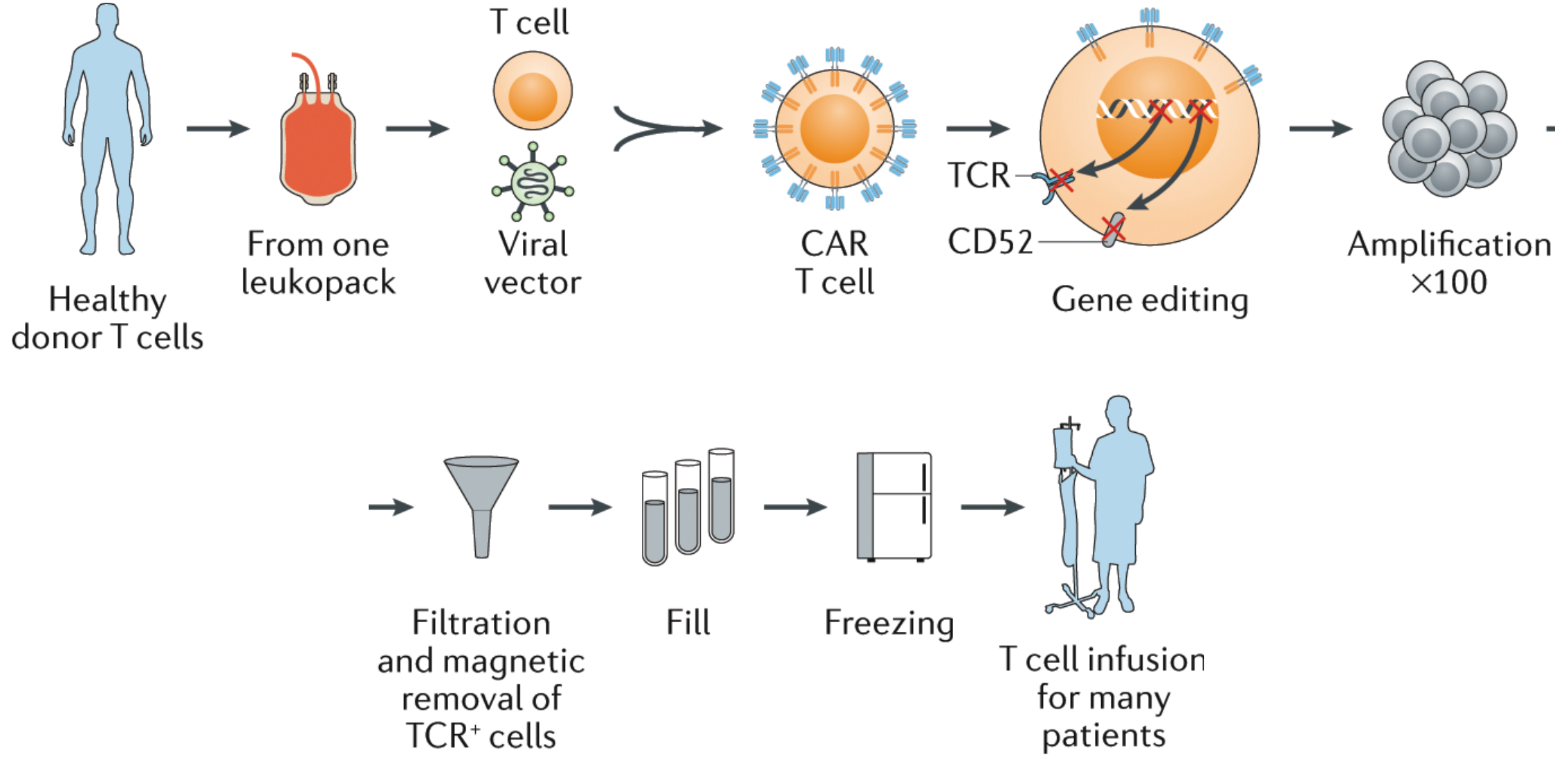
Healthy donor T cells → From one leukopack → T cell / Viral vector → CAR T cell → Gene editing (TCR, CD52) → Amplification ×100 → Filtration and magnetic removal of TCR+ cells → Fill → Freezing → T cell infusion for many patients

How do CAR-T fail?

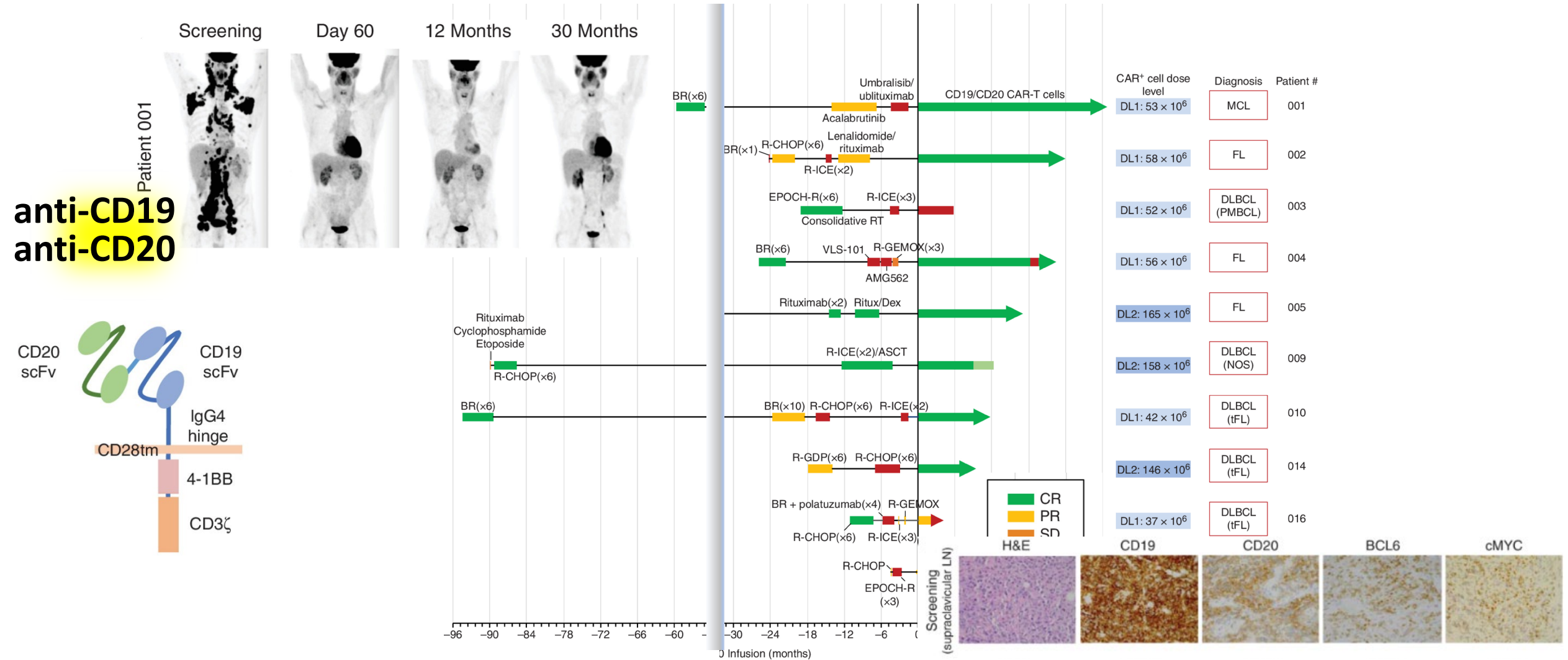## Tumor intrinsic causes e.g. Antigen Escape → Action Plan: switch target

anti-CD19
anti-CD22

Spiegel JY et al., *Nature Medicine* 2021.

How do CAR-T fail?
Tumor intrinsic causes e.g. Antigen Escape → Action Plan: switch target
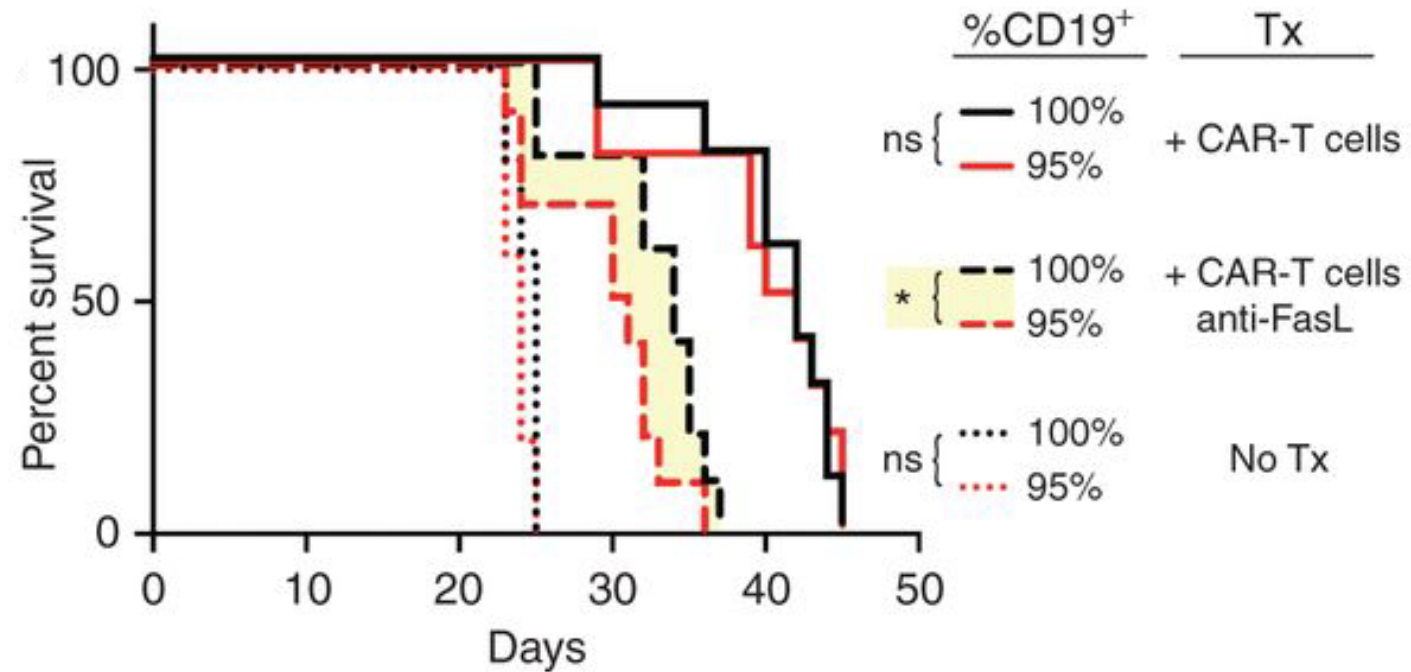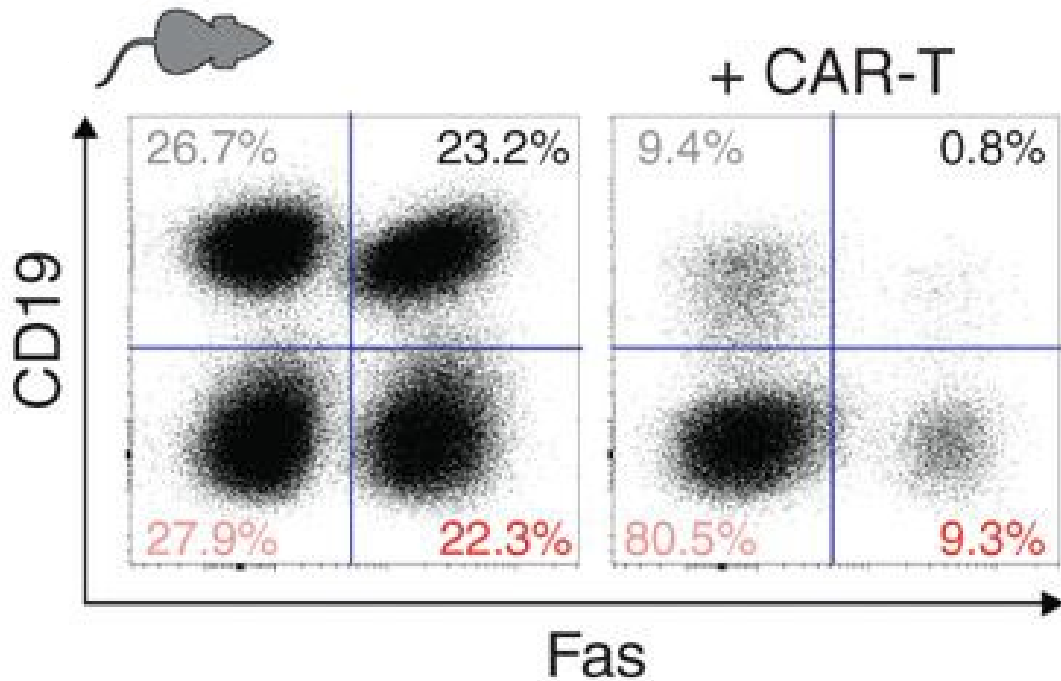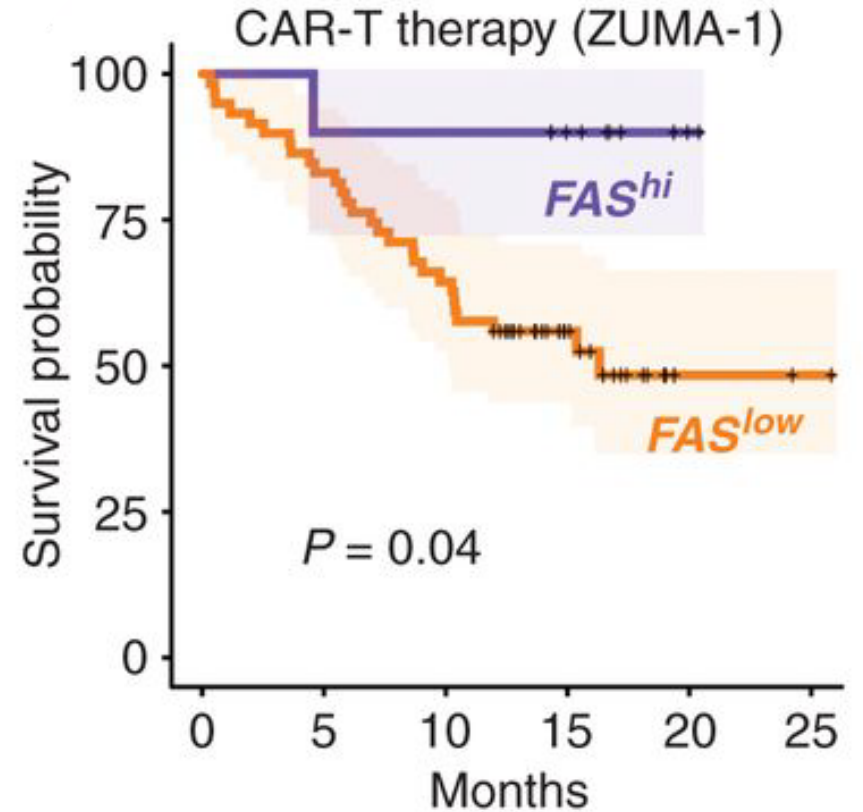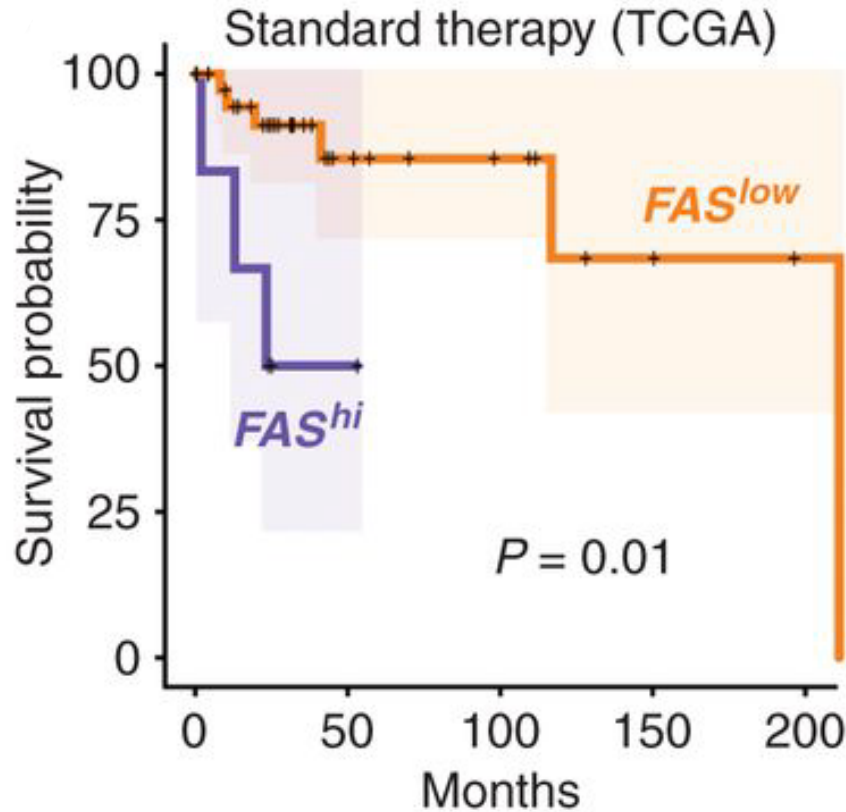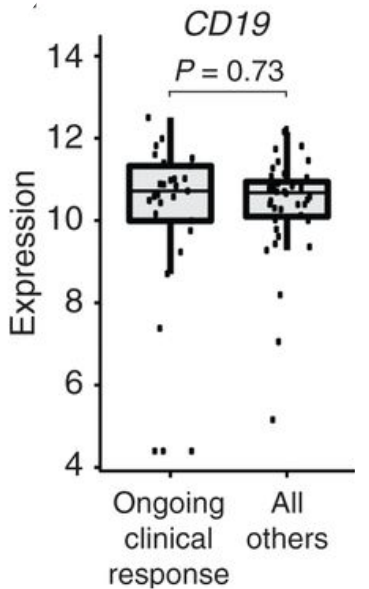

anti-CD19
anti-CD20
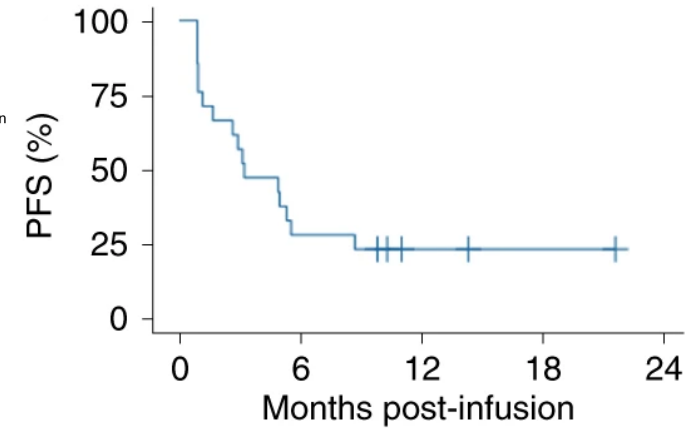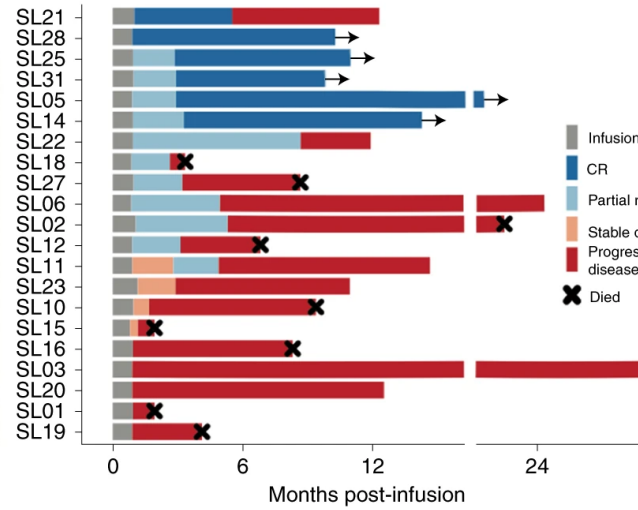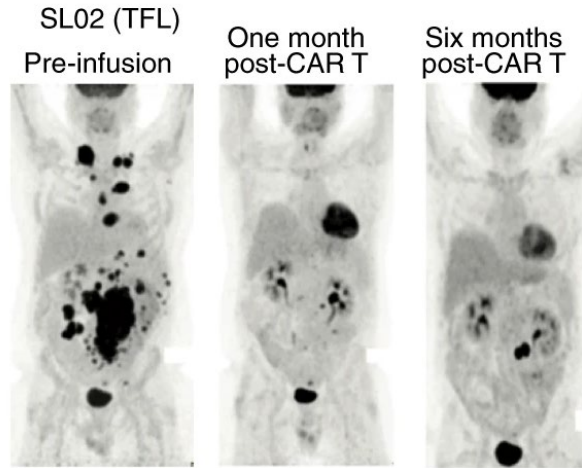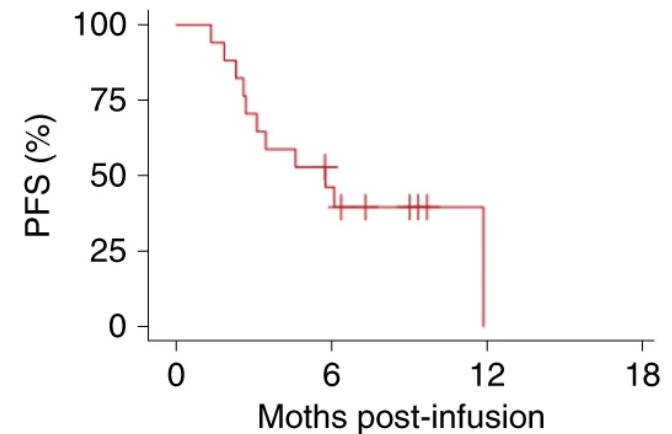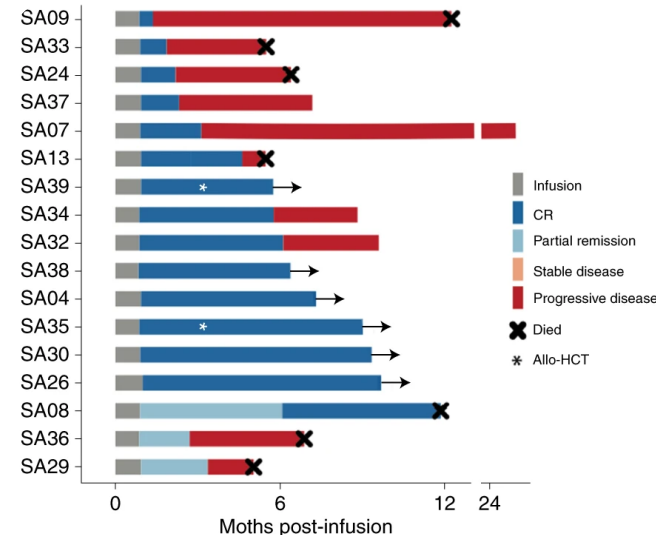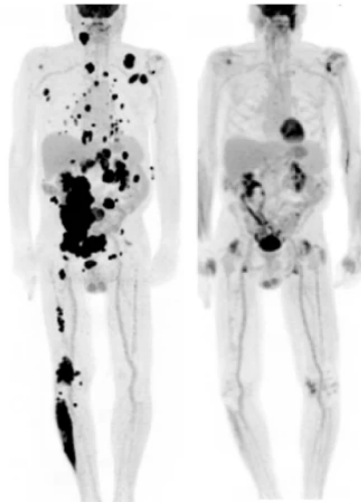
How do CAR-T fail?
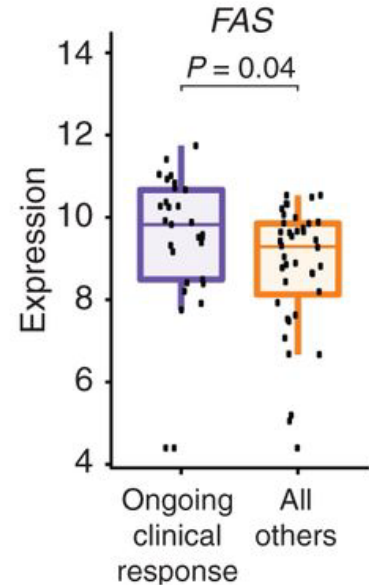## Tumor intrinsic causes e.g. Antigen Escape → Action Plan: 'Kill Bystanders'

How do CAR-T fail?
Tumor intrinsic causes e.g. Antigen Escape → Action Plan: 'Kill Bystanders'

Upadhyay R, et al., *Cancer Discovery*, 2021.

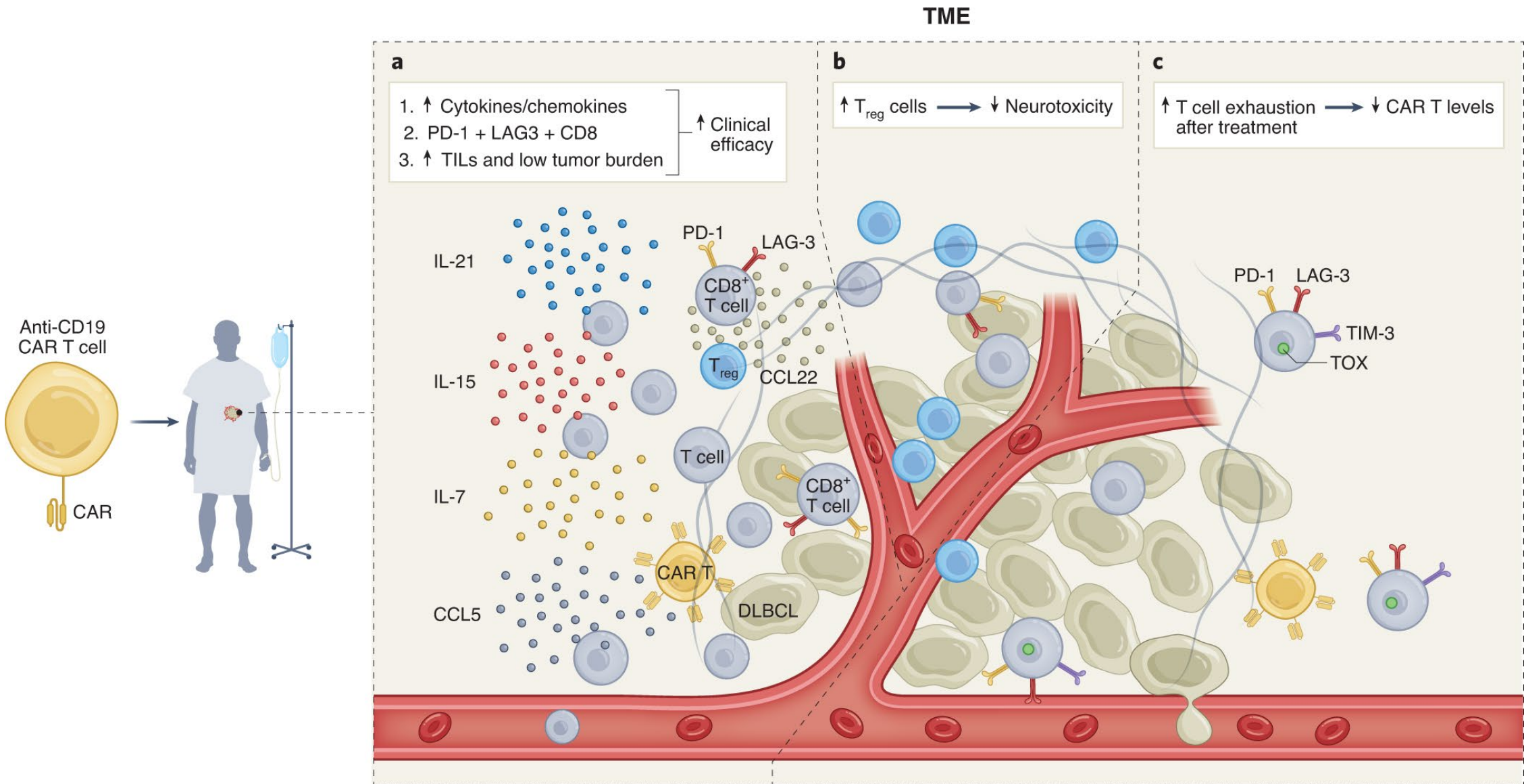# How do CAR-T fail?
Tumor intrinsic causes e.g. Antigen Escape → Action Plan: 'Kill Bystanders'

Upadhyay R, et al., *Cancer Discovery*, 2021.

Increasing Bystander Killing can *prevent* Antigen Escape

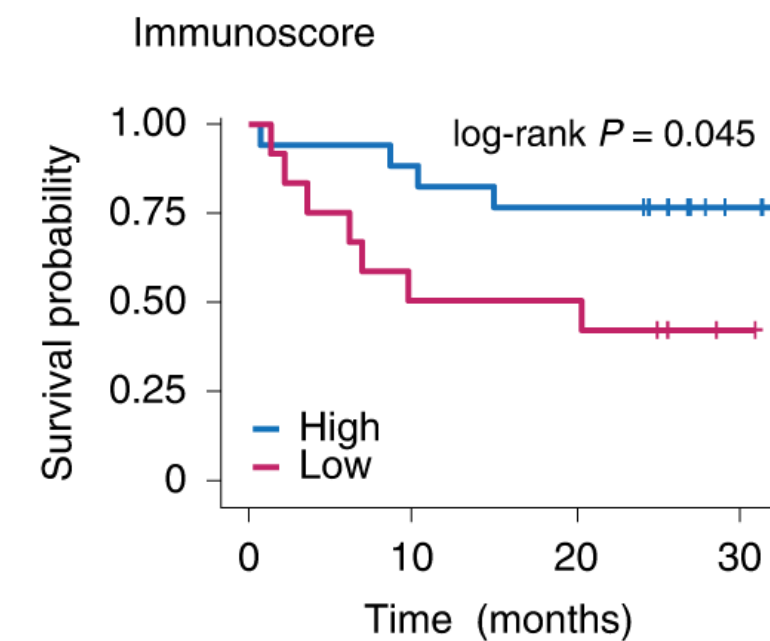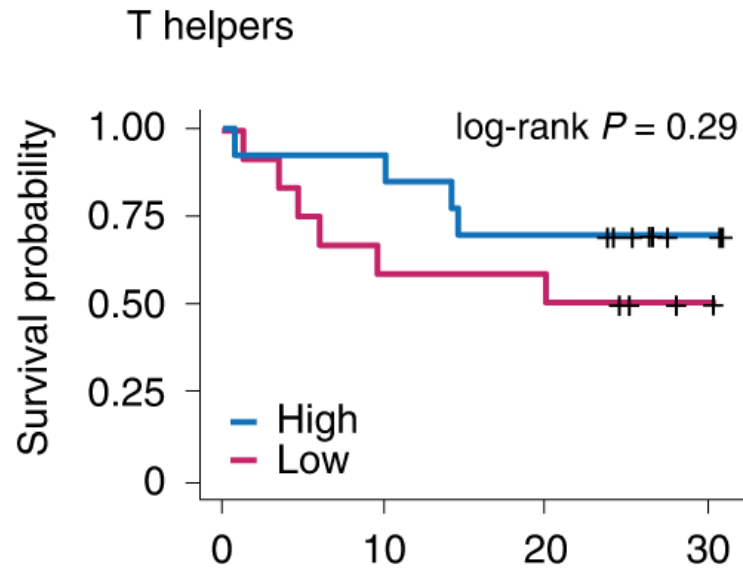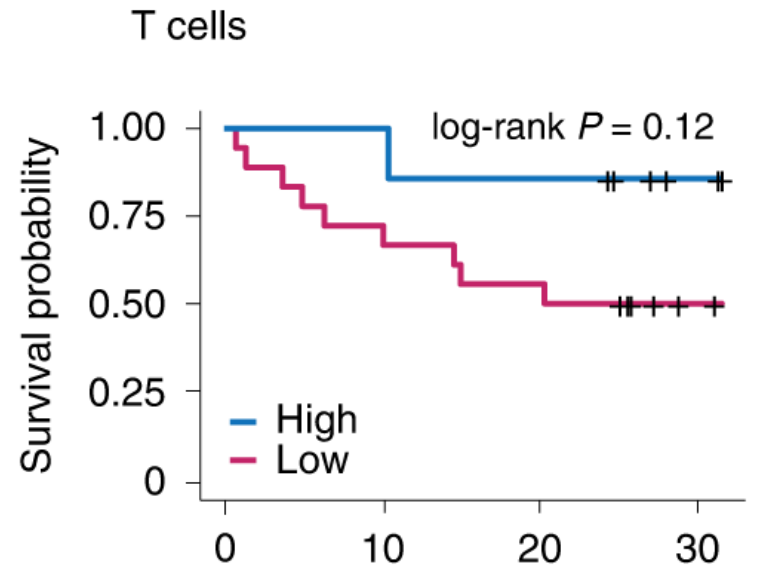Upadhyay R, et al., *Cancer Discovery*, 2021.
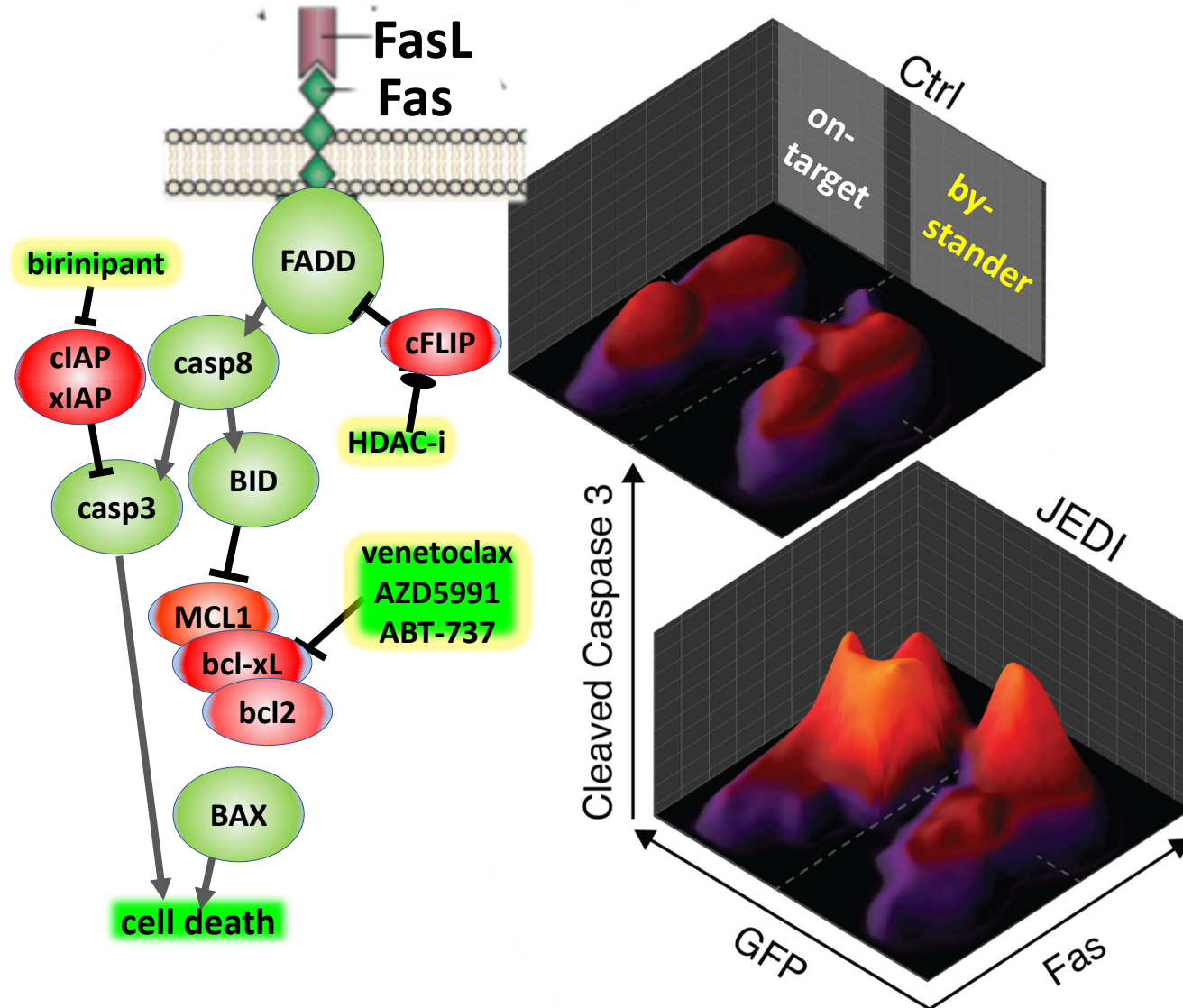
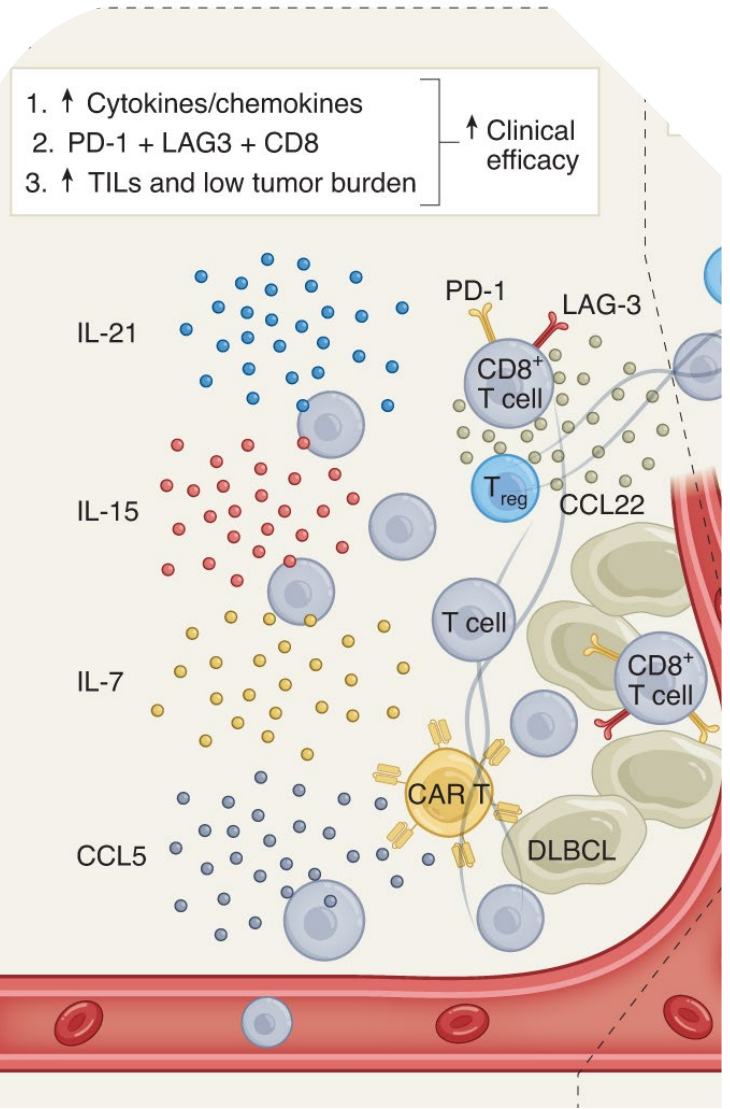# How do CAR-T fail?
TME intrinsic causes e.g. increased TILs correlate with CAR-T efficacy
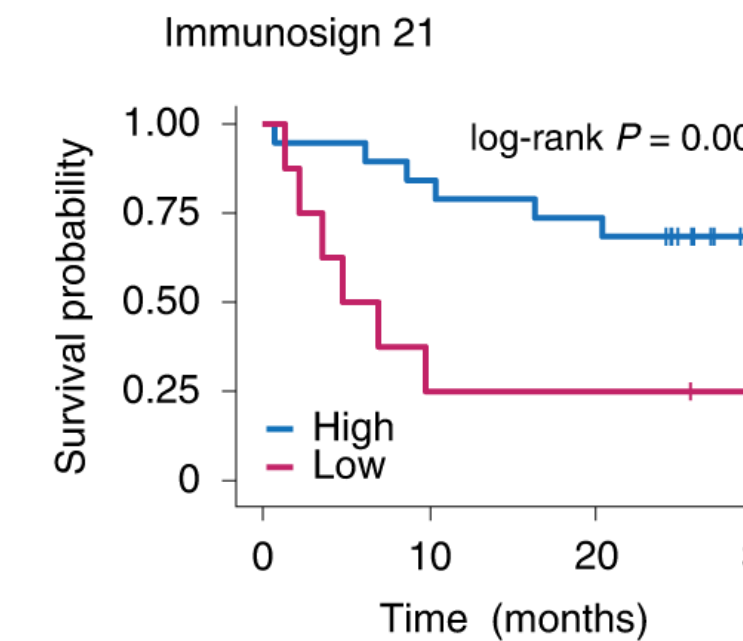
Anagnostou T et al., *Nature Medicine* 2022

Scholler N et al., Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. *Nature Medicine* 2022

How do CAR-T fail: TME

Scholler N et al., Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. *Nature Medicine* 2022

How do CAR-T fail?

TME intrinsic causes e.g. PDL1 → Action Plan: anti-PD1

Chong EA et al., *Blood* 2022
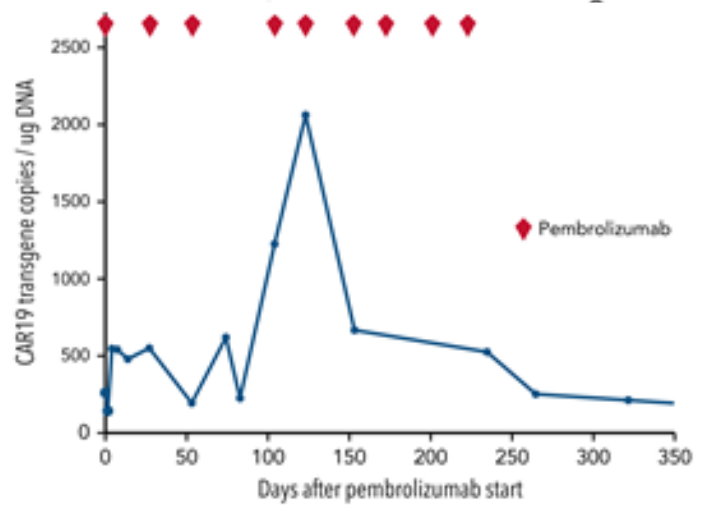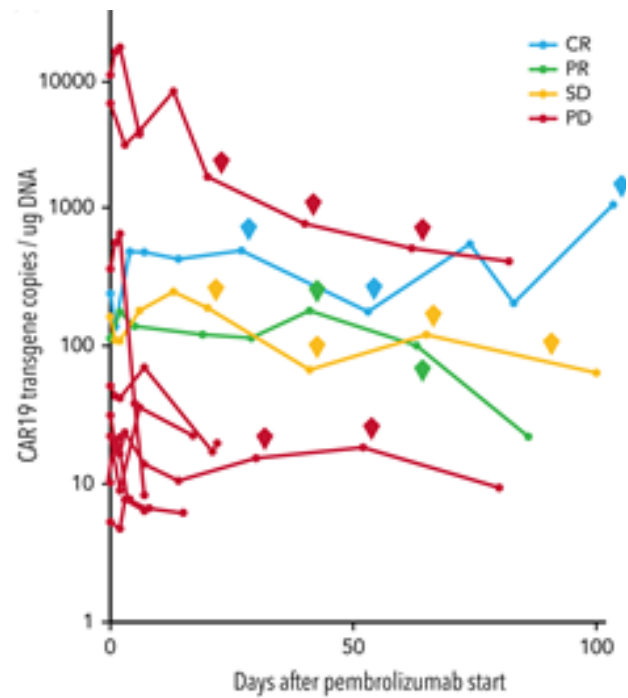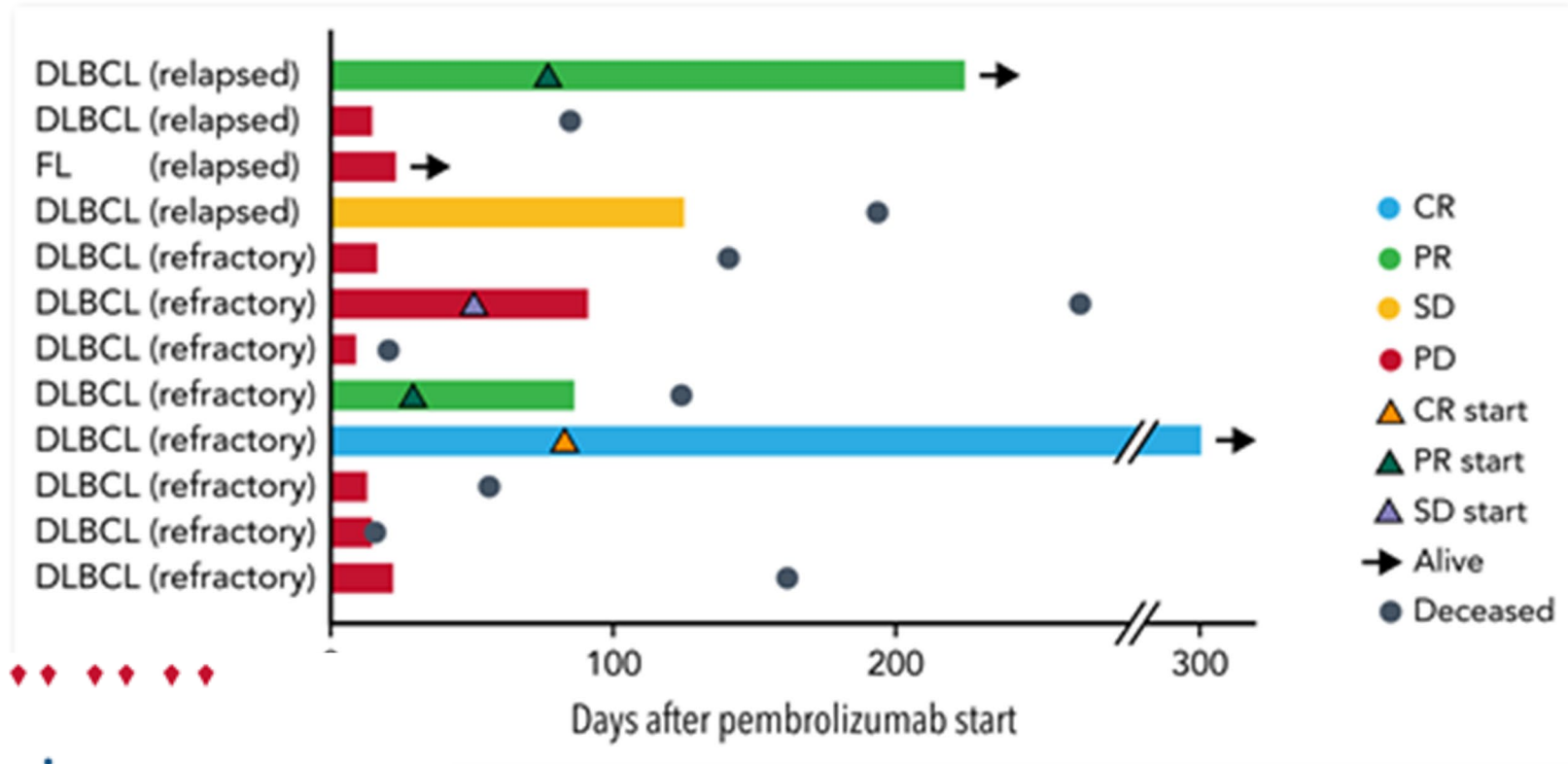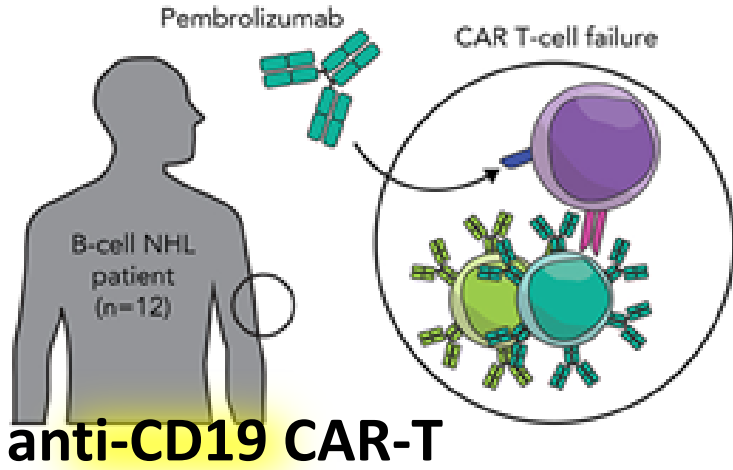
How do CAR-T fail?

TME intrinsic causes e.g. PDL1 → Action Plan: anti-PD1

anti-mesothelin CAR-T

Adusumilli PS et al., *Cancer Discov.* 2021

How do CAR-T fail: TME

anti-PSMA
TGFβ-insensitive 'armored' CAR T

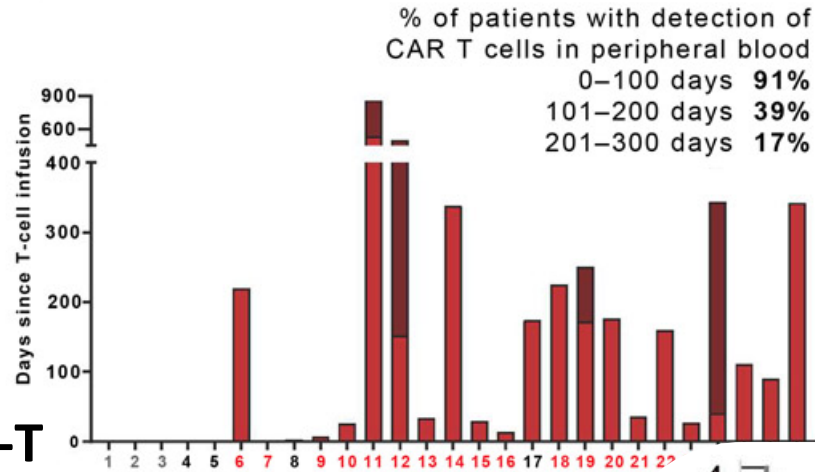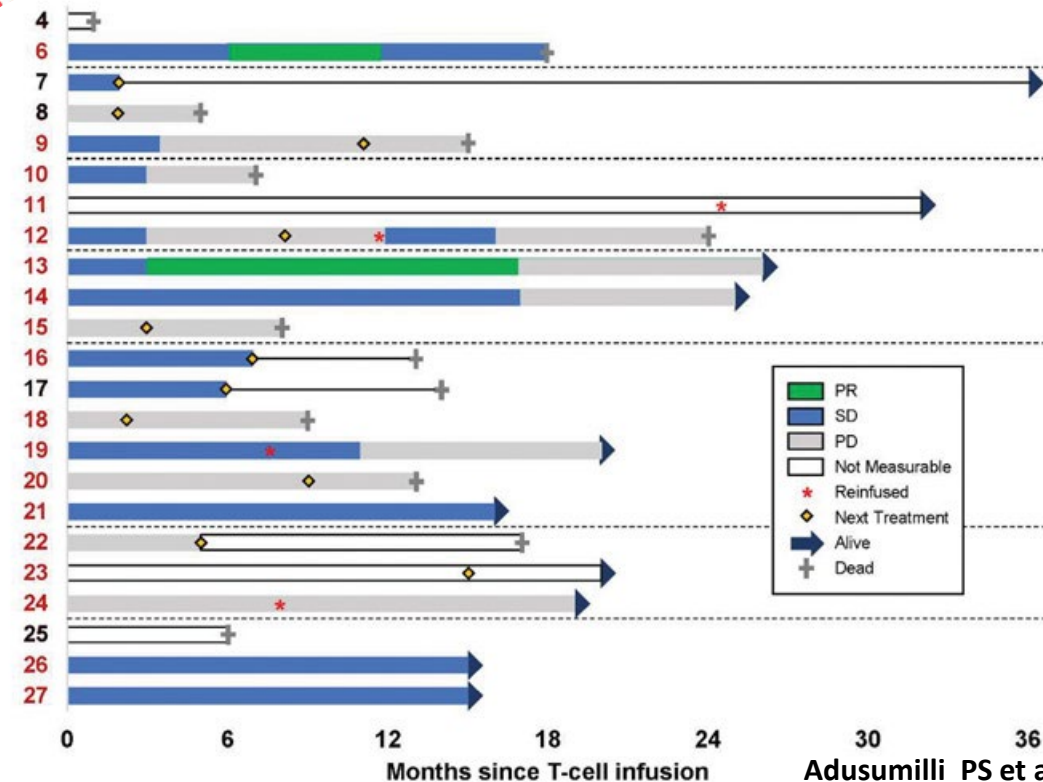Pretreatment/baseline

Post CAR T cell infusion (month 3)

Narayan V et al., *Nature Medicine* 2022

The future of CAR-T and T cell therapies:
Instead of making T-cell therapies in the lab, can we make them in our patients?

Brody J, et al., *Active and passive immunotherapy for lymphoma: proving principles and improving results*. J Clin Oncol. 2011 May 10;29(14):1864-75.

The future of CAR-T and T cell therapies:
Instead of making T-cell therapies in the lab, can we make them in our patients?

Hutchings M et al JCO 2022

*Israel Lowy MD,PhD will be speaking in more detail next hour…

The future of CAR-T and T cell therapies:

How can we measure CAR-T optimism?

Ivica NA et al., Healthcare 2021
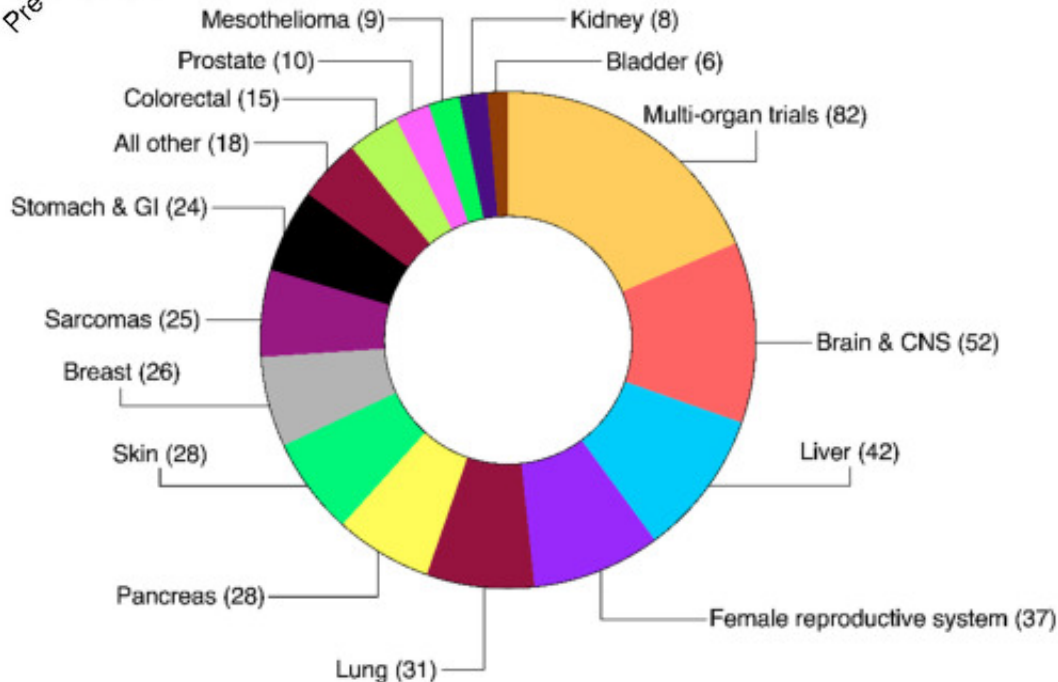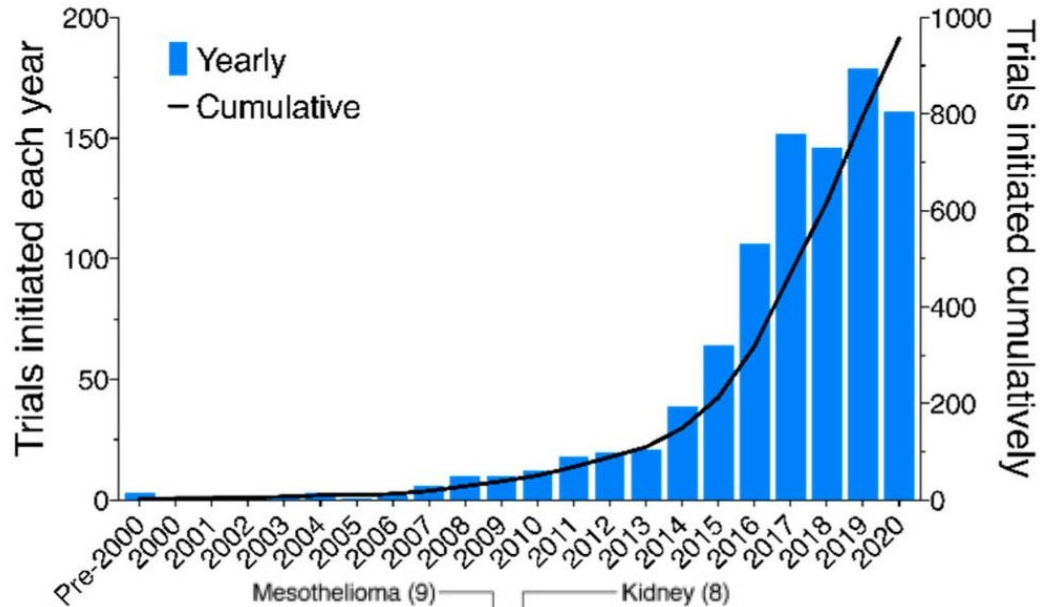
The future of CAR-T and T cell therapies:

EXTRA

CAR-T Resistance: Overall

Manufacturing starting material[4,7]

Conditioning and concomitant medications[2]

Tumor microenvironment (TME)[4,7]

Product features and fitness

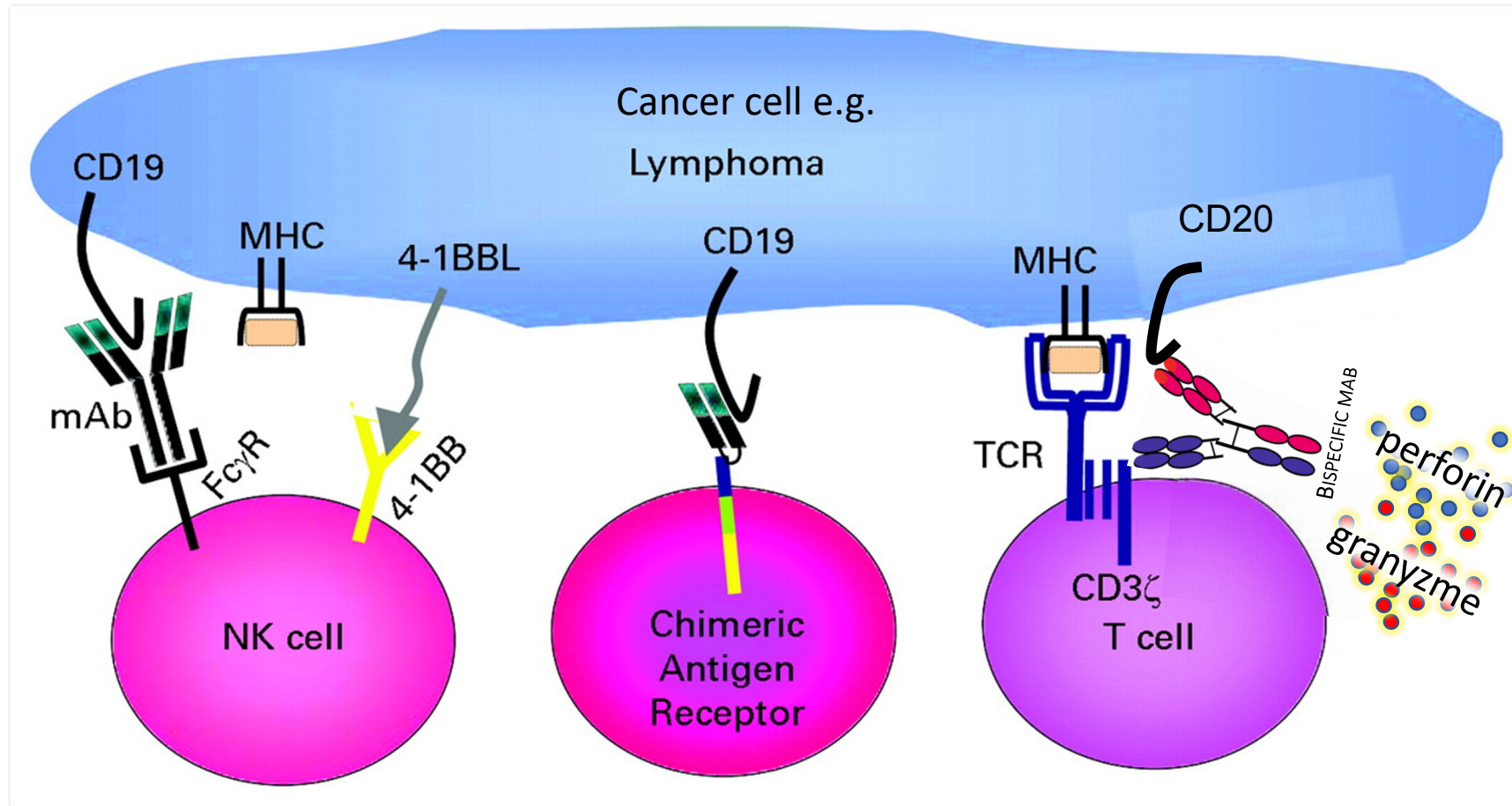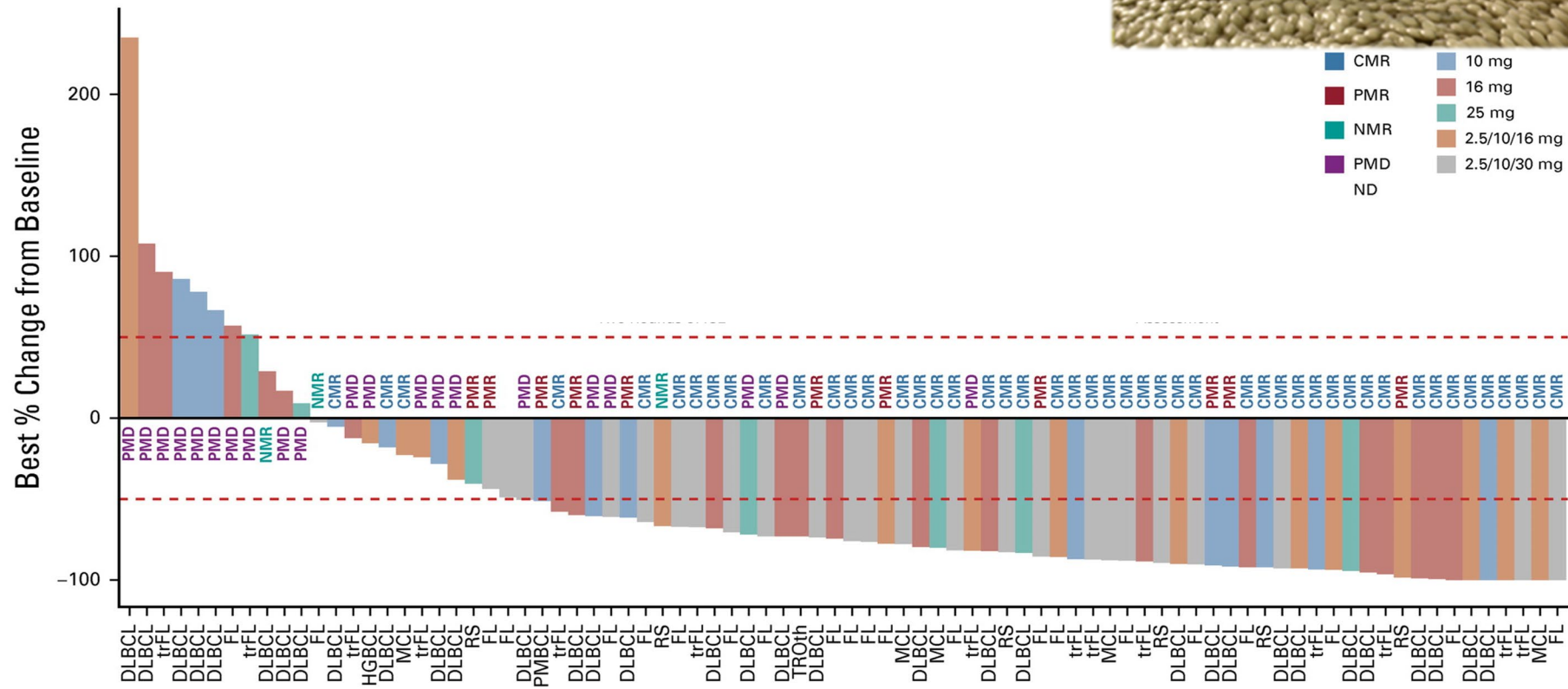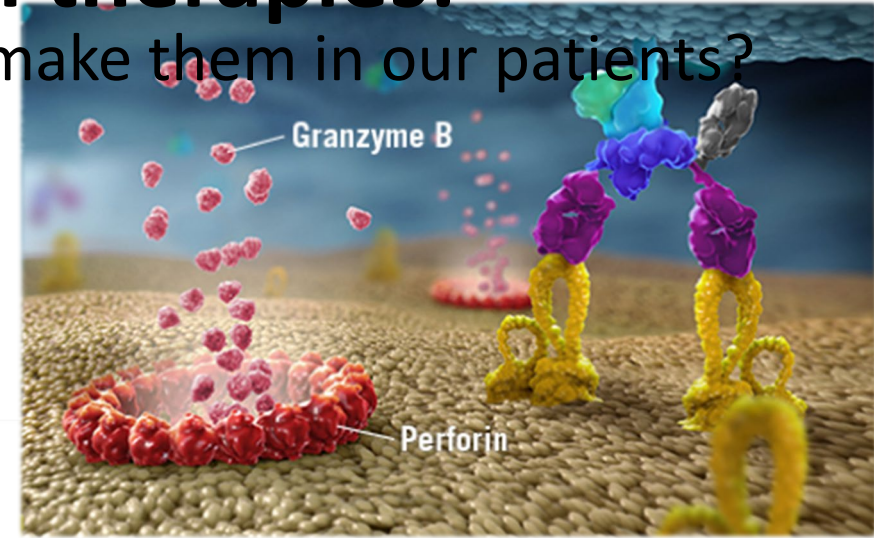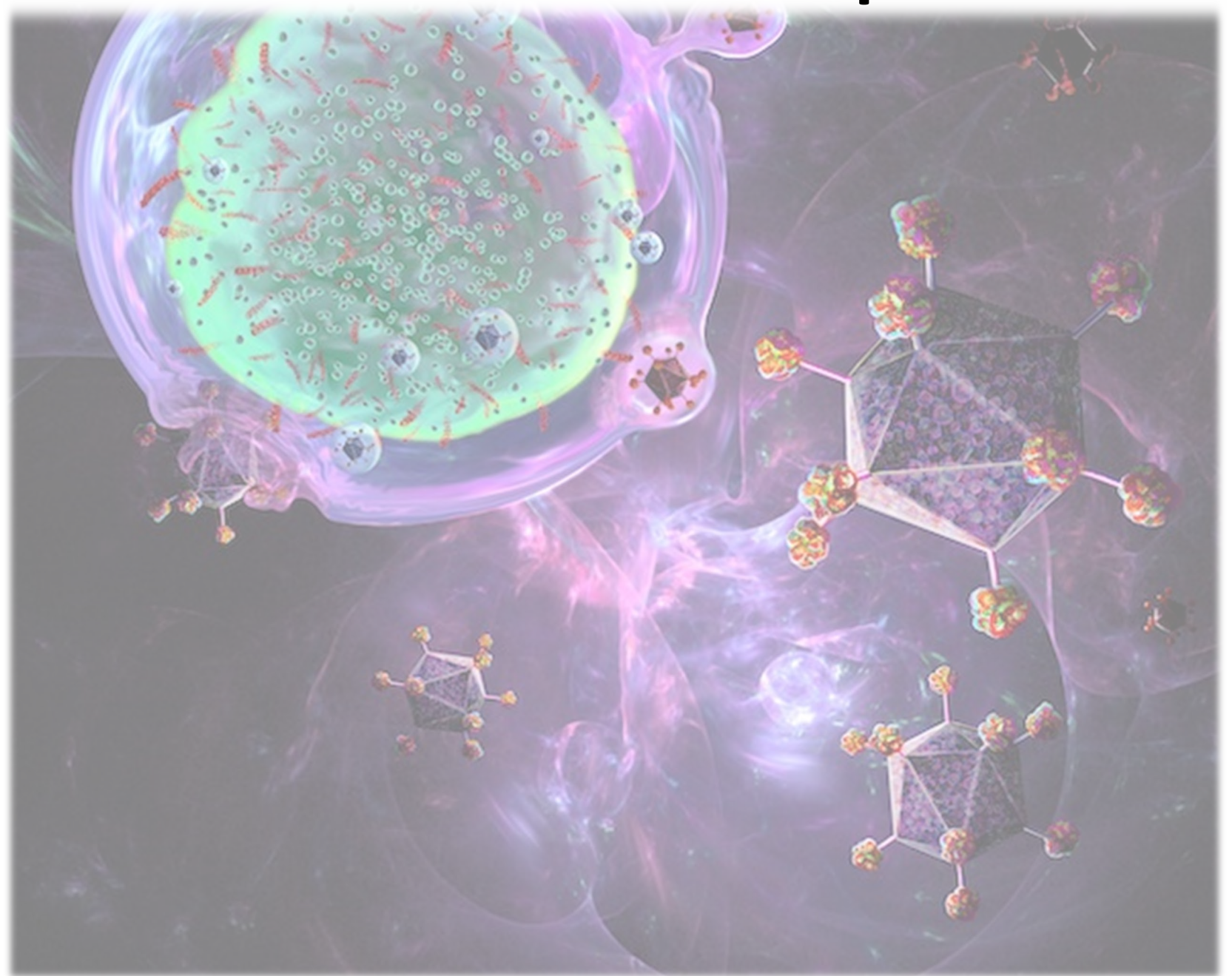- Number of specialized T cells
- T cell subsets / Phenotype
- Expansion capability
- Polyfunctionality[1]

Tumor burden[3]

Tumor cell biology[5]

Tumor immune contexture

PK & PD Profile[6, 7]

Systemic inflammatory state[7]

Clinical Efficacy and Toxicity

CAR, chimeric antigen receptor; PD, pharmacodynamics; PK, pharmacokinetics.

1. Rossi JM, et al. *Blood*. 2018. 2. Kochenderfer JN, et al. *J Clin Oncol*. 2017. 3. Locke FL, et al. ASCO 2018. #3039. 4. Rossi JM, et al. AACR 2018. #LB-016.
5. Neelapu SS, et al. ASH 2017. #578. 6. Neelapu SS, et al. *N Engl J Med*. 2017. 7.Locke FL et al. Blood Advances 2020

CAR-T Resistance: How and Who

CAR-T Resistance: Action Plan

Increase Efficacy / Overcome Resistance in B Cell Malignancies
- Increase in situ number and activity of CAR T cells
- Broaden up the category of immune effector mechanisms

Maximize applicability of current generation of CAR products
- Expand label to other histologies
- Earlier line, high risk patients

Current generation of CAR T

Next Generation Product Manufacturing
- Short autologous manufacturing
- Dual targeted products with optimized T cell composition
- Safe and effective off the shelf products

Optimize safety profile
- Optimized utilization of corticosteroids, IL-6R blockade
- Introduction of other targeted agents or product optimizations
- Optimized patient stratification and monitoring in outpatient setting

How do CAR-T fail: TME

Scholler N et al., Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. Nature Medicine (2022)

How do CAR-T fail: TME

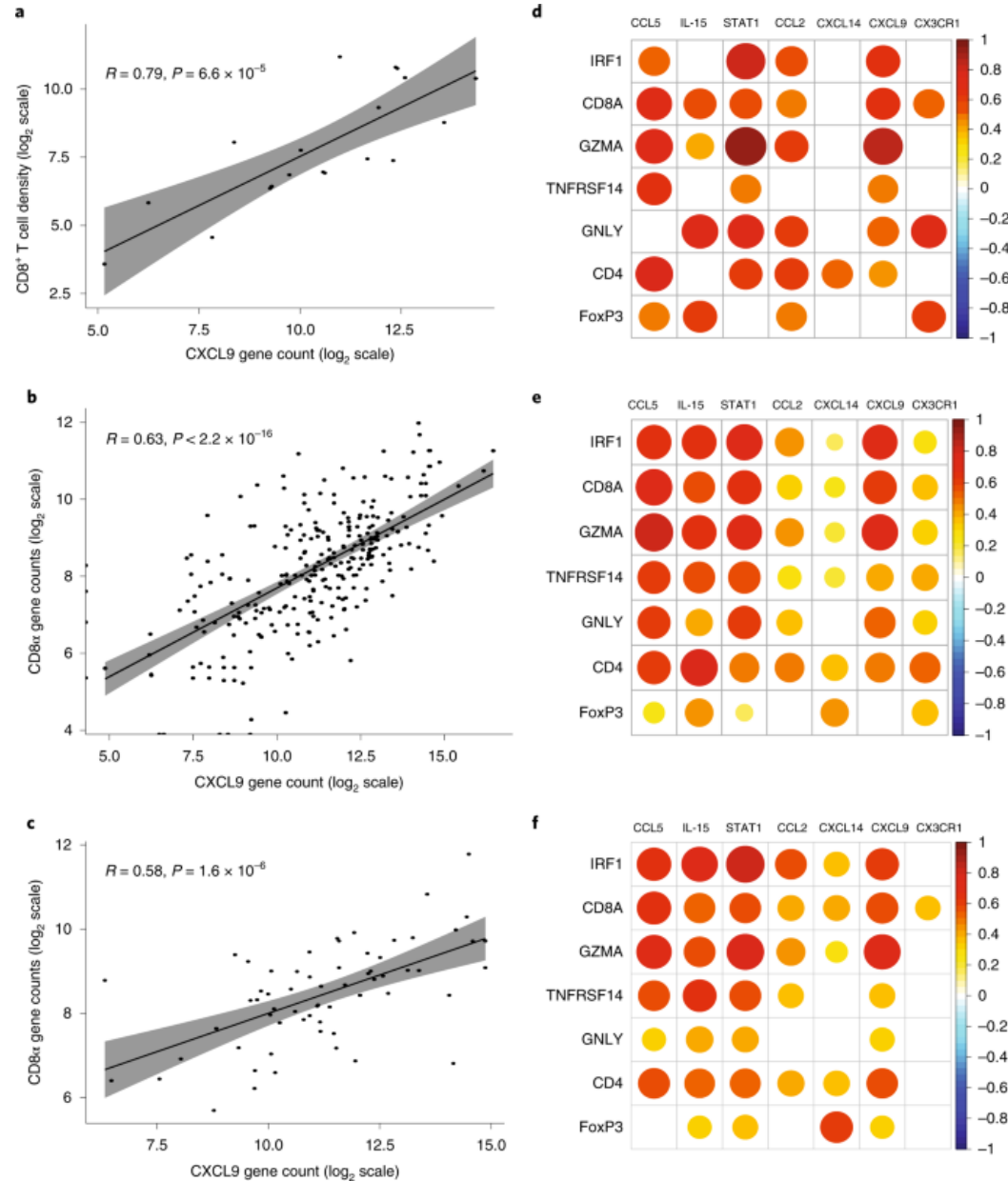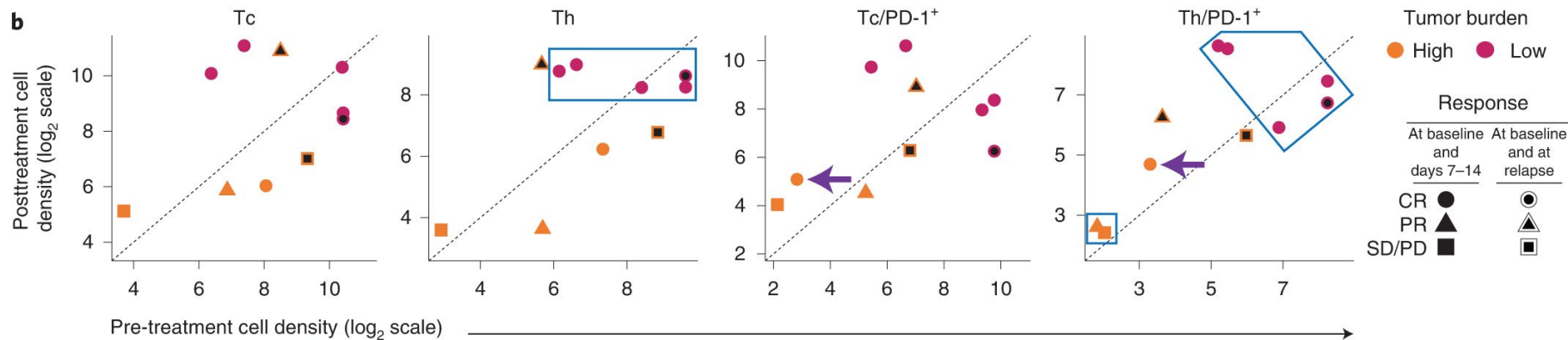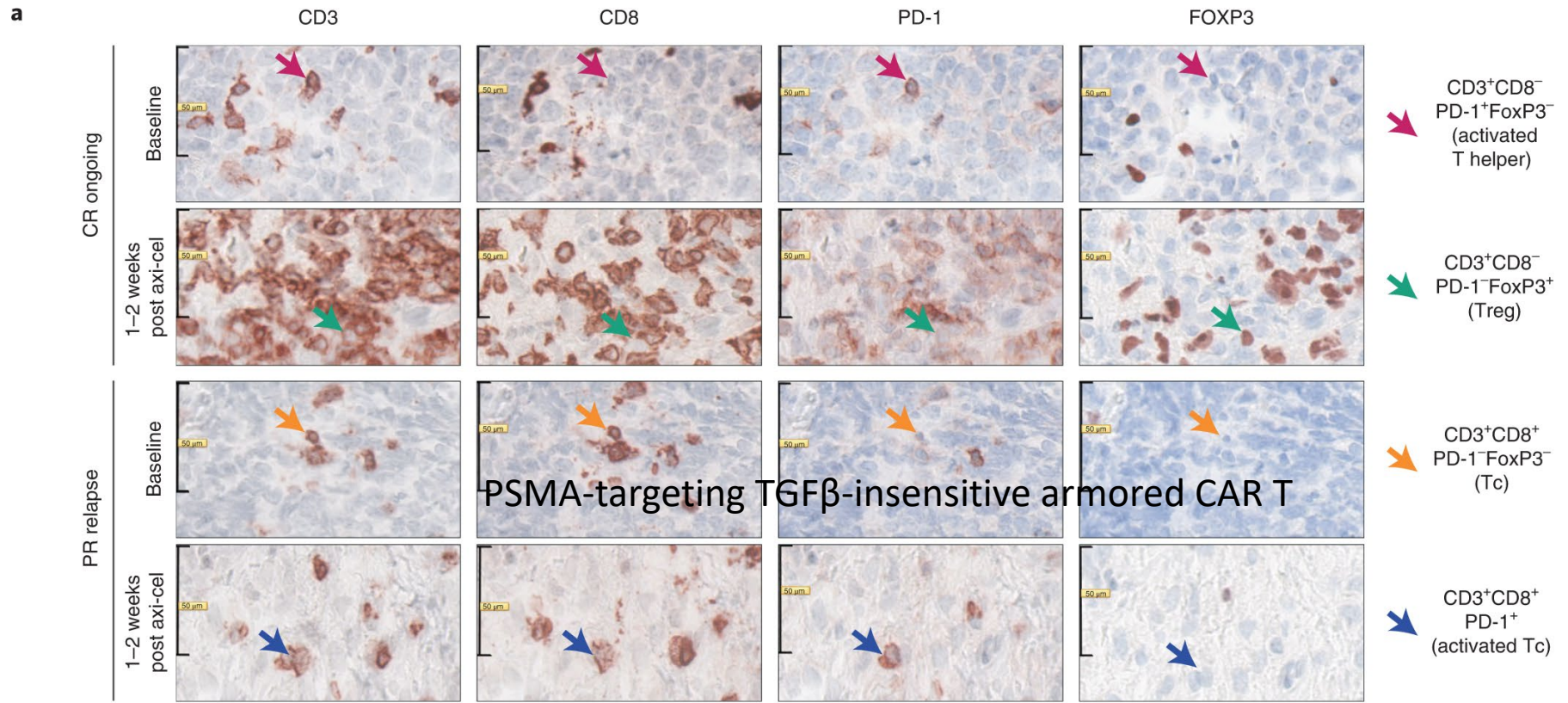**T cell subsets in pre-treatment tumor biopsies associated with myeloid-secreted chemokines.**

Scholler N et al., Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. Nature Medicine (2022)

How do CAR-T fail: TME

Evolution of T cell subset densities in the TME after axi-cel infusion

PSMA-targeting TGFβ-insensitive armored CAR T

Scholler N et al., Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. Nature Medicine (2022)

Making anti-tumor T cells in our patients: Bispecific Abs

Hutchings M et al JCO 2022