Chimeric antigen receptor T cells

- Tragedy, Perseverance, and Chance — The Story of CAR-T Therapy
- What are the CAR-T cells
- Indications
- Efficacy
- Adverse reactions

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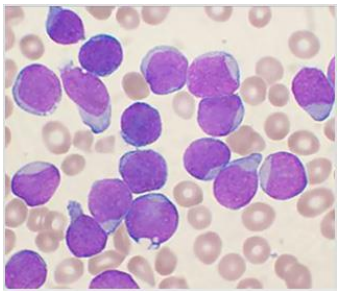
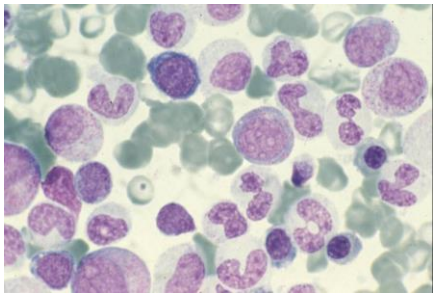
Tragedy, Perseverance, and Chance — The Story of CAR-T Therapy

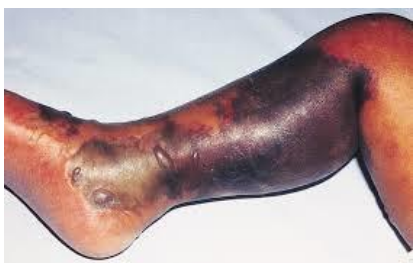
2010	5-year-old Emily Whitehead was diagnosed with acute lymphoblastic leukemia (ALL)
	• >80% chance of cure
	• Two rounds of chemotherapy, complete response • necrotizing fasciitis developed in both legs and she barely avoided amputation

Example of BM with ALL at diagnosis



Example of BM at remission





Example of Necrotizing faciitis
Fustes-Morales A
Arch Dermatol. 2002;138(7):893-899

Lisa Rosenbaum, M.D. national correspondent. N Engl J Med October 5, 2017 – Vol 377; pag 1313-1315

Tragedy, Perseverance, and Chance — The Story of CAR-T Therapy

2010	5-year-old Emily Whitehead was diagnosed with acute lymphoblastic leukemia (ALL)
16 months later	• Relapse. • Emily's leukemic cells were doubling daily. • Emily received another round of chemotherapy, which bought her 3 weeks but no remission
	• Out of options, one oncologist recommended hospice. • That just didn't make sense to us," says Tom Whitehead, Emily's father.
	• Her parents opted to enroll her in a study, and she became the first child to receive CART-19.

Emily is now a thriving 12-year-old and her survival helped reenergize a line of research that was nearing failure

Lisa Rosenbaum, M.D. national correspondent. N Engl J Med October 5, 2017 – Vol 377; pag 1313-1315

The Story of CAR-T Therapy: an incredible sequence of events (I)

1893	**William Coley's recognition of the immune system's potential for treating cancer when, in, he injected streptococcus into inoperable osteosarcoma and observed the tumor shrink**
1993	first CAR-T cells were generated by the Israeli immunologist Zelig Eshhar
1996	Carl June, the immunologist who led the development of Penn's CAR-T technology, recalled, "So many times, I almost had to quit." His 41-year-old wife was diagnosed with ovarian cancer June tried unsuccessfully to get a pharmaceutical company to provide the tools he needed to attempt immunotherapy
2001	June's his wife died The field of immunotherapy had been dogged by skepticism and setbacks, and the NIH wouldn't fund a clinical trial.

Lisa Rosenbaum, M.D. national correspondent. N Engl J Med October 5, 2017 – Vol 377; pag 1313-1315

The Story of CAR-T Therapy: an incredible sequence of events (I)

2001	**June's his wife died** **The field of immunotherapy had been dogged by skepticism and setbacks, and the NIH wouldn't fund a clinical trial.**
2001	Barbara and Edward Netter, having watched their daughter-in-law die of breast cancer, started the Alliance for Cancer Gene Therapy (ACGT), hoping to develop alternative approaches.
2008	ACGT granted June and his coinvestigator David Porter $1 million, enough to treat their first three patients with relapsed CLL with CART-19. Two of the three patients achieved complete remission, but the investigators ran out of funding
	The National Cancer Institute offered June a grant, and Novartis licensed Penn's CAR-T technology

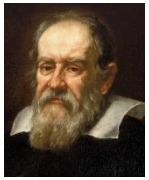
Lisa Rosenbaum, M.D. national correspondent. N Engl J Med October 5, 2017 – Vol 377; pag 1313-1315

The Story of CAR-T Therapy: an incredible sequence of events (II)

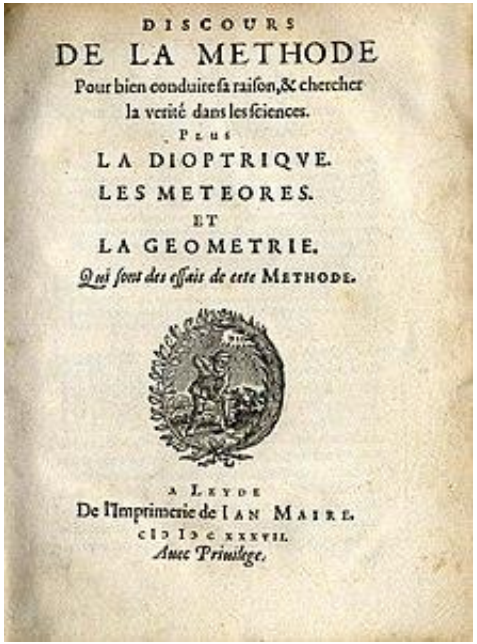
2011	**June acknowledges the tenuous nature of anecdote:** **“Were we lucky? Were they representative? Would it be durable?”**

Lisa Rosenbaum, M.D. national correspondent. N Engl J Med October 5, 2017 – Vol 377; pag 1313-1315

Niente nasce per caso: le radici culturali del pensiero scientifico moderno



- Sensata esperienza
- Necessaria dimostrazione


DISCOURS
DE LA METHODE
Pour bien conduire sa raison, & chercher
la verité dans les sciences.
Plus
LA DIOPTRIQVE.
LES METEORES.
ET
LA GEOMETRIE.
Qui sont des essais de cette METHODE.
A LEYDE
De l'Imprimerie de IAN MAIRE.
CIↃ IↃ C XXXVII.
Avec Privilege.

L'evidenza:	**«Non prendere mai niente per vero, se non ciò che io avessi chiaramente riconosciuto come tale; ovvero, evitare accuratamente la fretta e il pregiudizio, e di non comprendere nel mio giudizio niente di più di quello che fosse presentato alla mia mente così chiaramente e distintamente da escludere ogni possibilità di dubbio».**
L'analisi:	«Dividere ognuna delle difficoltà sotto esame nel maggior numero di parti possibile, e per quanto fosse necessario per un'adeguata soluzione» .
La sintesi:	«Condurre i miei pensieri in un ordine tale che, cominciando con oggetti semplici e facili da conoscere, potessi salire poco alla volta, e come per gradini, alla conoscenza di oggetti più complessi; assegnando nel pensiero un certo ordine anche a quegli oggetti che nella loro natura non stanno in una relazione di antecedenza e conseguenza.»
L'enumerazione	«E per ultimo, di fare in ogni caso delle enumerazioni così complete, e delle sintesi così generali, da poter essere sicuro di non aver tralasciato nulla».

1ª ed. originale 1637

The Story of CAR-T Therapy: an incredible sequence of events (II)

2011	**June acknowledges the tenuous nature of anecdote:** **"Were we lucky? Were they representative? Would it be durable?"**
	Anecdote can easily break a field rather than make it: • Had Emily died, the CAR-T field would probably have died with her. • The death of Jesse Gelsinger in a trial at Penn had set the field of gene therapy back at least a decade
	• Emily Whitehead was a case in point. • After receiving her third dose of CART-19, she developed high fevers, respiratory failure, and shock necessitating the use of three pressors. • Though Emily was experiencing what's now understood to be cytokine-release syndrome, which occurred in 78% of patients in Novartis's phase 2 trial, it wasn't clear at the time what was driving this response, much less how to treat it

Lisa Rosenbaum, M.D. national correspondent. N Engl J Med October 5, 2017 – Vol 377; pag 1313-1315

The Story of CAR-T Therapy: an incredible sequence of events (III)

2011	“Chance favors the prepared mind.”
	Per protocol, participants’ blood was sent for cytokine analysis
	But as Emily rapidly deteriorated
	Two hours later, in time for his 3 p.m. lab meeting, Grupp learned that Emily’s level of IL-6 was elevated 1000-Fold
	“No one thought we should be thinking about this thing, IL6,” “It isn’t even made by T cells.”
	• June, giving a talk in Seattle, received the results and had an idea. • His daughter, who has juvenile rheumatoid arthritis, had recently started taking tocilizumab, a monoclonal antibody that targets interleukin-6
	Once again, they got lucky. Tocilizumab was on the hospital’s formulary for rheumatologic indications, which meant that rather than having to wait for up to 2 days, by 8 p.m. that evening
	Emily received a dose. Within hours, she began to improve, so dramatically that her doctors could barely wean the pressors fast enough
	On her seventh birthday, Emily woke up. Eight days later, on the basis of a bone marrow biopsy, Grupp reported that the treatment had worked.

Lisa Rosenbaum, M.D. national correspondent. N Engl J Med October 5, 2017 – Vol 377; pag 1313-1315

Chimeric antigen receptor T cells

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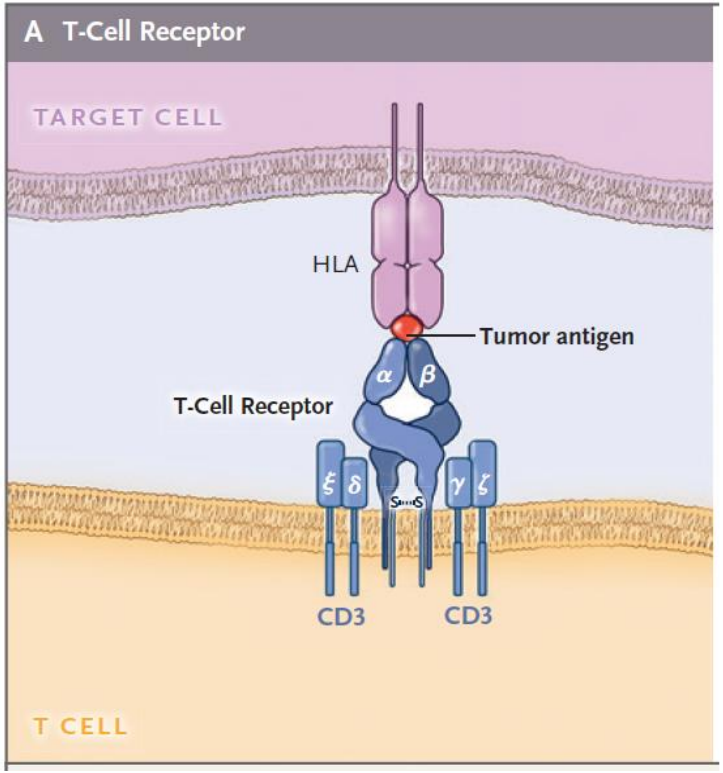
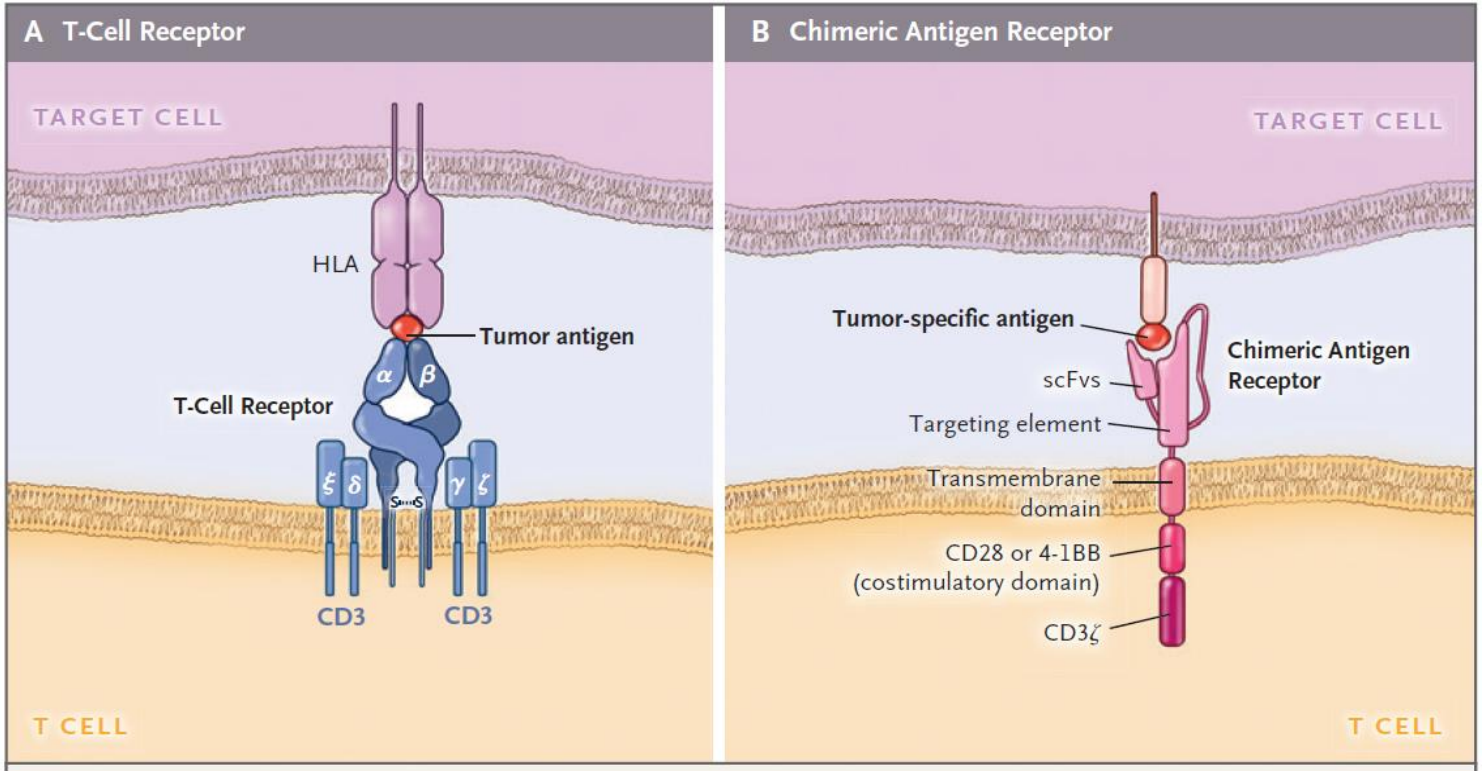
- **What are the CAR-T cells**
- Indications
- Efficacy
- Adverse reactions



Structure of a normal T-cell receptor, which consists of heterodimeric and antigen-specific α and β chains that closely associate with the invariant ε, δ, γ, and ζ chains of the CD3 complex.

The T-cell receptor binds to the HLA allele that has a bound peptide derived from a tumor antigen on the target cell.

Carl H. June, N Engl J Med 2018;379:64-73.



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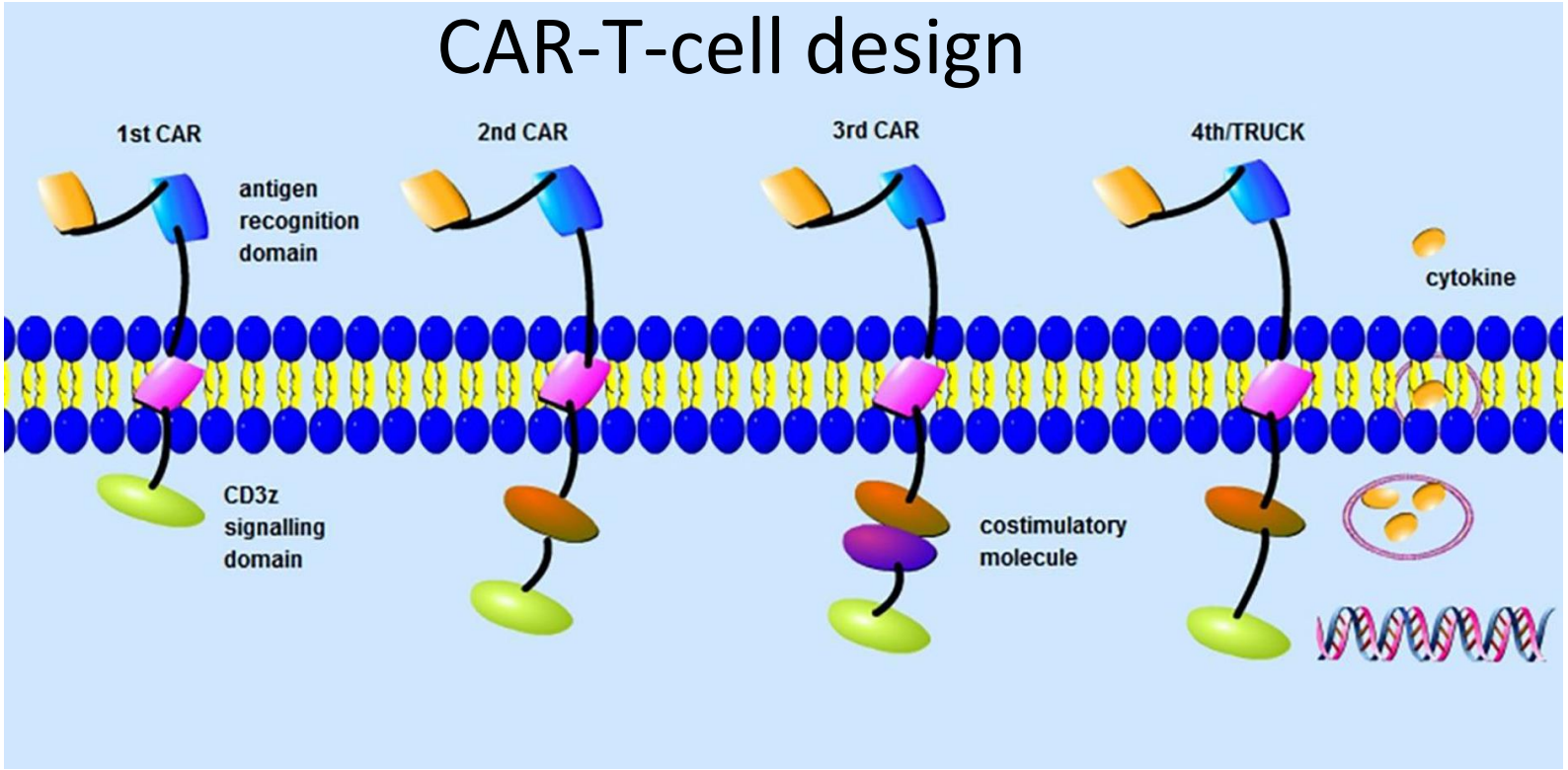
The T-cell receptor binds to the HLA allele that has a bound peptide derived from a tumor antigen on the target cell.

Structure of the CAR, which includes the single-chain variable fragment (scFv) that binds to tumor antigens, fused to a spacer and transmembrane domain. The intracellular domain contains costimulatory domains, such as CD28 and 4-1BB and the CD3ζ chain, which drive signal activation and amplification of CAR T cells

Carl H. June, N Engl J Med 2018;379:64-73.

CAR-T-cell design

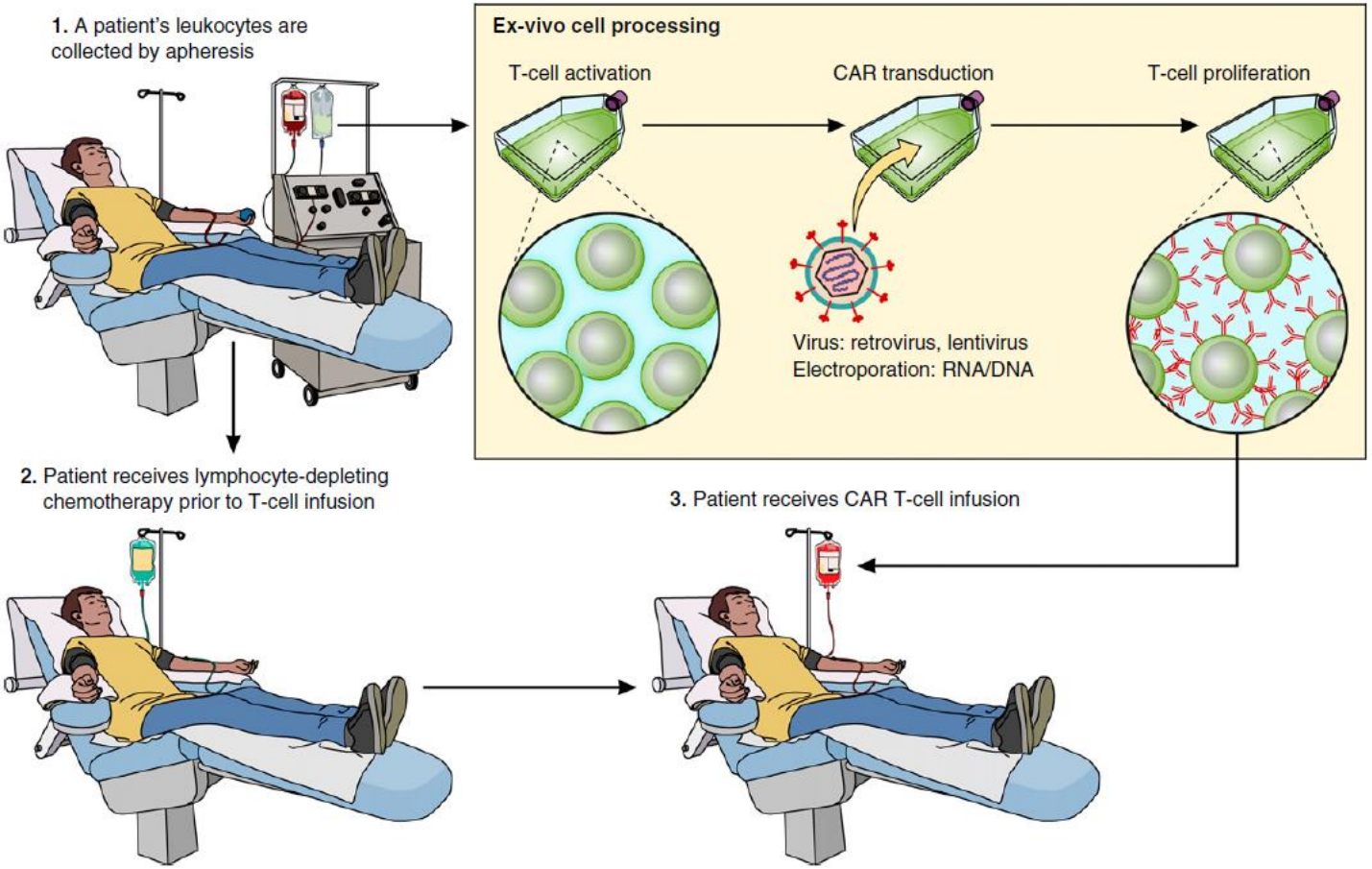


Chimeric antigen receptors (CARs) are composed of an extracellular domain, a transmembrane domain and an intracellular signaling domain

- First generation CARs only have a CD3z signalling domain.
- Second generation CARs have a costimulatory signalling domain to enhance the signal function of the CD3z signalling domain.
- In third generation CARs, two costimulatory signalling domains are added to amplify anti-tumor effect of second generation CARs.
- In the fourth generation CARs (TRUCKs), cytokine genes are added

Xu D etal, Oncotarget, 2018

Steps required



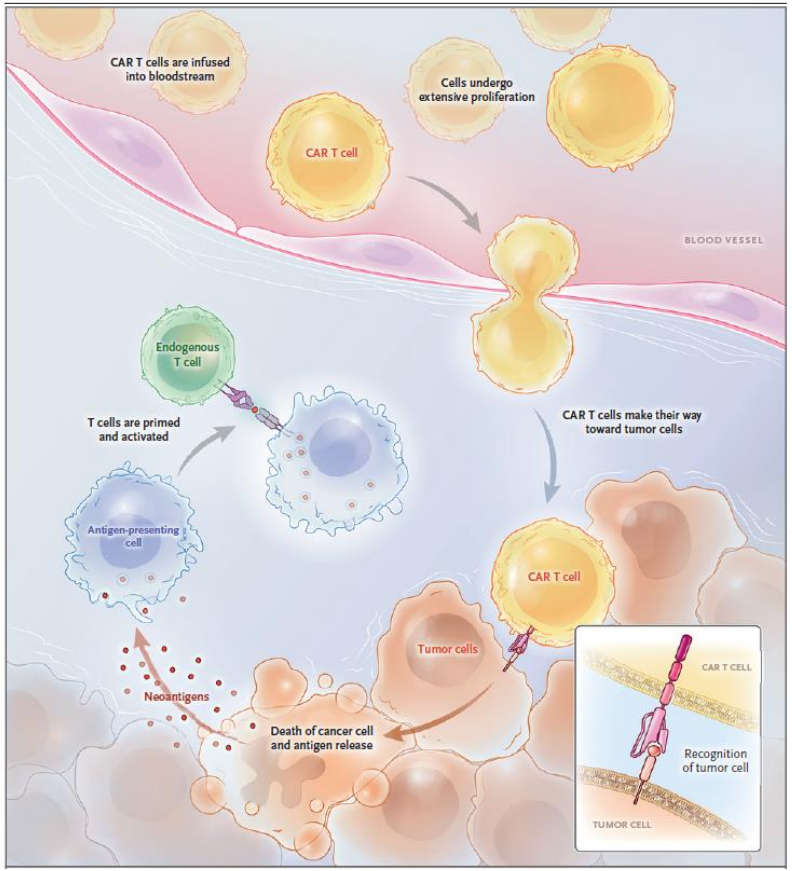
Some critical factors for the design of CAR-T cells (I)

- Choice of target antigen: it is important that a selected antigen is uniformly expressed on the malignancy targeted by CAR-T
- Targeted antigen should be absent on essential normal tissues to avoid toxicity
- CD19 is an ideal antigen for some B-cell malignancies, i.e. B-ALL, NHL, because of its broader and higher expression relative to other B-cell markers
- Its expression in normal tissues, which is confined to the B-cell lineage, predicted that on-target and off-tumor activity would be limited to B-cell aplasia, a side effect that can be mitigated with immunoglobulin replacement therapy.
- B-cell depletion may preempt a potential antibody response to the CAR, especially its murine components

Some critical factors for the design of CAR-T cells (II)

- More than 1000 patients have received CD19-targeted CAR T cells in the United States
- The defining characteristic of the responses is that they tend to be durable
- The inclusion of mouse sequences can trigger rejection of the CAR T cells by the host immune system
- The lack of immunogenicity, and hence persistence of CAR T cells, is associated with improved relapse-free survival among patients with leukemia
- Thus, CAR designs that are composed of fully human sequences have become preferable

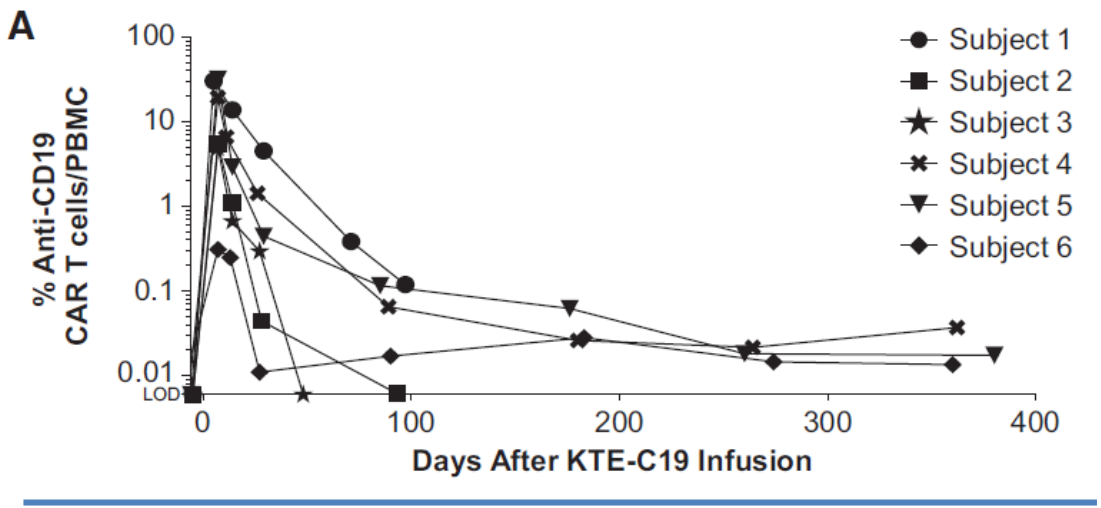
CAR-T cells: basic mechanisms of action



Carl H. June, N Engl J Med 2018;379:64-73.

PCR data demonstrates exponential expansion and persistence of CD19 CAR T cells in blood

- Expansion occurs rapidly with peak levels achieved within the first 7–14 days post-KTE-C19 infusion
- Persisting CD19 CAR T cells were detectable
- in six of six (100%) patients at week 4 and
- in four of five (80%) patients with samples available for testing at month 3.
- Three patients with ongoing CR had detectable CAR T cells at 12 months



Locke FL et al. Molecular Therapy 2017: 25; 285-294

Chimeric antigen receptor T cells

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- **What are the CAR-T cells**
- **Indications**
- **Efficacy**
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- In August 2017, the FDA approved the first chimeric antigen receptor T-cell (CAR-T) therapy, Novartis's tisagenlecleucel, which uses the Penn developed technology

- Patients up to 25 years of age with relapsed or refractory ALL.

- Though the indication is narrow, the results are striking in a patient population with limited options
- 83% of the 63 evaluable children who received tisagenlecleucel had complete elimination of malignant cells at 3 months

- ***On 28 June 2018 the EMA- CHMP recommended granting for marketing authorization for tisagenlecleucel**
- **young patients up to 25 years of age with B-ALL that is refractory, in relapse post-transplant or in second or later relapse.**
- **Adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy."**

- ***On 28 June 2018 the EMA- CHMP recommended granting for marketing authorization for axicabtagene ciloleucel for refractory DLBCL and primary mediastinal large B-cell lymphoma (PMBCL), after two or more lines of systemic therapy**

*http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion_-_Initial_authorisation/human/004090/WC500251211.pdf

Chimeric antigen receptor T cells

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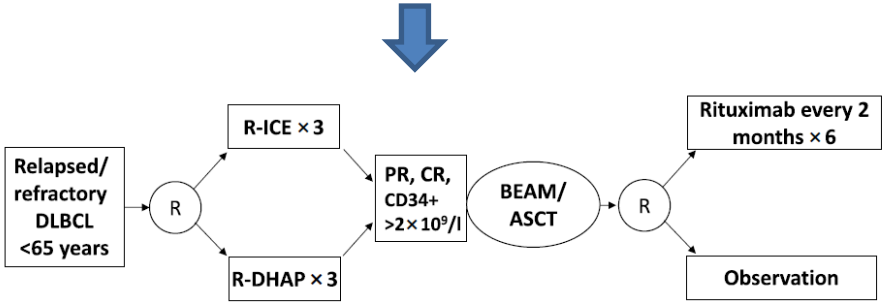
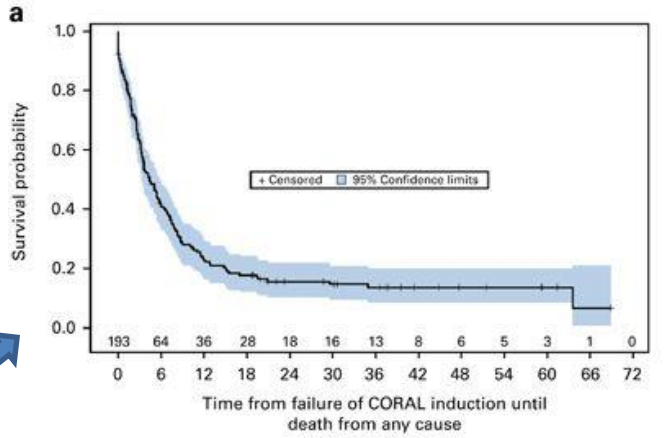
Efficacy of traditional chemotherapeutic approches as second salvage treatment in DLBCL

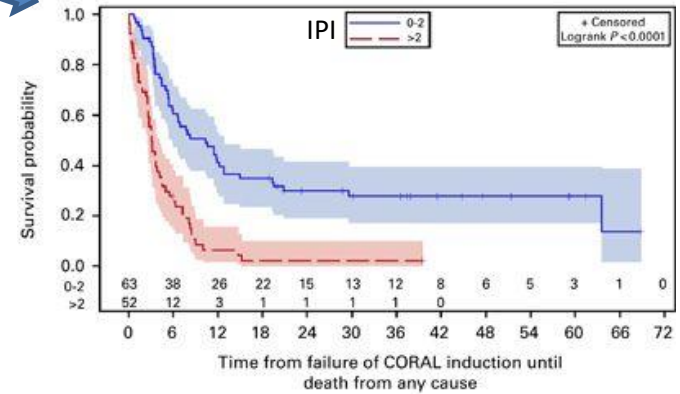
396 pts in first relapse or refractory after first-line therapy



203 off study
- 170 treatment failure
- 19 toxicity
- 14 other reasons

166 had information on 3° line
- CR 33,1%
- PR 14,5%
- ORR 47,6%
- Median OS 4,1 months
- Alive at 12 mos: 23%
- Alive at 24 mos: 15,7%





Gisselbrecht C et al, J Clin Oncol 2010; 28:4184-90
Van Den Nesten E et al, Bone Marrow Transplantation 2016: 51, 51–57

Response to Axicabtagene Ciloleucel in Patients With Refractory DLBCL

CCO Independent Conference Highlights*

of the *2018 ASCO Annual Meeting; June 1-5, 2018; Chicago, Illinois*

*CCO is an independent medical education company that provides state-of-the-art medical information to healthcare professionals through conference coverage and other educational programs.

This activity is supported by educational grants from Amgen, AstraZeneca, Celgene Corporation, Eisai, Genentech, Janssen, and Merck & Co., Inc.

CLINICAL CARE OPTIONS®
ONCOLOGY

ZUMA-1 Long-term Follow-up: Study Design

- ZUMA-1 phase II portion (N = 101)
  - Cohort 1: patients with refractory DLBCL (n = 77)
  - Cohort 2: patients with refractory PMBCL or transformed FL (n = 24)

- Primary/secondary endpoints:

  CR+PR/ DOR, PFS, OS, AE, cytokine and blood levels of cytokine and CAR-T

- Key inclusion criteria
  - No response to last CT or relapsed within 12 mos of ASCT
  - Prior treatment with anthracycline and anti-CD20 monoclonal antibody

Locke FL, et al. ASCO 2018. Abstract 3003.

Key eligibility criteria

Hematologic criteria

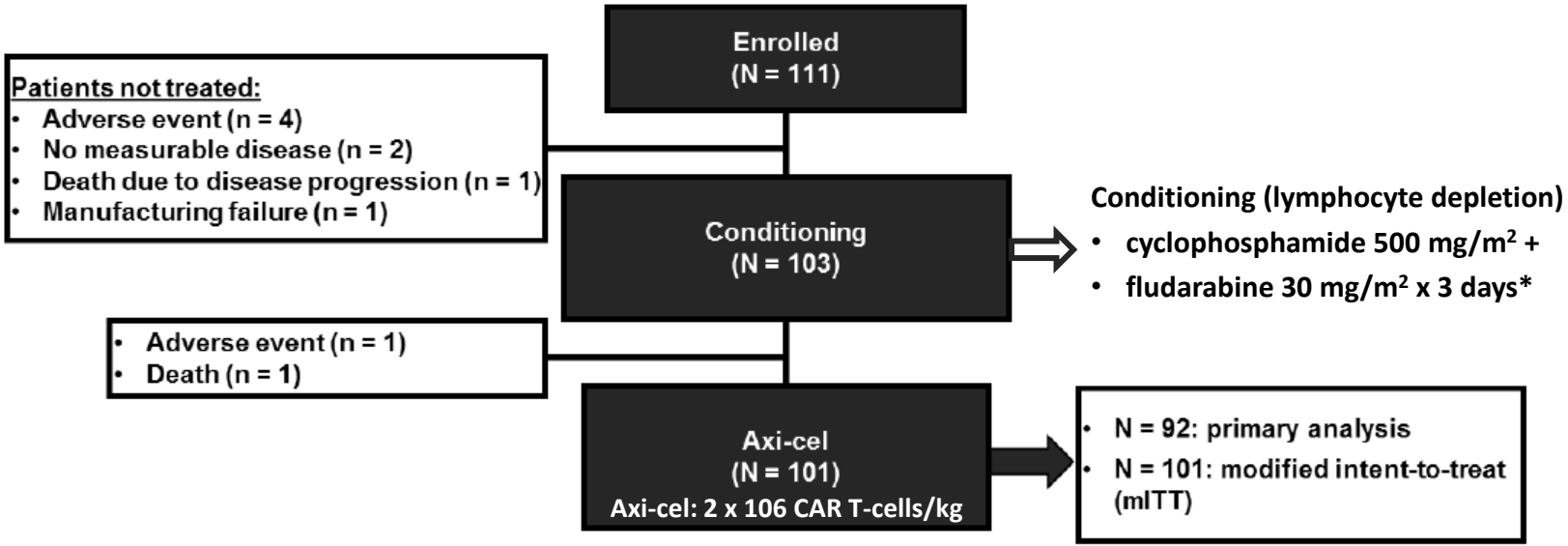
- absolute neutrophil count greater than 1,000 cells per microliter,
- absolute lymphocyte count greater than 100 cells per microliter,
- platelet count greater than 75,000 cells per microliter,

Adequate organ function, no CNS involvement, and no active infection

- Eastern cooperative oncology group (ECOG) performance status of 0 or 1
- Creatinine clearance (as estimated by Cockcroft Gault) ≥ 60 mL/min
- Serum ALT/AST ≤ 2.5 ULN o Total bilirubin ≤1.5 mg/dl,
- Cardiac ejection fraction ≥ 50% with no evidence of pericardial effusion as determined by an ECHO,
- no clinically significant ECG findings
- No clinically significant pleural effusion
- Baseline oxygen saturation >92% on room air

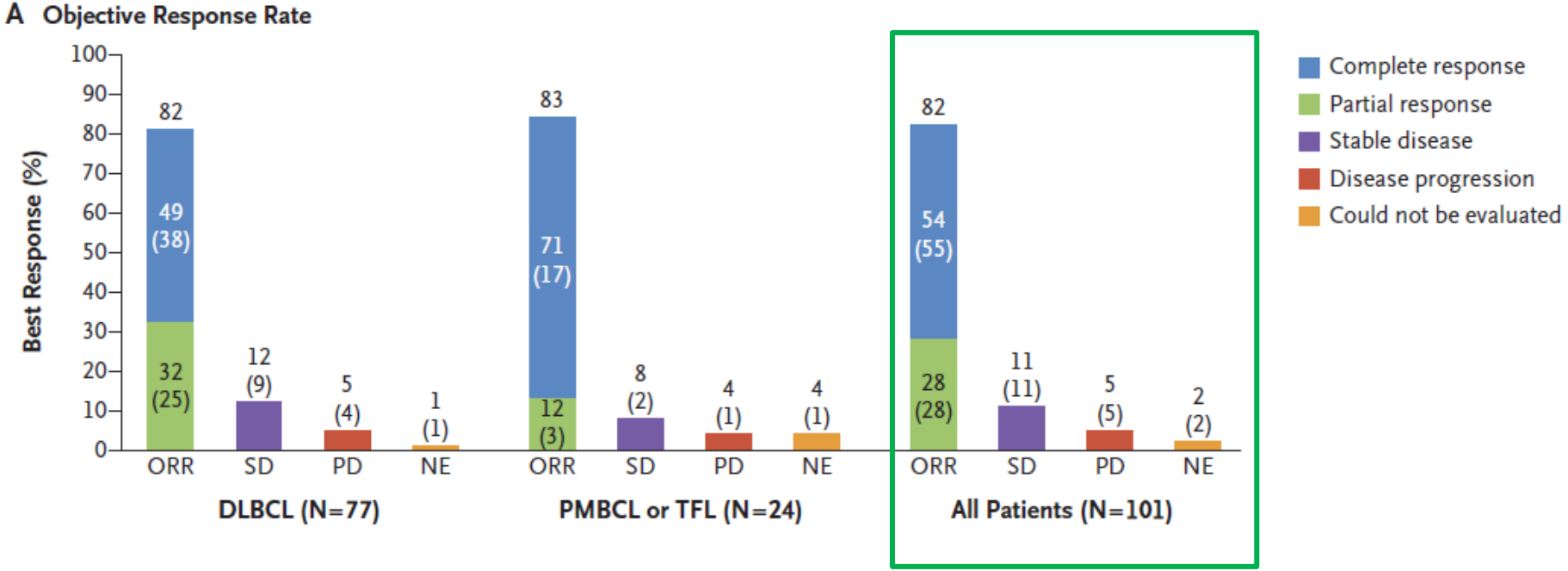
Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

Figure S1. Consort Diagram for Overall Population (N=111).



Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

Objective response rate (ORR = CR plus PR) at a minimum follow-up of 6 months among the patients who received axicabtagene

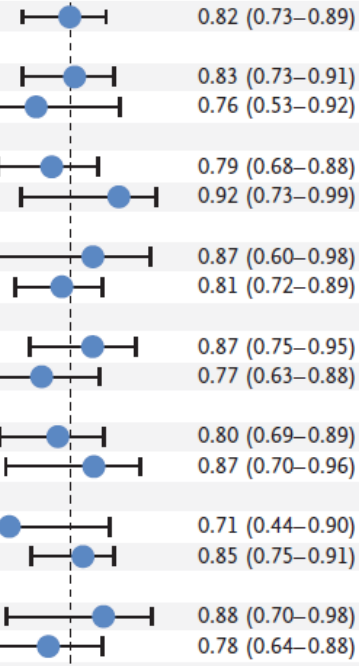


Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

Objective response rate for key baseline and clinical covariates

B Subgroup Analysis

Subgroup	No. of Patients Who Could Be Evaluated	No. of Patients with Event	Objective Response Rate (95% CI)
Overall	101	83	0.82 (0.73–0.89)
Refractory subgroup			
Refractory to ≥second-line therapy	78	65	0.83 (0.73–0.91)
Relapse after ASCT	21	16	0.76 (0.53–0.92)
Age			
<65 yr	77	61	0.79 (0.68–0.88)
≥65 yr	24	22	0.92 (0.73–0.99)
Disease stage			
I or II	15	13	0.87 (0.60–0.98)
III or IV	86	70	0.81 (0.72–0.89)
IPI risk score			
0–2	53	46	0.87 (0.75–0.95)
3 or 4	48	37	0.77 (0.63–0.88)
Extranodal disease			
Yes	70	56	0.80 (0.69–0.89)
No	31	27	0.87 (0.70–0.96)
Bulky disease (≥10 cm)			
Yes	17	12	0.71 (0.44–0.90)
No	84	71	0.85 (0.75–0.91)
Treatment history			
Primary refractory disease	26	23	0.88 (0.70–0.98)
Refractory to two consecutive lines	54	42	0.78 (0.64–0.88)

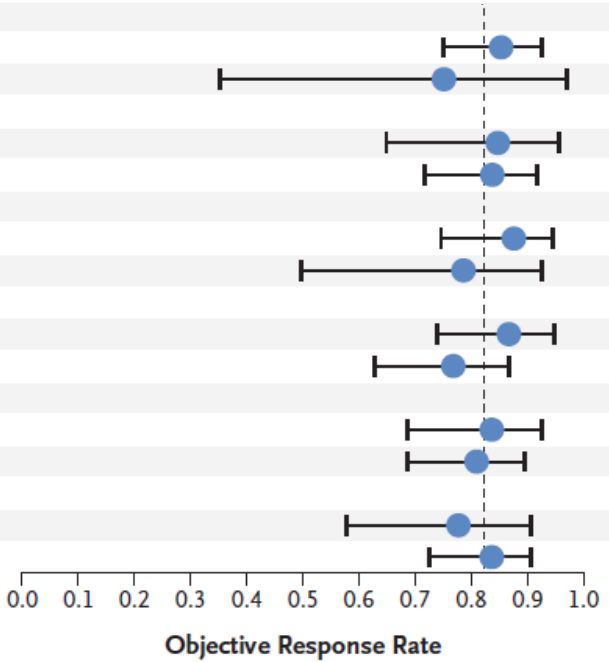


Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

Objective response rate for key baseline and clinical covariates

B Subgroup Analysis

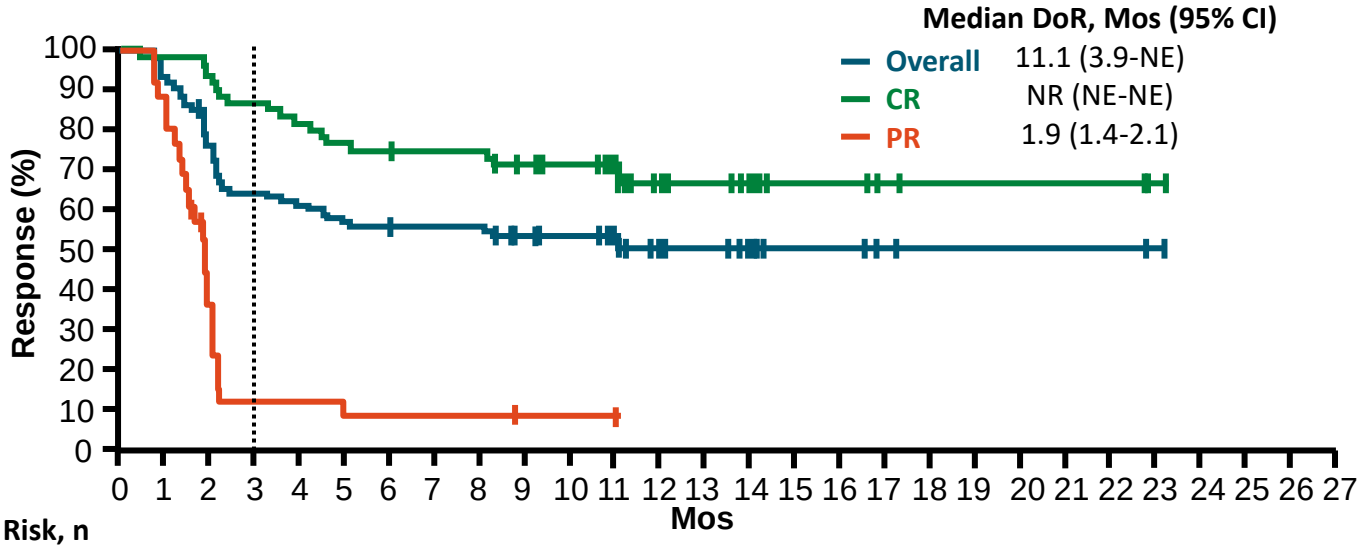
Subgroup	No. of Patients Who Could Be Evaluated	No. of Patients with Event	Objective Response Rate (95% CI)
CD19 status			
Positive	74	63	0.85 (0.75–0.92)
Negative	8	6	0.75 (0.35–0.97)
CD19 histologic score			
≤150	26	22	0.85 (0.65–0.96)
>150	56	47	0.84 (0.72–0.92)
Cell of origin			
Germinal center B-cell–like subtype	49	43	0.88 (0.75–0.95)
Activated B-cell–like subtype	17	13	0.76 (0.50–0.83)
CD4:CD8 ratio			
>1	47	41	0.87 (0.74–0.95)
≤1	52	40	0.77 (0.63–0.87)
Tocilizumab use			
Yes	43	36	0.84 (0.69–0.93)
No	58	47	0.81 (0.69–0.90)
Glucocorticoid use			
Yes	27	21	0.78 (0.58–0.91)
No	74	62	0.84 (0.73–0.91)



Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

Duration of Response by Best Objective Response (Primary Analysis) in the 89 pts who attained PR or CR

- More than one half of patients with PR progressed by Month 3, defining Month 3 as a clinically relevant timepoint.

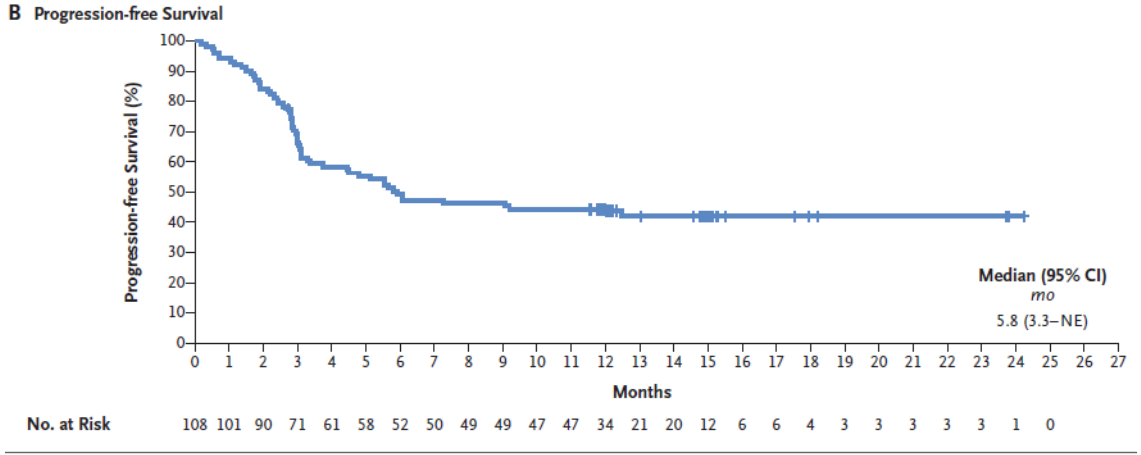


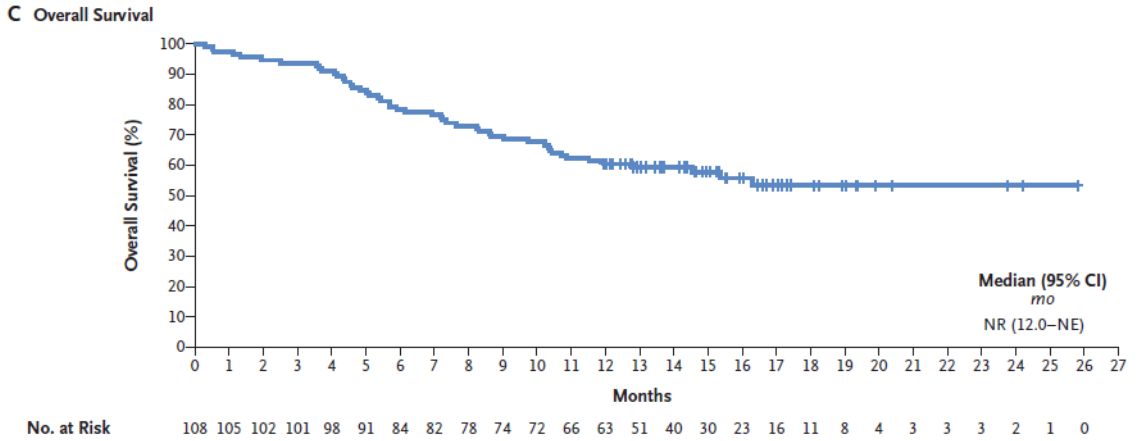
Patients at Risk, n

Overall	89	82	67	56	53	49	48	47	47	42	38	31	19	16	12	6	6	4	3	3	3	3	3	1	0
CR	63	61	58	53	50	47	46	45	45	41	37	30	19	16	12	6	6	4	3	3	3	3	3	1	0
PR	26	21	9	3	3	2	2	2	2	1	1	1	0												

Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

PFS and OS in the modified intention to treat population





Median PFS, Mos (95% CI)
5,8 (3.3-NE)

PFS rate
- 49% (95% CI, 39 to 58) at 6 months
- 44% (95% CI, 34 to 53) at 12 months
- 41% (95% CI, 31 to 50) at 15 months

Median OS, Mos (95% CI)
not reached (12-NE)

OS rates of
- 78% (95% CI, 69 to 85) at 6 months
- 59% (95% CI, 49 to 68) at 12 months
- 52% (95% CI, 41 to 62) at 18 months

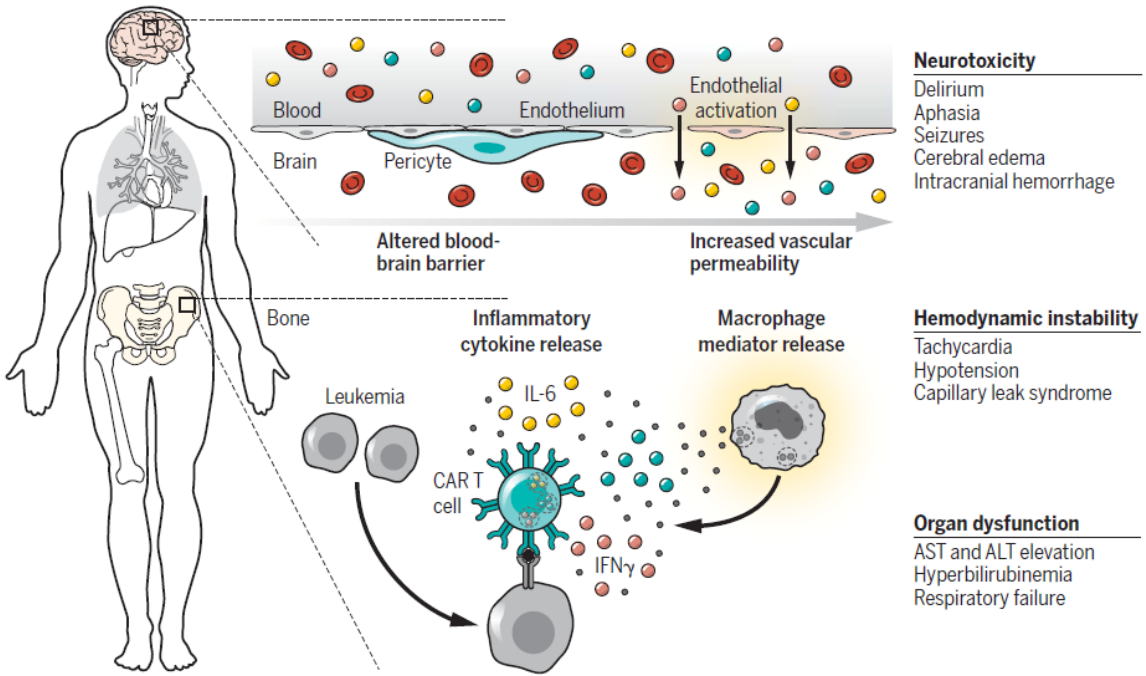
Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

Chimeric antigen receptor T cells

- **Tragedy, Perseverance, and Chance — The Story of CAR-T Therapy**
- **What are the CAR-T cells**
- **Indications**
- **Efficacy**
- **Adverse reactions**

Pathophysiology of the cytokine release syndrome

1. Release a variety of cytokines and chemokines.
2. Macrophages and other cells of the innate immune system also become activated and contribute to the release of soluble mediators.
3. CAR T cells are routinely observed in cerebral spinal fluid,
4. Cytokines may increase permeability to soluble mediators and permit increased trafficking of CAR T cells and other lymphocytes to CNS parenchyma



June et al., Science 359, 1361–1365 (2018)

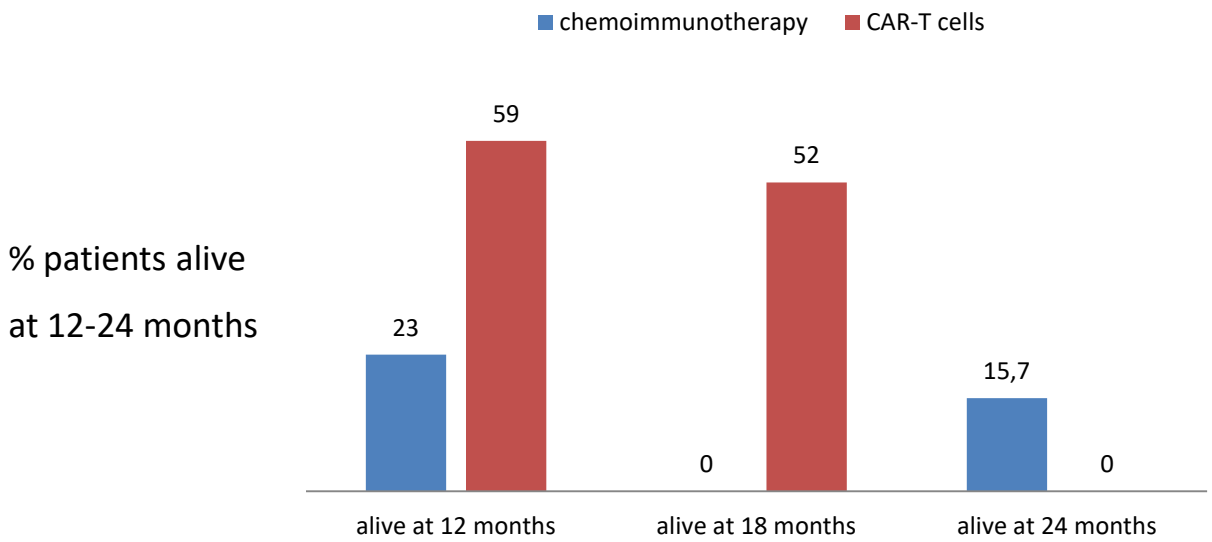
ZUMA-1 Long-term Follow-up: Conclusions

- Long-term analysis of phase II ZUMA-1 trial demonstrated high, durable response rates in patients with refractory large B-cell lymphoma treated with axi-cel
- ORR and CR rate increased during long-term follow-up, with patients achieving CRs up to 1 yr after a single infusion of axi-cel
- Patients with an ongoing response at 3 months had ~ 80% probability of maintaining response at 12 months
- Investigators concluded that achieving PR or CR by 3 months may be prognostic of long-term response to axi-cel

Locke FL, et al. ASCO 2018. Abstract 3003.

OS in third line with chemoimmunotherapy

as compared with OS in pts who received CAR-T cells



Gisselbrecht C et al, BJH 2018; 182, 633–643
Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544