**Antonello Pinto**

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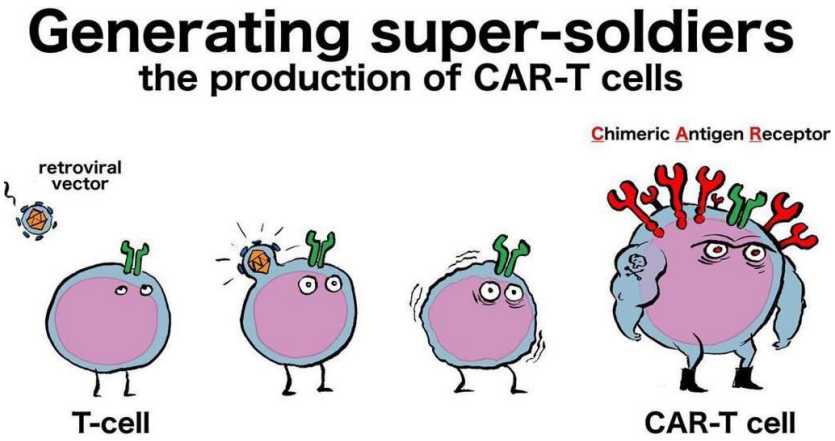
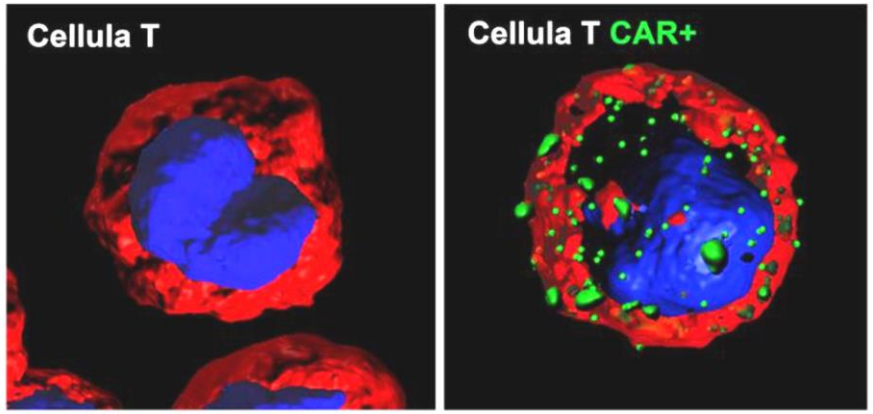
*Department of Hematology and Developmental Therapeutics*

*National Cancer Institute, Fondazione G. Pascale, IRCCS, Naples, Italy*




ISTITUTO NAZIONALE TUMORI
IRCCS - Fondazione Pascale

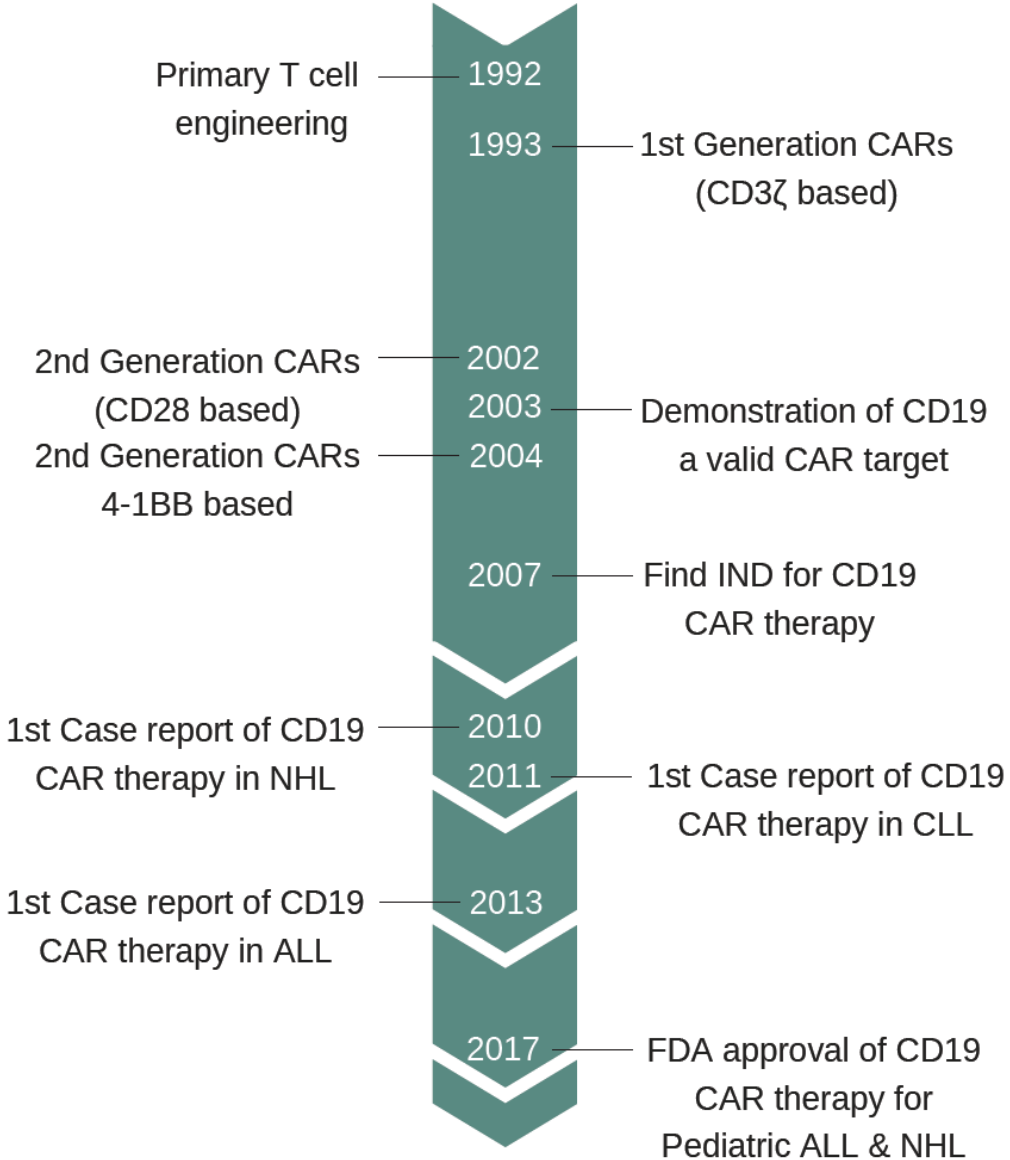
# *CAR-T Cells-based treatments: Development Timeline*

***CAR T-cells represent an autologous cellular immunotherapy using gene transfer to reprogram T cells to recognize and eliminate cancerous cells by targeting tumor-associated antigens***

*JC Chavez, C Bachmeier, MA Kharfan-Dabaja. Ther Adv Hematol 2019, Vol. 10: 1–20.*

# CAR-T Cells-based treatments: Development Timeline

## Discovery to FDA approval ~ 25 years



# *CAR-T Cells-based treatments: opportunities and challenges*

- **The clinical *scenario***
  - *The unmet needs*
- **CAR-T cells as *a living drug***
  - The idea
  - How it works
  - The logistics
- **The Clinical Results**
  - Registered indications
- **The Toxicity & Safety Management**
  - CRS
  - ICAN (Immune effector Cells Associated Neurotoxicity)
  - Other
- **The Future (*tomorrow*)**

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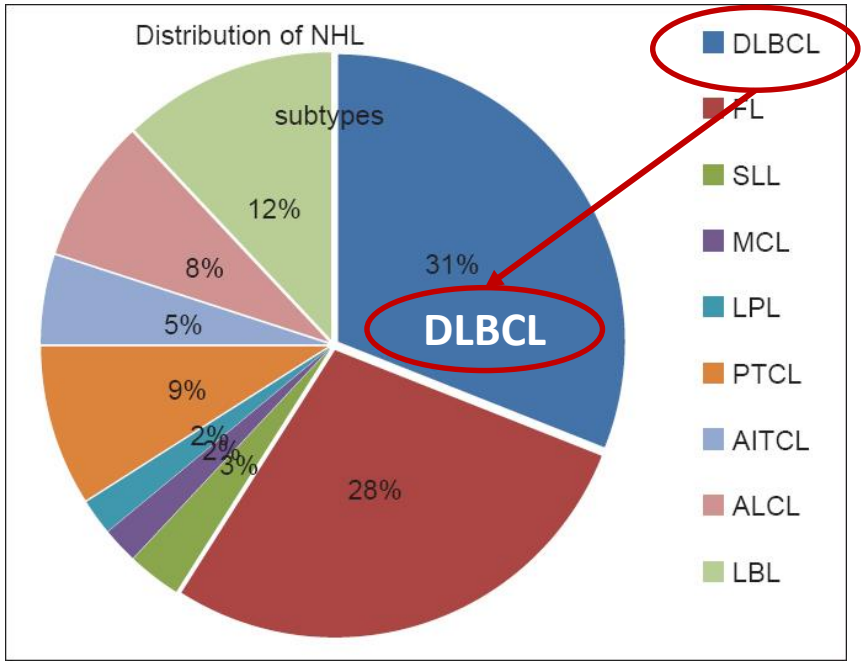
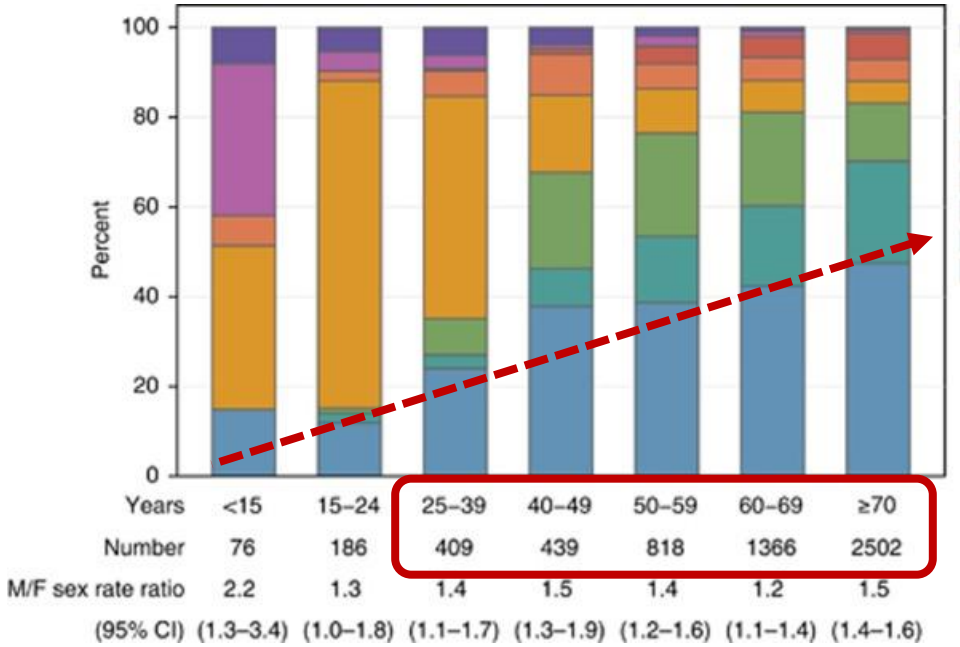
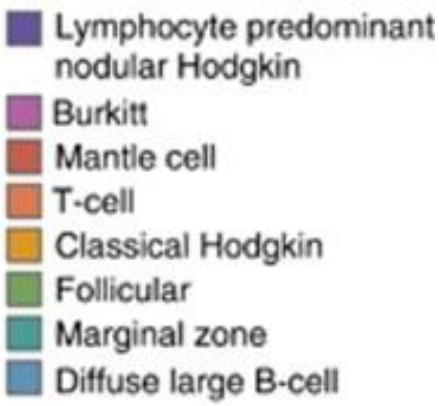
# Diffuse Large B-cell Lymphoma



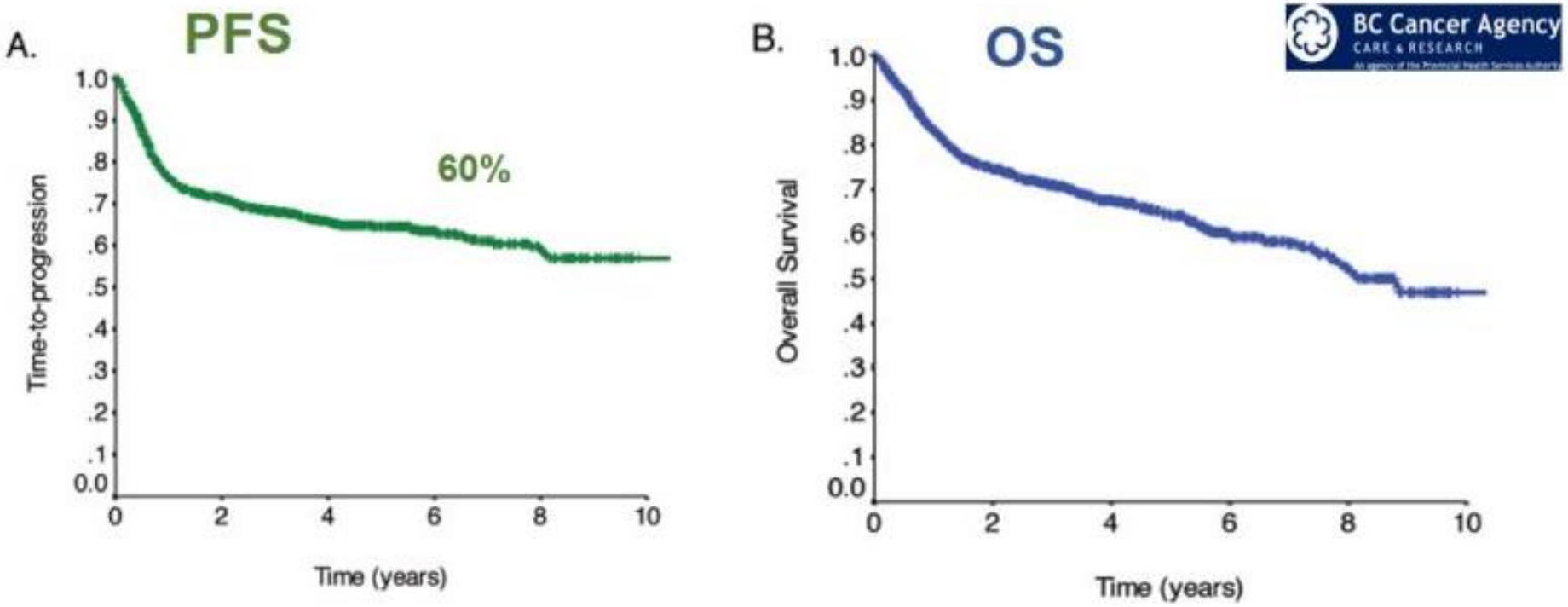

- **DLBCL: 30% - 32% of NHL**
- ***Age-standardized incidence rate:***

  **5-6 *per* 100,000 persons-year**
- **[Campania: 420-480 new cases/year]**
- ***Upfront cure rate:* 50-55% [60% ?]**



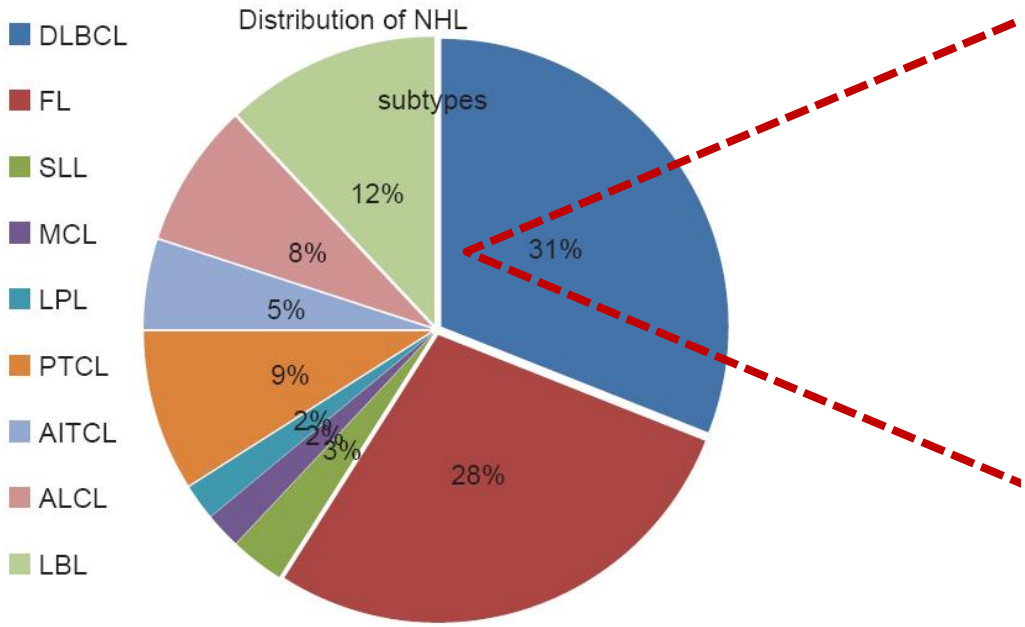
# Evolution of aggressive NHL subtypes

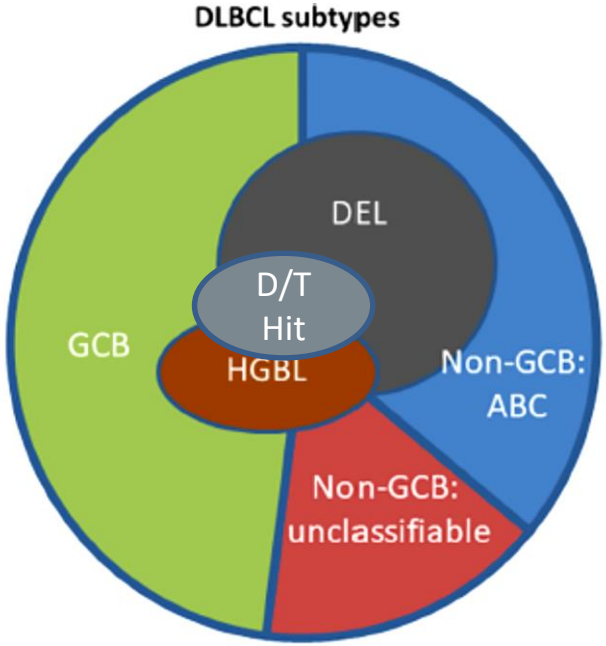
## Patients with DLBCL treated with R-CHOP-21 at BCCA (n=1476)



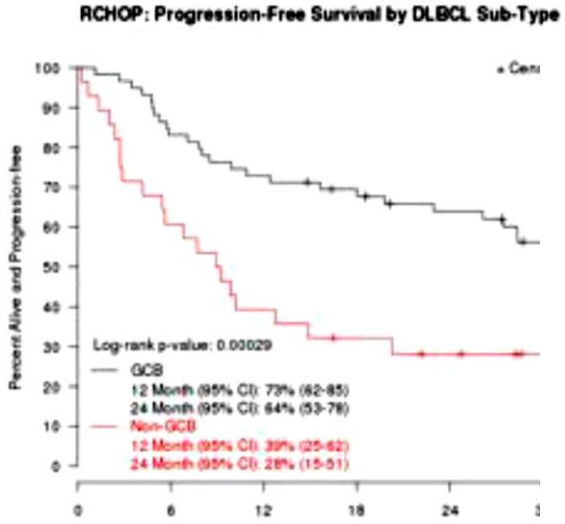

*BC Cancer Agency Database Sehn Hematology 2012*

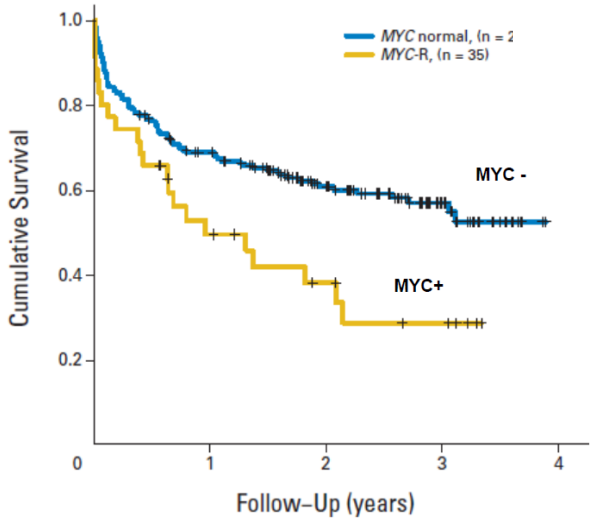
# DLBCL & Aggressive NHL: 2019

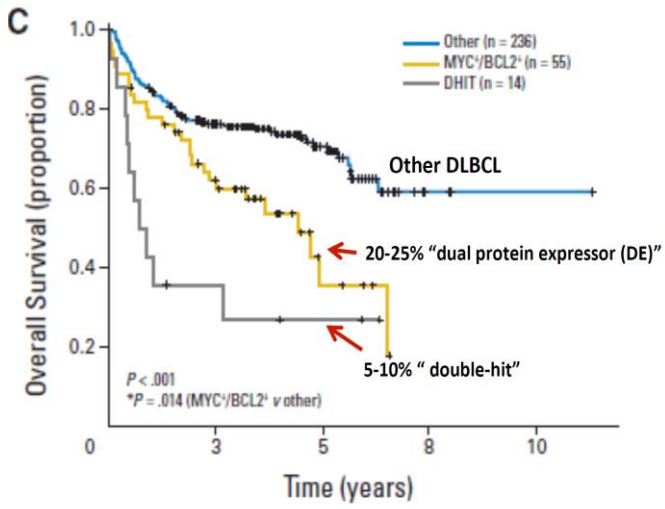



Liu & Barta (2019; modified)



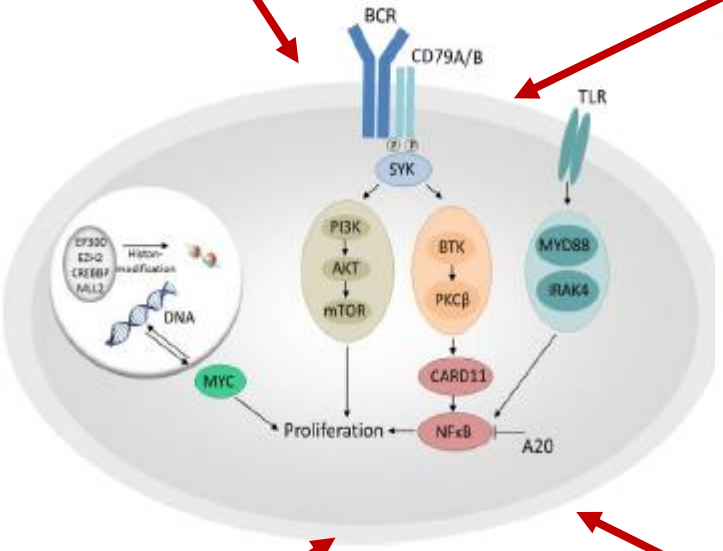



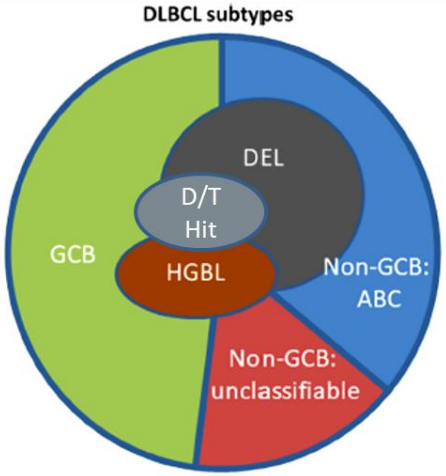
# Evolution of Treatment for DLBCL

**_Work_ on Rituximab**

**_Change_ anti-CD20**

- ~~G-CHOP~~
- Polatuzumab R-CHOP




**_Add_ maintenance**

- ~~Lenalidomide~~
- ~~Rituximab~~
- ~~Everolimus~~
- ~~Enzastaurin~~

**_Add_ targeted agent (X)**

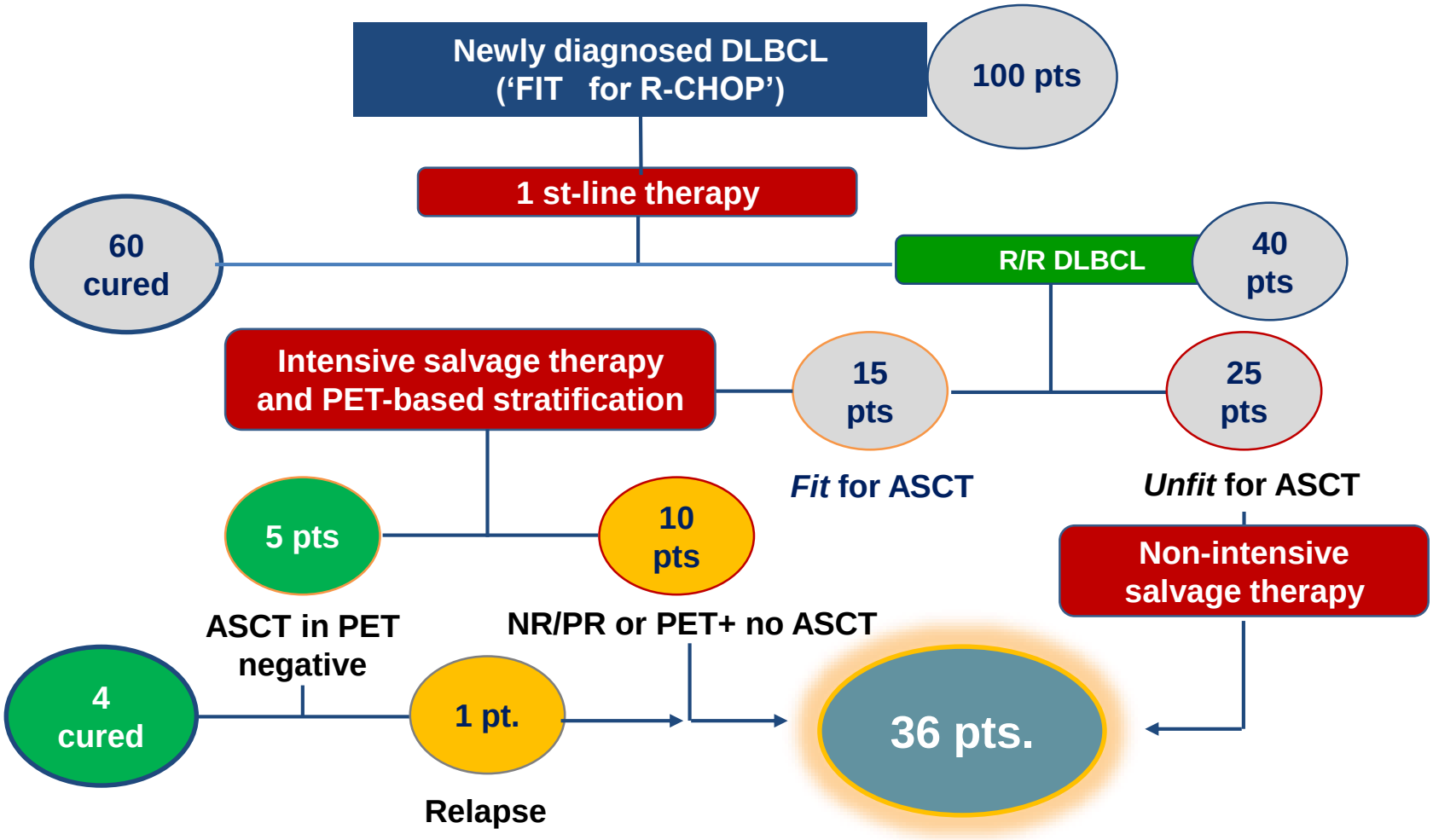
- ~~Bortezomib~~
- ~~Lenalidomide~~
- ~~Ibrutinib~~
- Venetoclax

**Intensify Chemotherapy**

- ~~R-CHOP14~~
- ~~DA-EPOCH-R~~
- ~~HDC-ASCT~~
- ~~R-ACVBP~~

(DEL/D/TH)

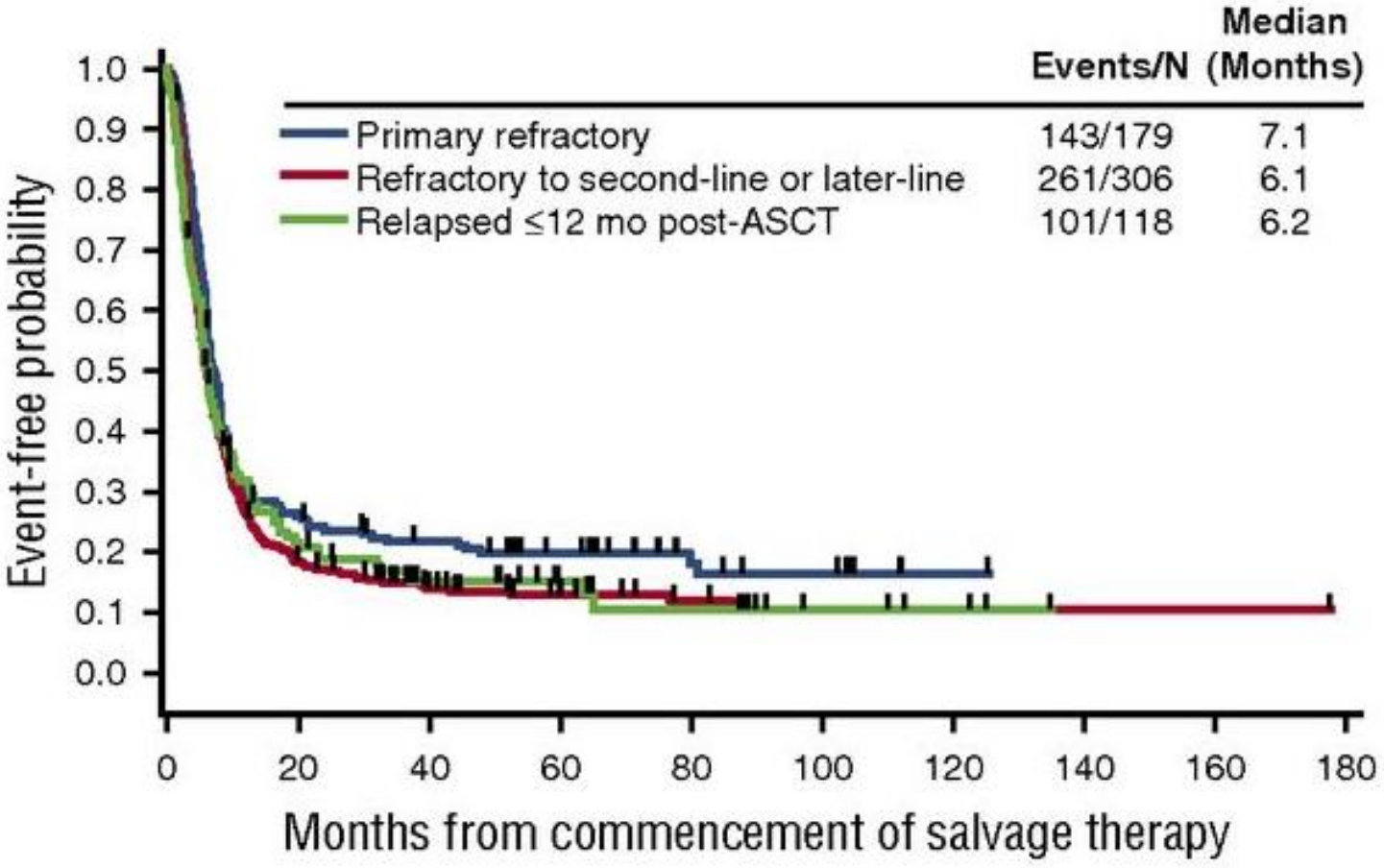
# R/R DLBCL: new options are needed in different settings




***'Uncured' Refractory/relapsed DLBCL***

Modified from Friedberg J.W. (2011)

# Overall Survival in Refractory DLBCL: Historical Outcomes data – SCHOLAR-1




**N = 636**

- ORR = 26%; CR rate = 7%
- Median OS = 6.3 months

*Blood.* 2017;130(16):1800-1808

# *CAR-T Cells-based treatments: opportunities and challenges*

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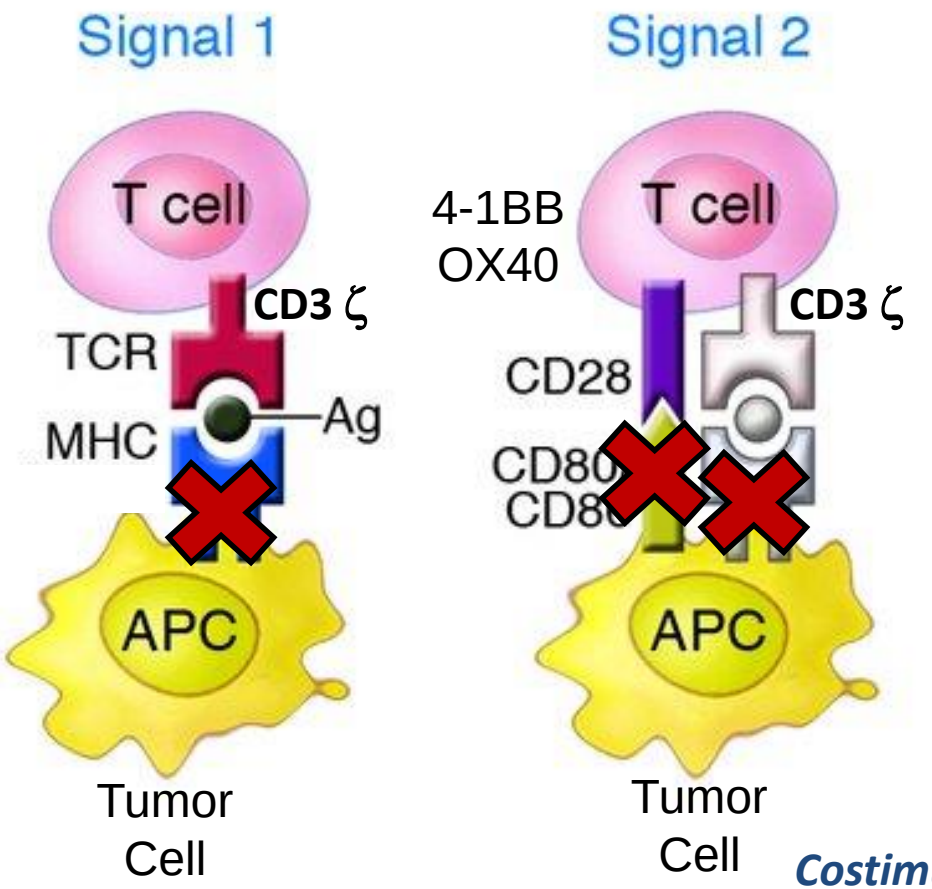
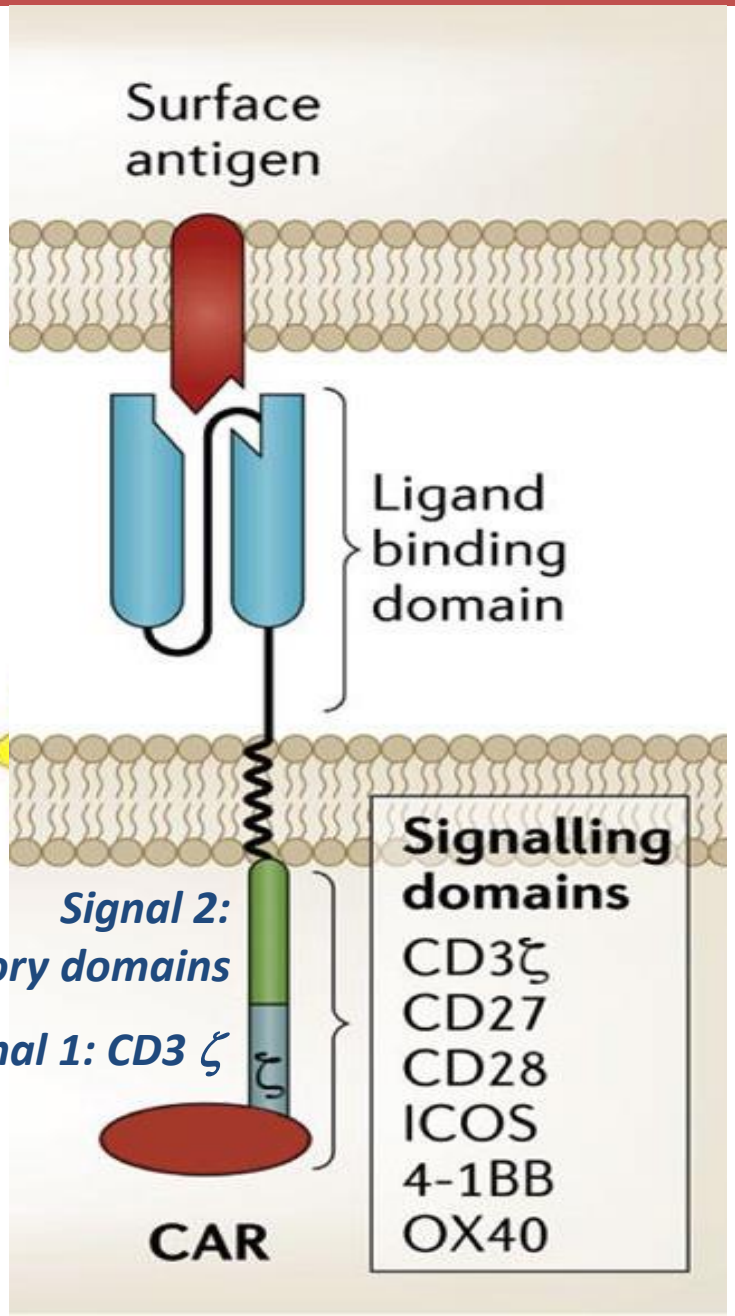
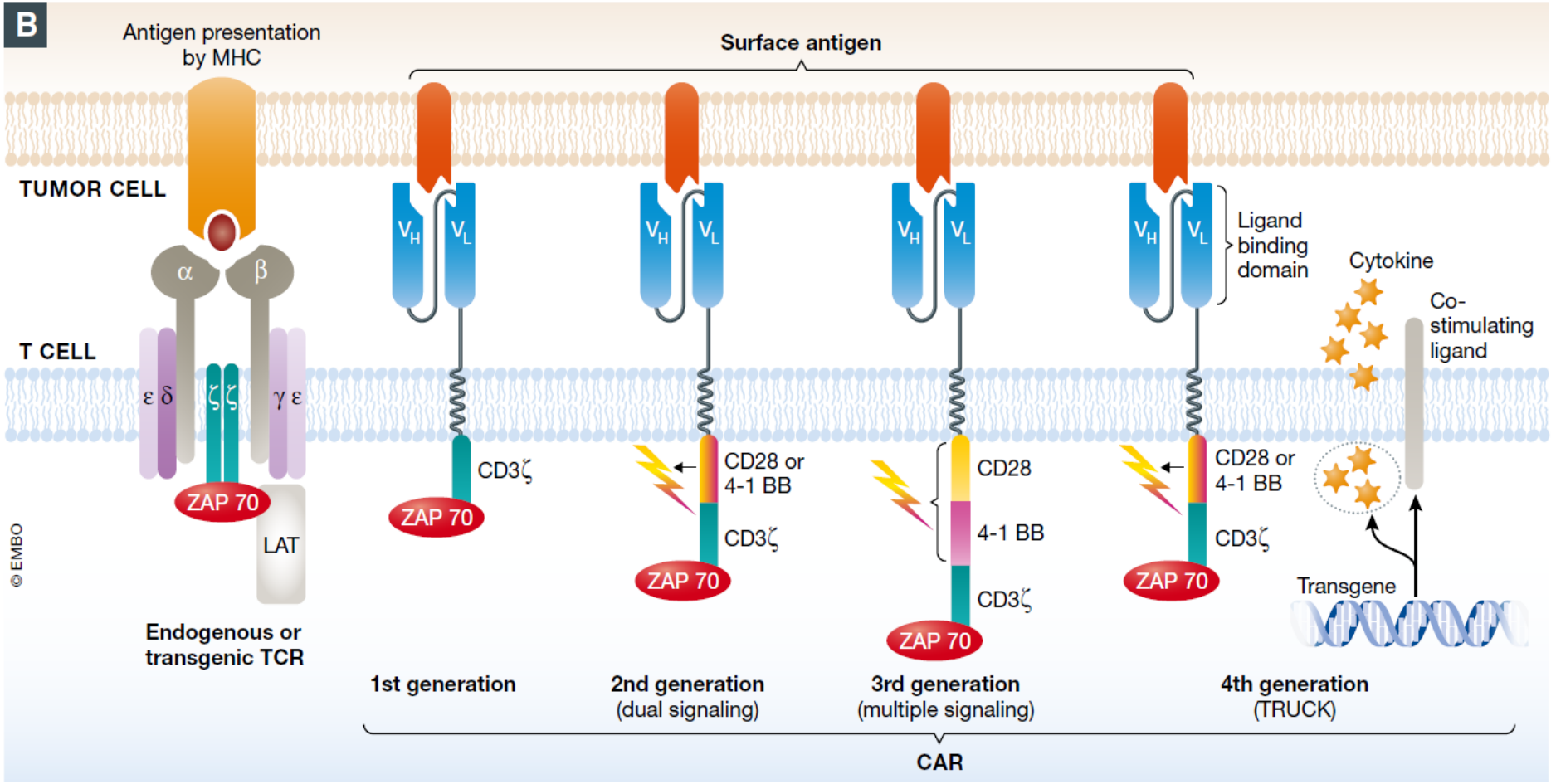


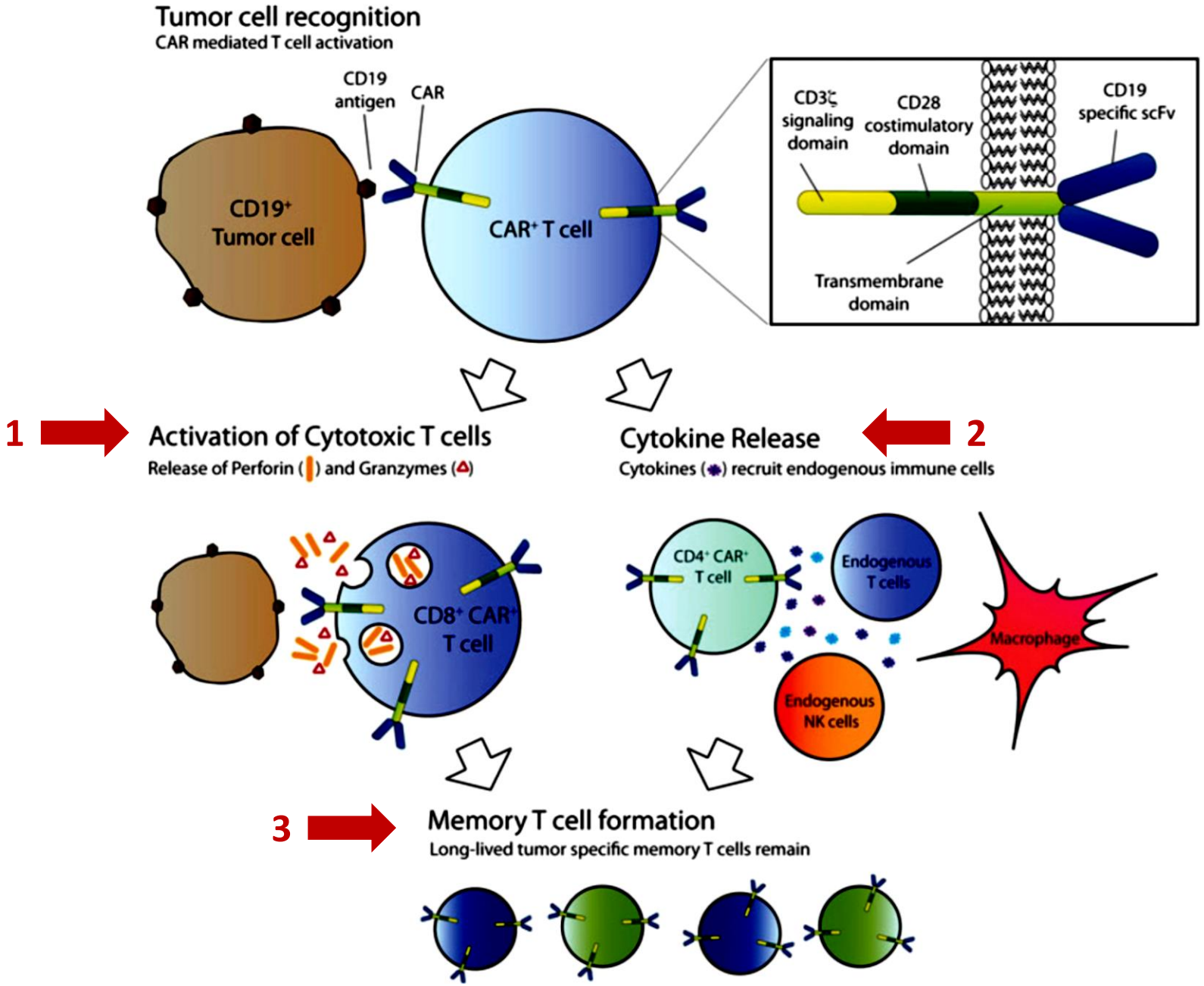



# CAR-T Cells-based treatments: opportunities and challenges

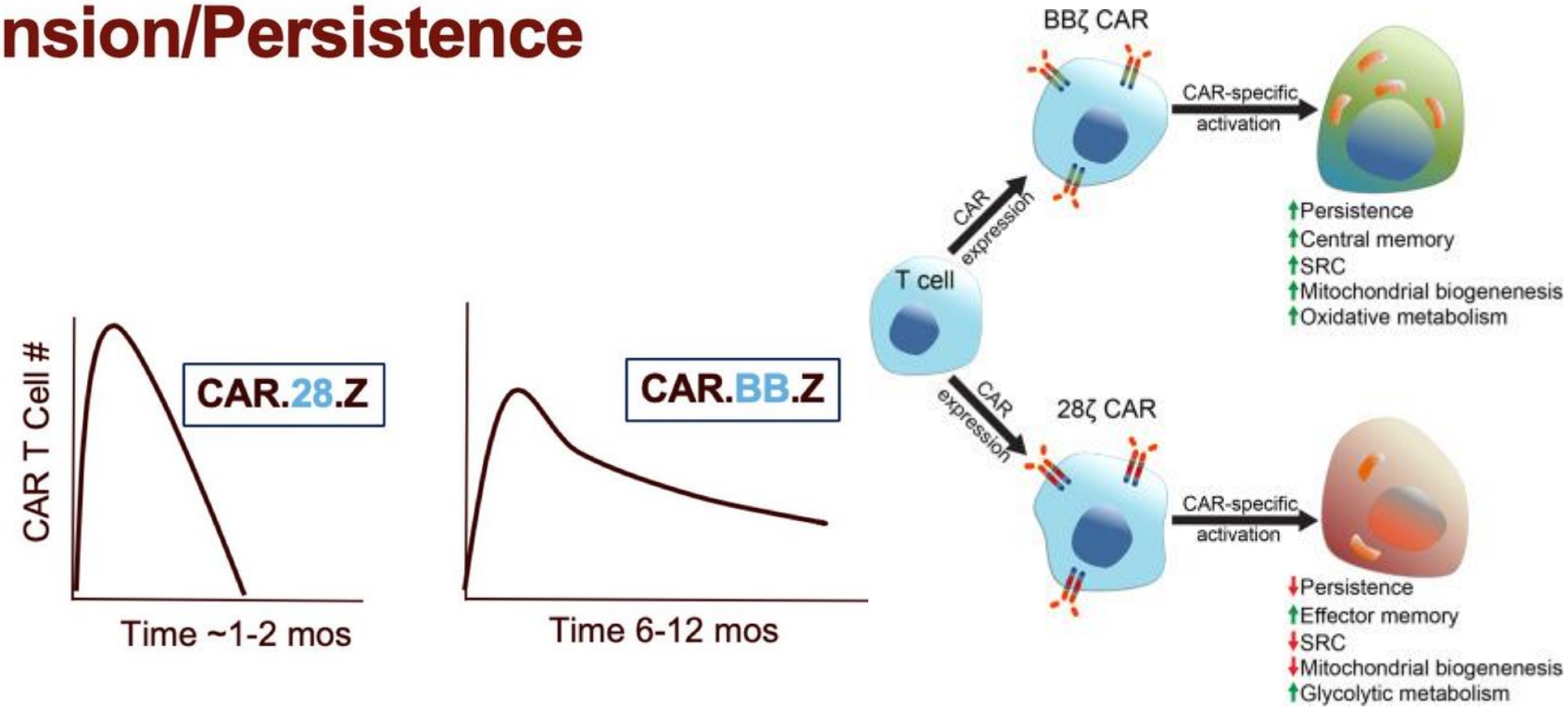



| | | | |
|---|---|---|---|
| Killing ability | + | + | ++ |
| Ability to multiply | | + | ++ |
| Cytokine secretion | | + | ++ |
| Persistence | | + | ++ |

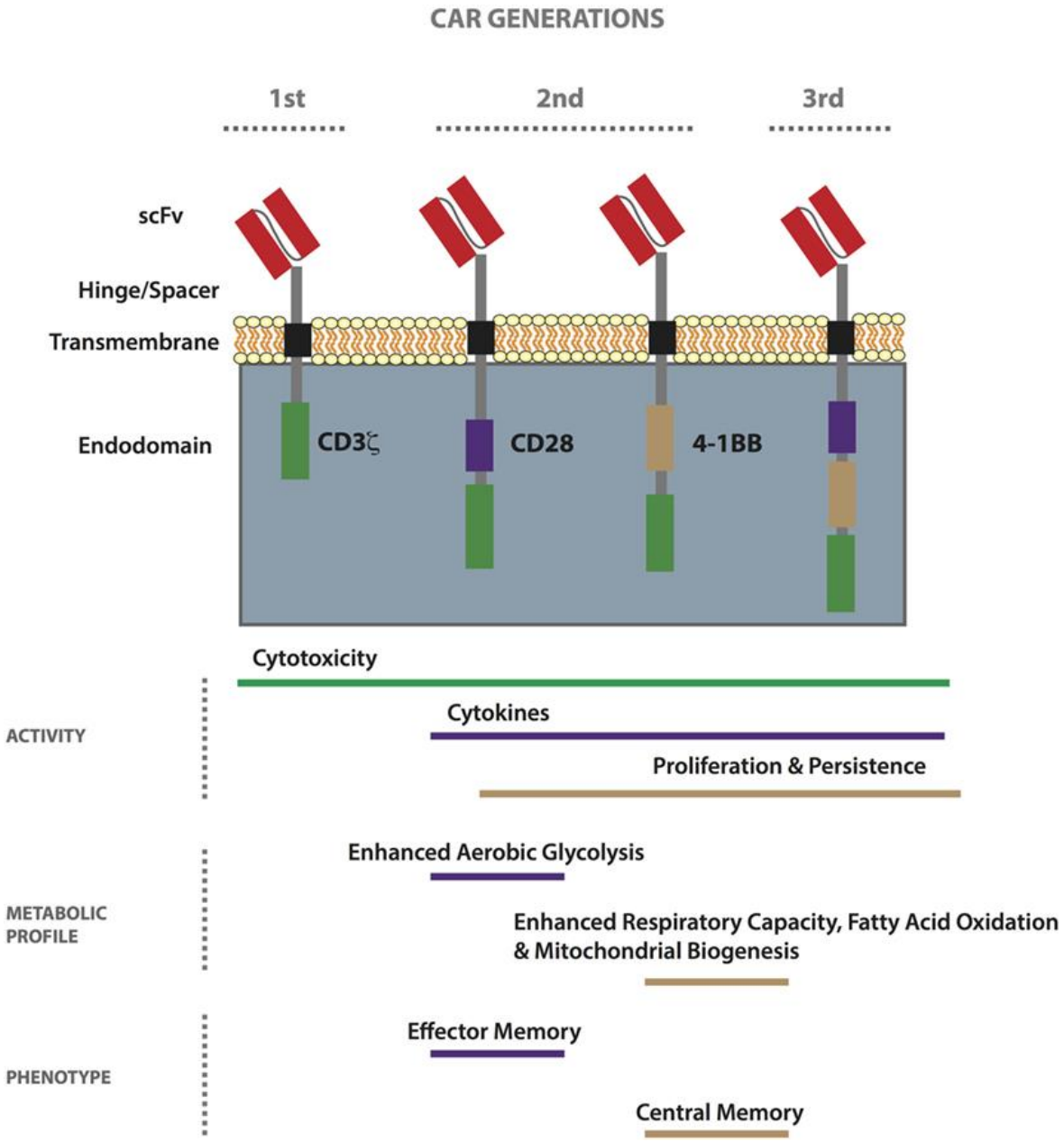
# CAR-T Cells-based treatments: opportunities and challenges



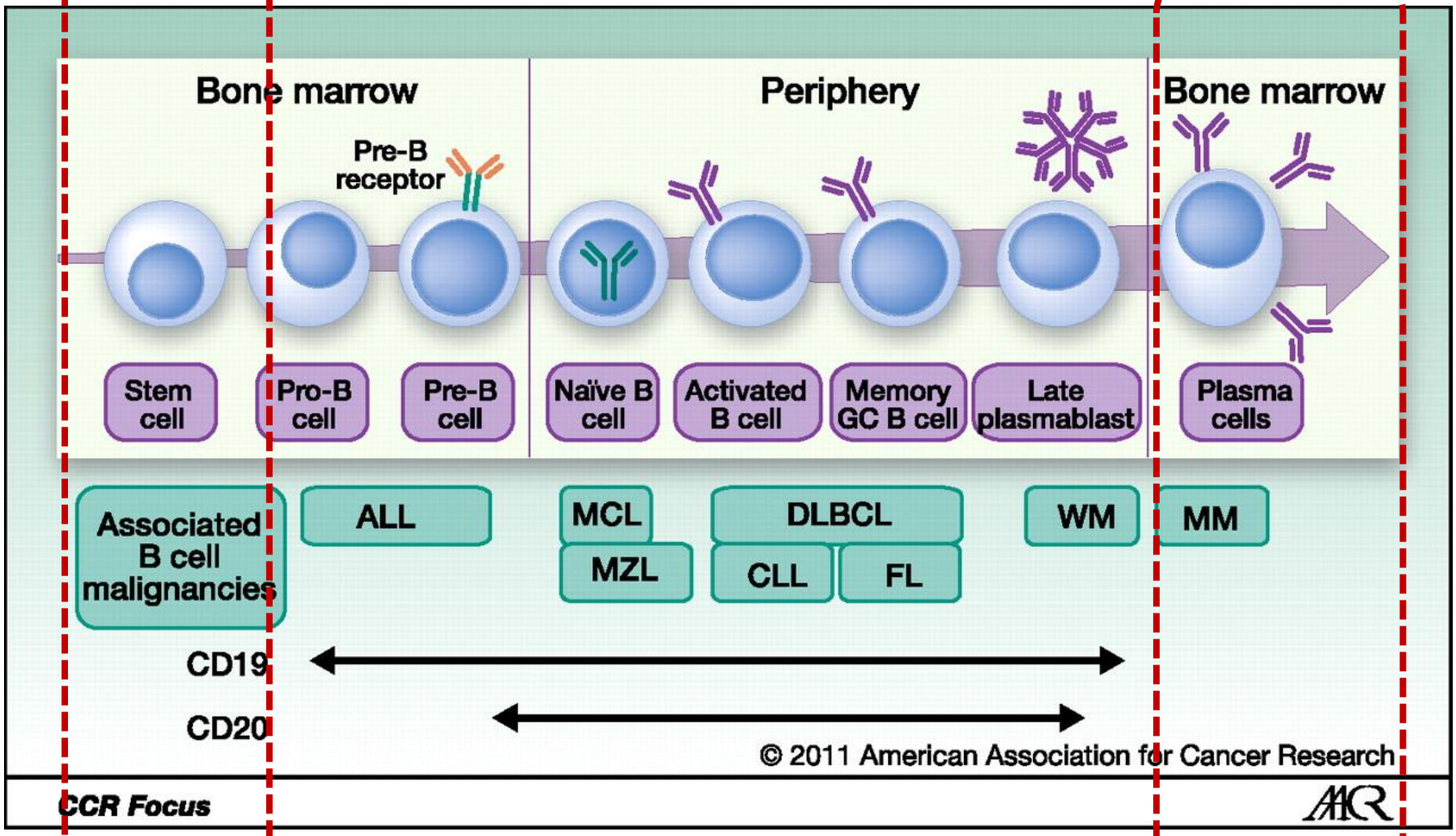
# Costimulation and T-cell Expansion/Persistence



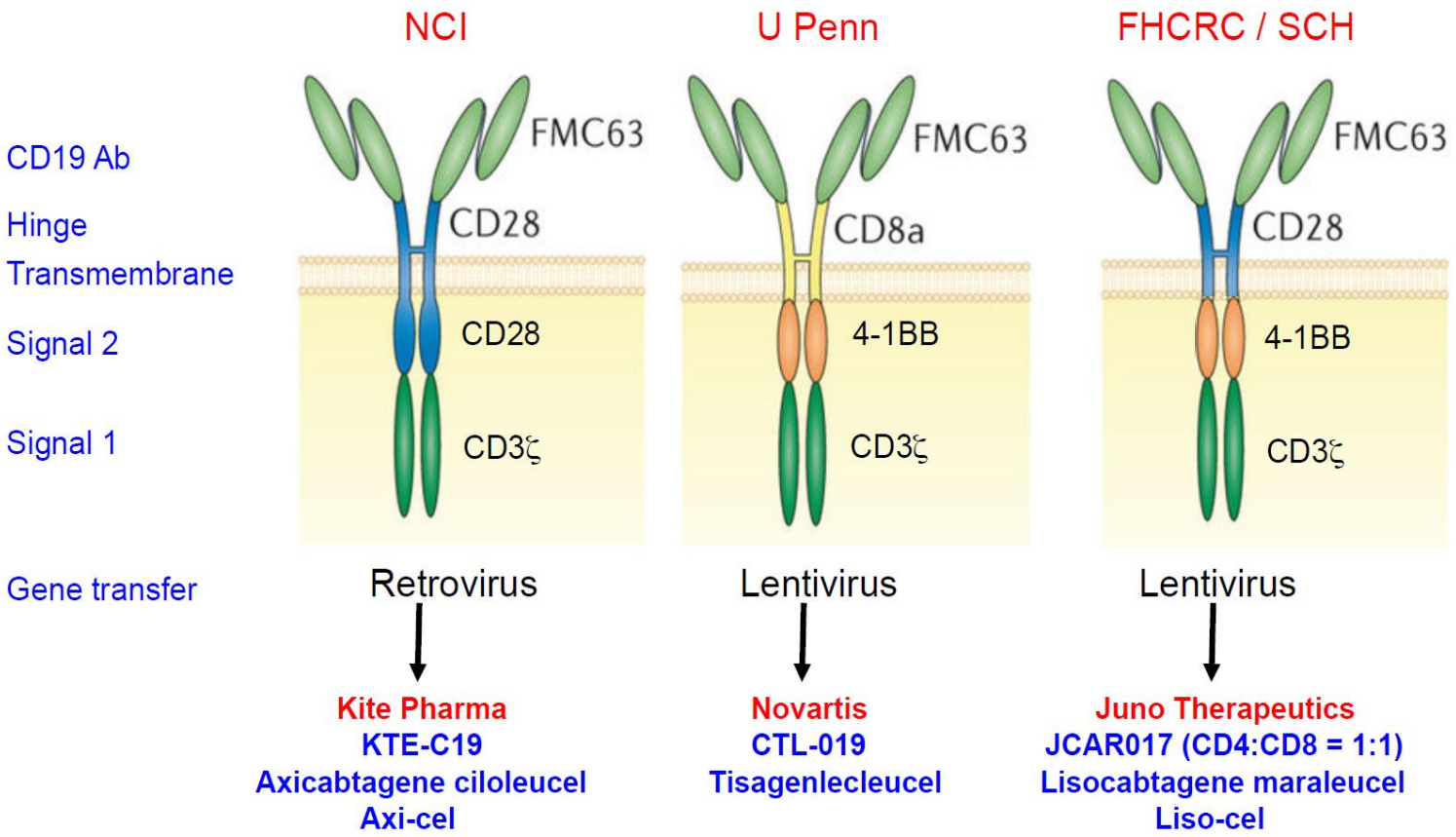
# CAR-T Cells-based treatments: opportunities and challenges



# CAR-T Cells-based treatments: opportunities and challenges



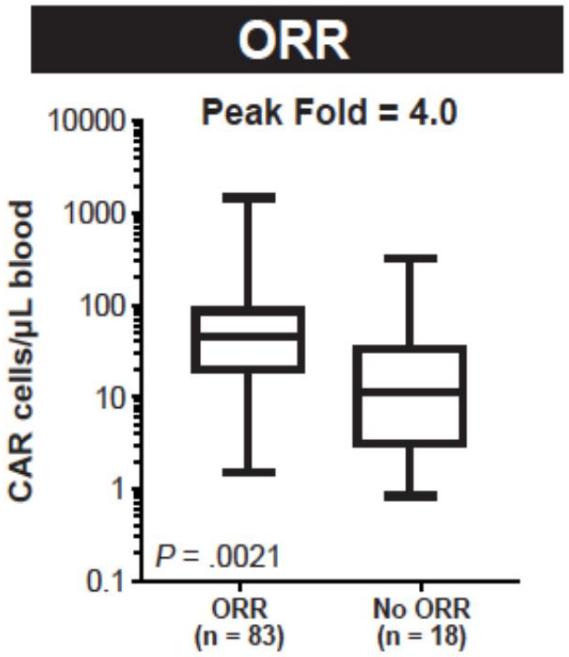
# CD19 CAR T products in pivotal trials in NHL

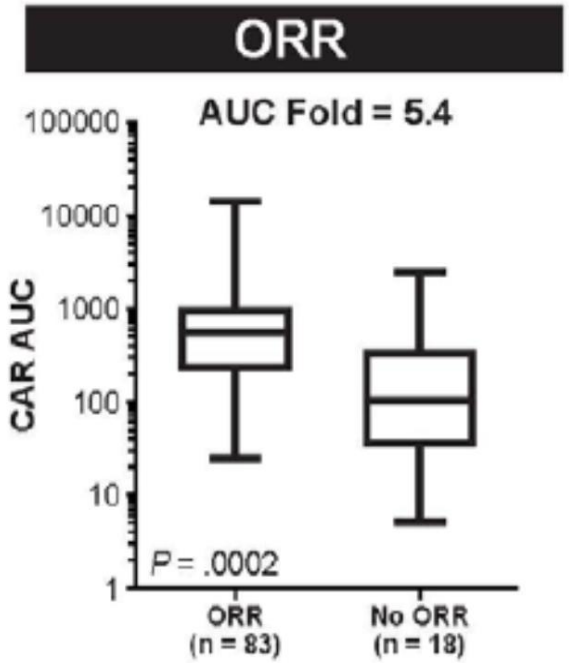



Adapted from van der Steegen et al. Nat Rev Drug Discov, 2015

# ZUMA1: CAR T-cell expansion after axi-cel infusion is associated with response

CD28

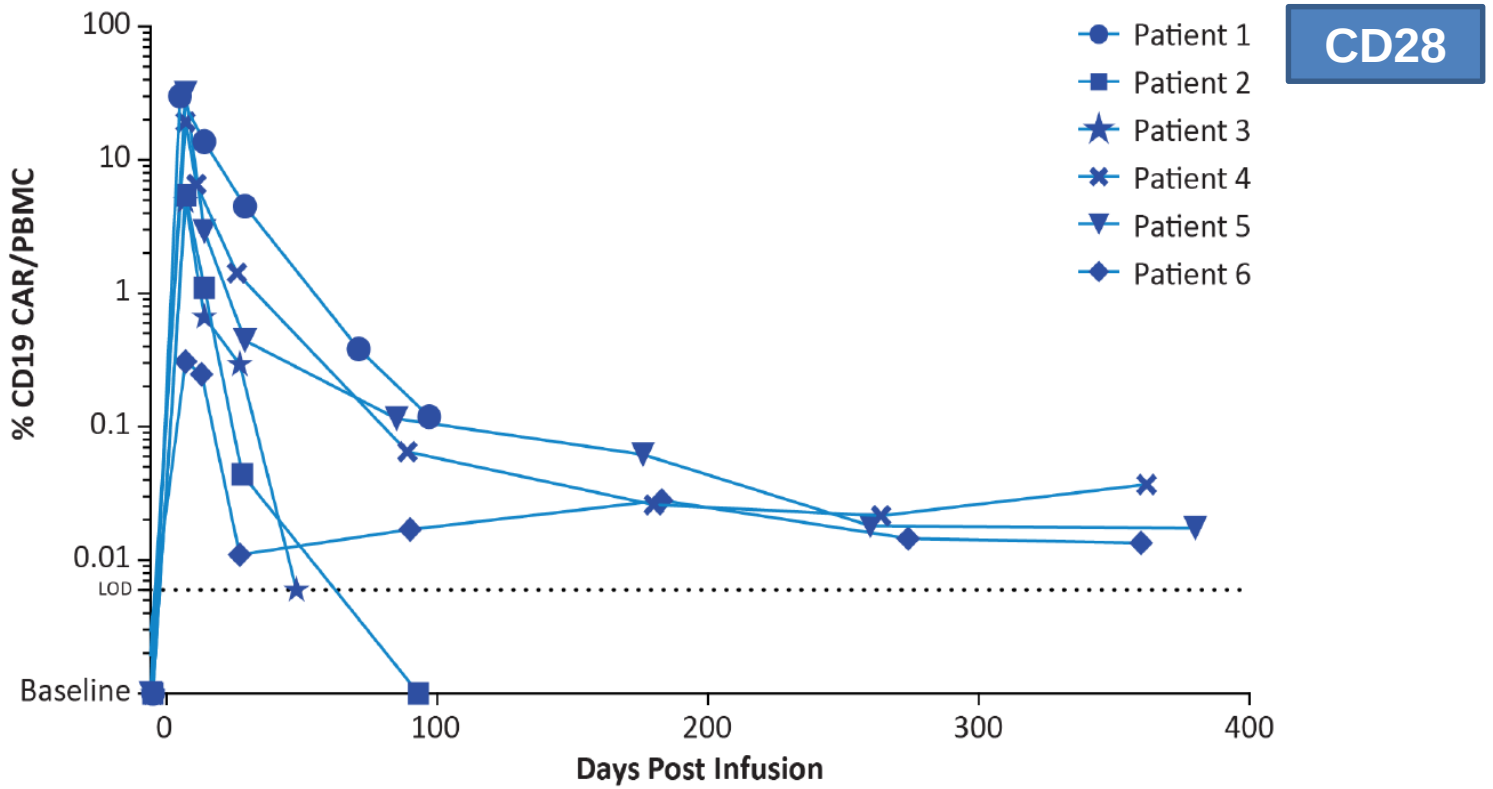



Neelapu et al. N Eng J Med 2017

# CAR-T Cells-based treatments: opportunities and challenges

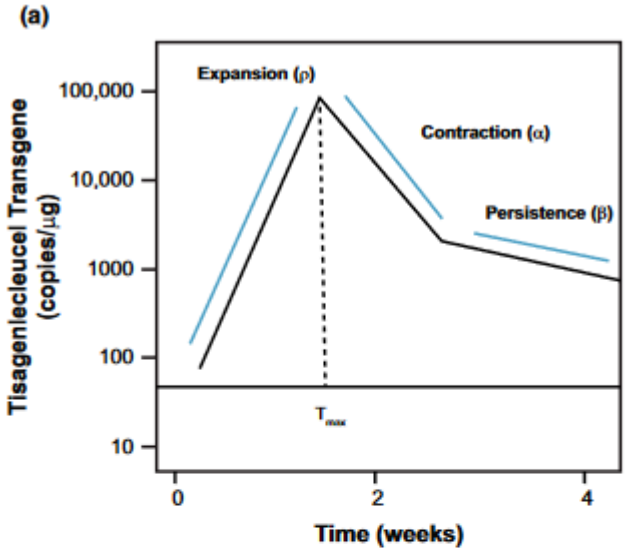
## CAR T cell expansion and persistence after KTE-C19 infusion

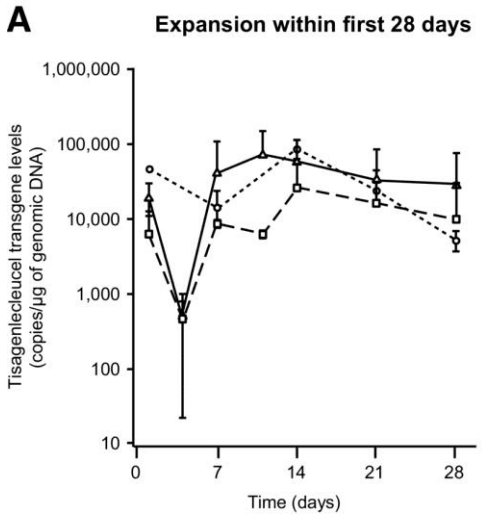


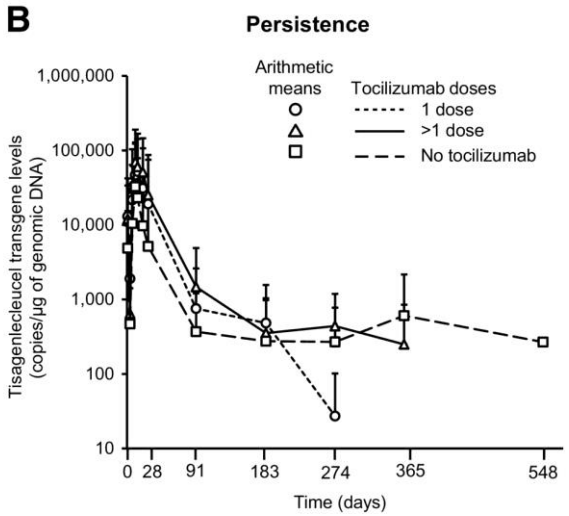

- Peak expansion of CAR T cells observed within 2 weeks
- CAR T cells detectable one year after infusion

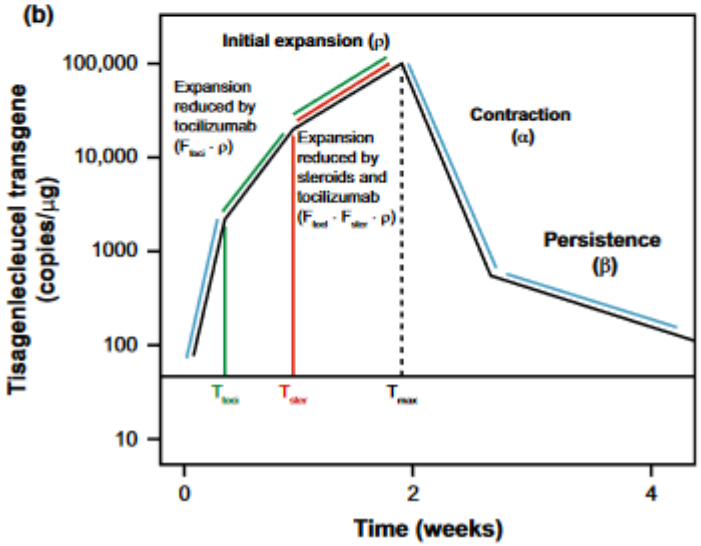
*Locke, Neelapu et al, Mol Ther (In press)*

# Tisagenlecleucel (CTL019; Novartis): JULIET Trials



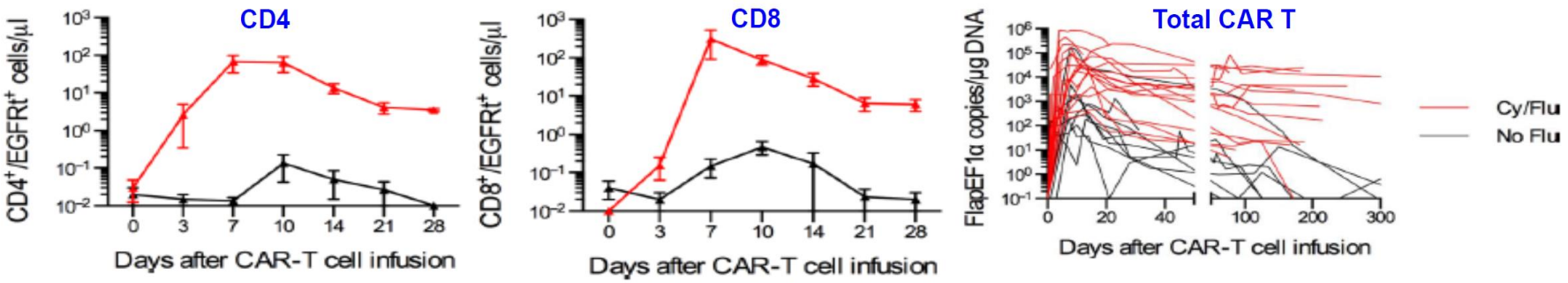



## Conditioning chemotherapy affects CAR T cell expansion, persistence, and clinical outcome

DLBCL, transformed LBCL, FL, MCL (**CD19/CD3ζ/4-1BB)**



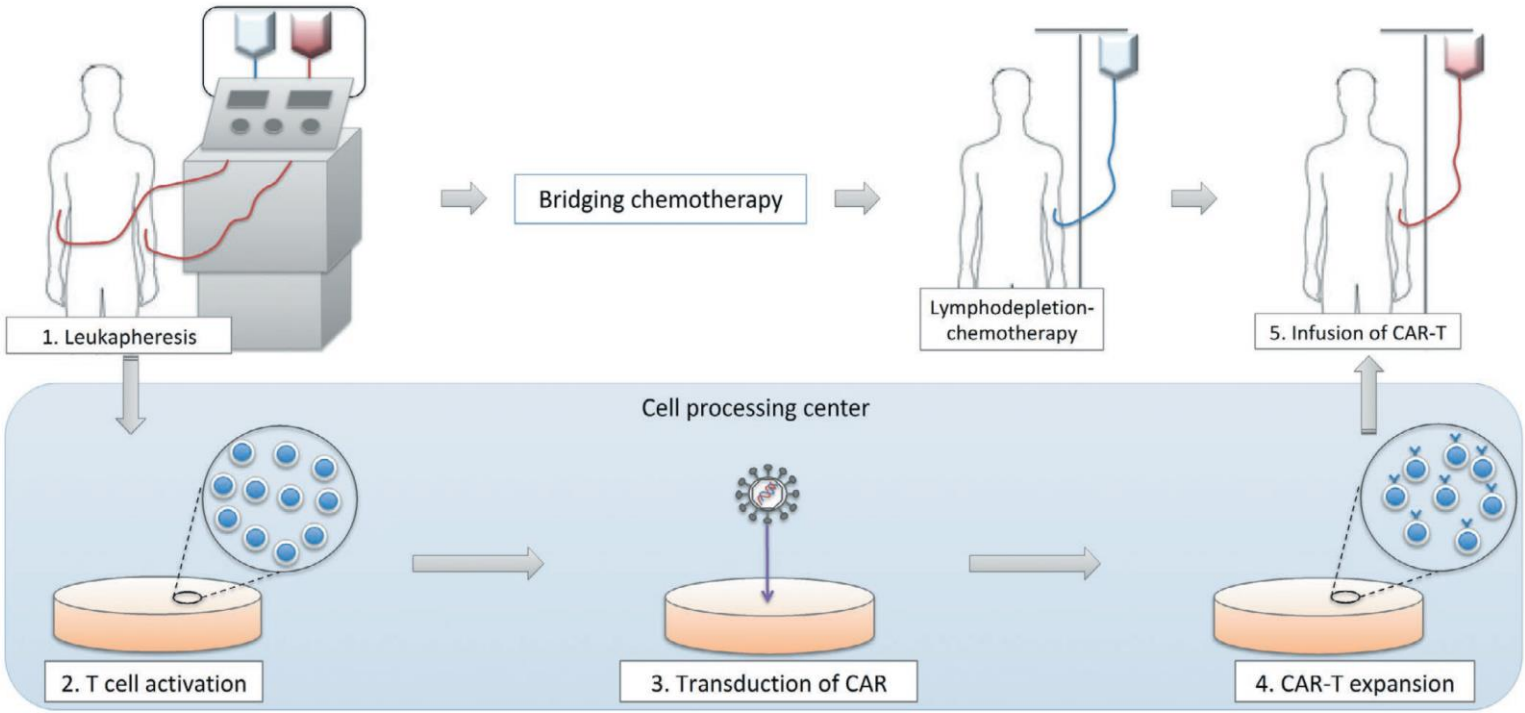

- CAR T cells reached higher peaks and persisted longer with Cy/Flu conditioning regimen compared with Cy regimen

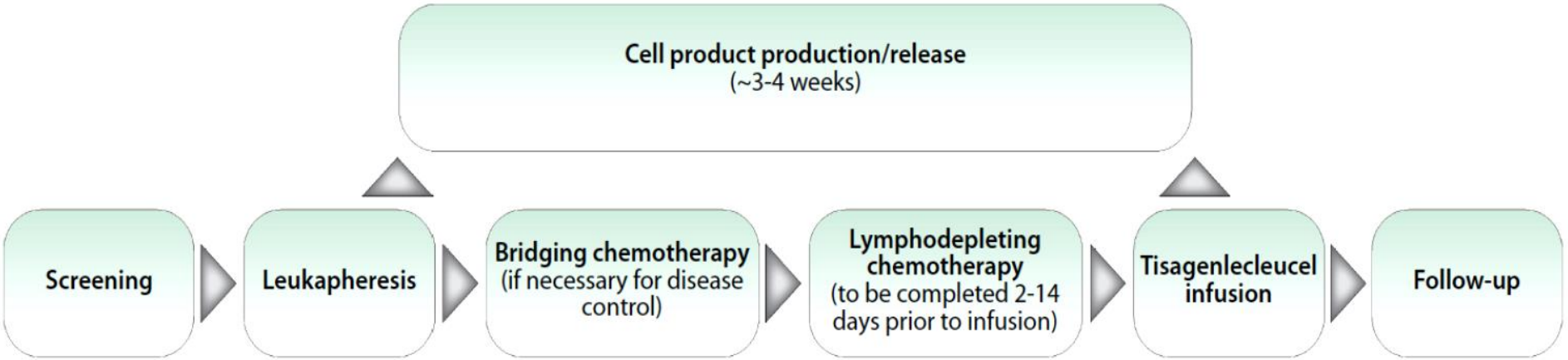
| Subgroup | N | ORR | CR |
|---|---|---|---|
| Cy or Cy/E | 12 | 50% | 8% |
| Cy/Flu | 18 | 72% | 50% |

Turtle et al, Science Trans Med, 2016

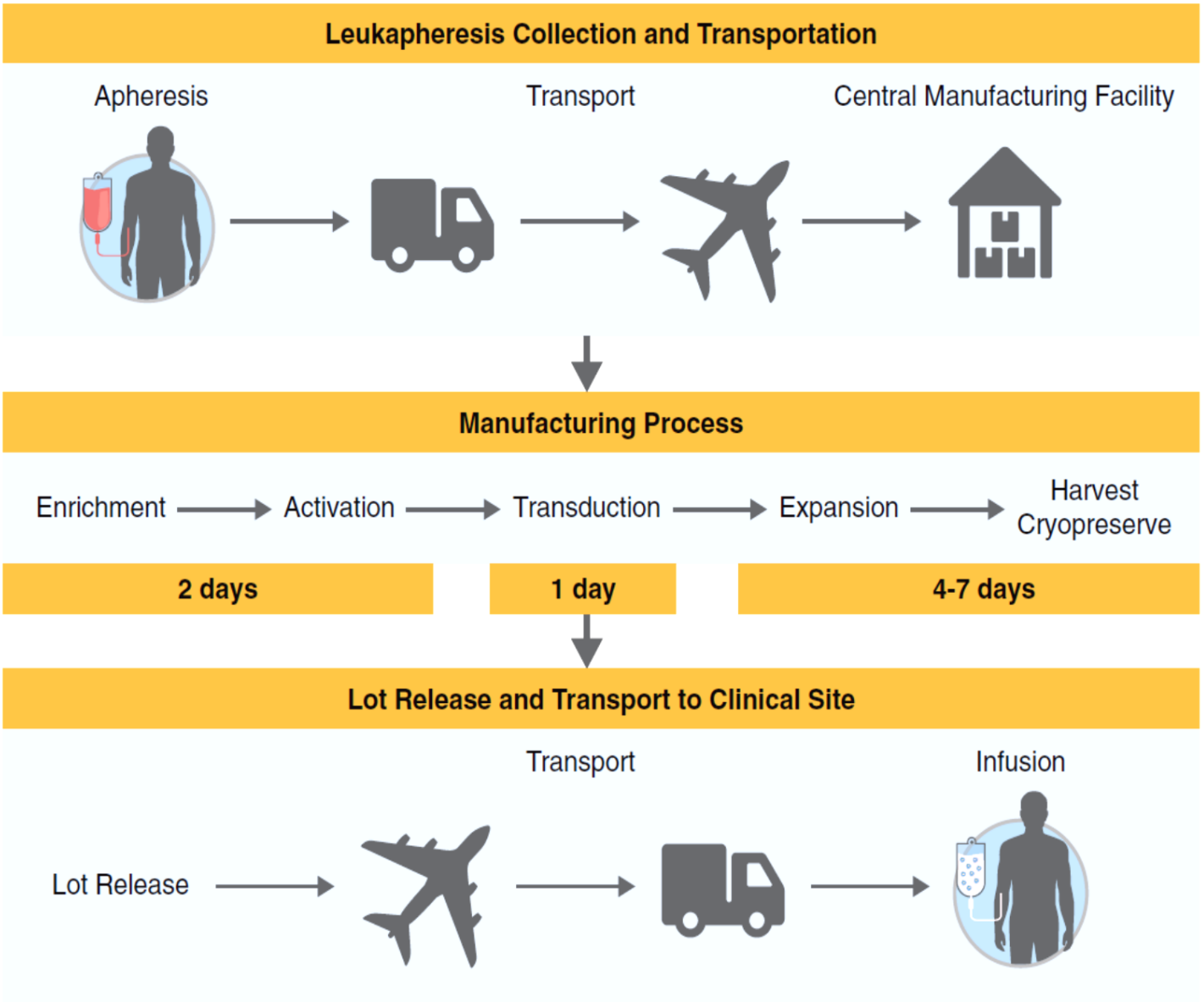
# CAR-T Cells-based treatments: opportunities and challenges

**Figure 2. Outline of CAR T-cell therapy.**



## LE CAR-T POSSONO ESSERE CONSIDERATE COME UN *'FARMACO IMMUNOLOGICO AUTOLOGO PERSONALIZZATO'*



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# *New frontiers in the treatment of NHL: Selected CAR-T Cell Products*

| | **Axicabtagene Ciloleucel (axi-cel)** *Kite Pharma (GILEAD)* | **Tisagenlecleucel (CTL019)** *Novartis* | **Lisocabtagene Maraleucel (liso-cel)** *Juno Therapeutics* |
|---|---|---|---|
| **CAR Type** | CD19/CD28/CD3z | CD19/4-1BB/CD3z | CD19/4-1BB/CD3z |
| **Costimulatory Domain** | CD28 | 4-1BB (CD 137) | 4-1BB (CD 137) |
| **scFv** | FMC63 | FMC63 | FMC63 |
| **Vector Delivery** | Retrovirus | Lentivirus | Lentivirus |
| **Defined Cells** | No | No | CD4:CD8 |
| **Trial in NHL** | ZUMA-1 | JULIET | TRANSCEND |

# PRE-JULIET: Tisagenlecleucel Activity in DLBCL

- Single-center, pilot study at the University of Pennsylvania using CTL019 (tisagenlecleucel)
- Transient encephalopathy developed in approximately one in three patients and severe cytokine-release syndrome developed in one in five patients
- Durable remissions with a single infusion of CTL019 in r/r DLBCL and FL

Key eligibility criteria

- Adult histologically proven CD19+ relapsed or refractory DLBCL, FL or MCL
- DLBCL R/R after ASCT or ineligible for ASCT; transformation from CLL/SLL or FL allowed
- FL with ≥2 prior CIT regimens and PD <2 years after prior therapy
- ECOG PS 0 or 1

**Response Rates in r/r DLBCL (N = 14)**

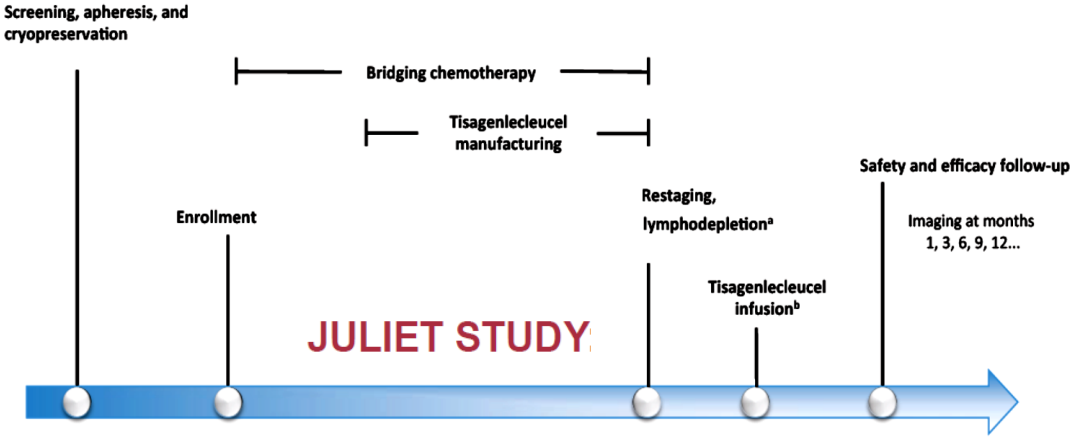
| | Month 3 | Month 6 |
|---|---|---|
| ORR | 50% | 50% |
| CR | 36% | 43% |
| PR | 14% | 7% |

CR, complete response; ORR, overall response rate; PR, partial response

- Median response duration: not reached
- No patient in CR at 6 months had relapsed at median follow-up, 28.6 months

Schuster, Svoboda, Chong *et al*. N Engl J Med. 2017 Dec 28;377(26):2545-2554.

# Tisagenlecleucel (CTL019; Novartis): JULIET Trials




[a] To be completed 2 to 14 days prior to tisagenlecleucel infusion:
-fludarabine (25 mg/m2)/cyclophosphamide (250 mg/m2) x 3 days or bendamustine (90 mg/m2) x 2 days
[b] Infusion conducted on an in- or outpatient basis at investigator discretion.

| | Patients (N = 111) |
|---|---|
| Age, median (range), years | 56 (22-76) |
| ≥ 65 years, % | 23 |
| ECOG performance status 0/1, % | 55/45 |
| Central histology review | |
| Diffuse large B-cell lymphoma, % | 79 |
| Transformed follicular lymphoma, % | 19 |
| Double/triple hits in *CMYC/BCL2/BCL6* genes, % | 17[a] |
| Cell of origin[b] | |
| Germinal/Nongerminal center B-cell type, % | 57/41 |
| Number of prior lines of antineoplastic therapy, % | |
| 2/3/4-6 | 44/31/21 |
| IPI ≥ 2 at study entry, % | 72 |
| Refractory/relapsed to last therapy, % | 55/45 |
| Prior auto-SCT, % | 49 |
| **Bridging chemotherapy, n** | **102** |
| **Lymphodepleting chemotherapy, n** | **103** |

## Key eligibility criteria

- ≥ 18 years of age
- Central confirmation of histology
- ≥ 2 prior lines of therapy for DLBCL
- PD after or ineligible for auto-SCT
- No prior anti-CD19 therapy
- No active CNS involvement

## Endpoints

- **Primary endpoint:** best overall response rate (ORR: CR + PR)
  - Lugano criteria used for response assessment by IRC[1]
- **Secondary endpoints:** DOR, OS, safety

Schuster, Bishop, Tam *et al*, N Engl J Med. 2019 Jan 3;380(1):45-56.

# *Tisagenlecleucel (CTL019; Novartis): JULIET Trials*

| Response Rate, % | Best Overall Response Rate (N = 93) | Response at 3 Months (N = 93 ) | Response at 6 Months (n = 93) |
|---|---|---|---|
| ORR (CR + PR) | 52[a] | 38 | 33 |
| CR | 40 | 32 | 29 |
| PR | 12 | 5 | 3 |

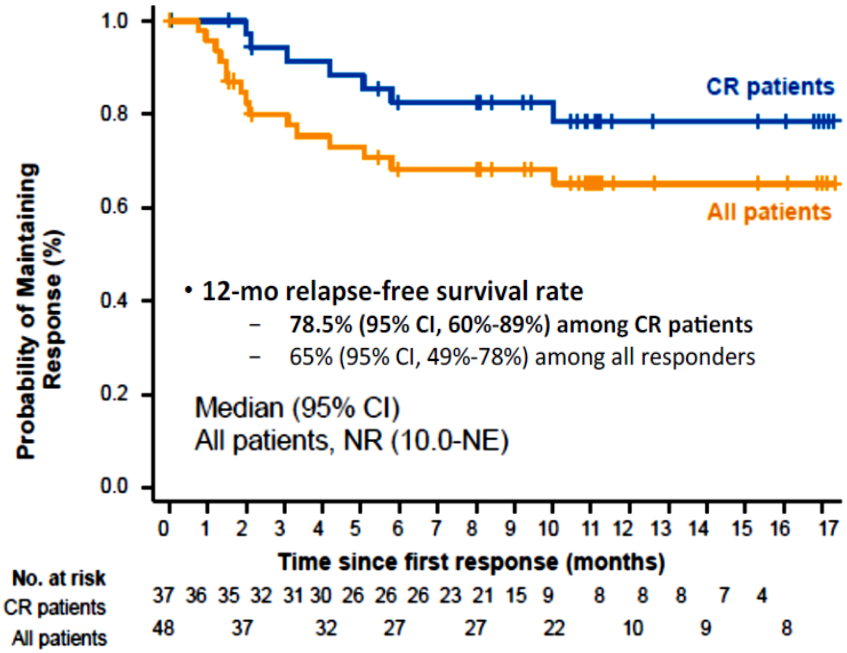
[a] *P* < .0001; (95% CI, 42%-64%). Null hypothesis of ORR ≤ 20%.

- Durability of responses is shown by the stability between 3 and 6 month response rates
- Response at 3 months is indicative of the long term benefit of this treatment
- No differences in outcomes based on lymphodepleting therapy used

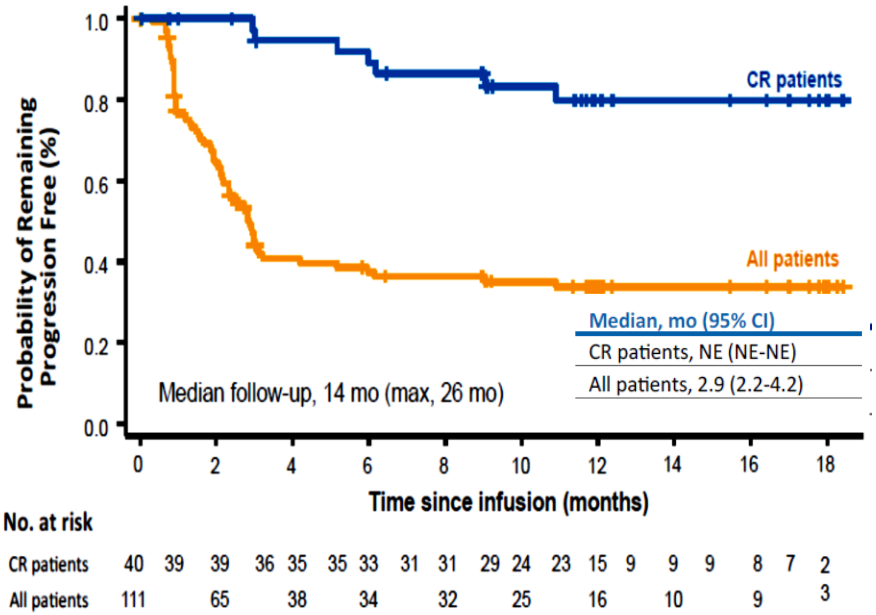
Schuster, Bishop, Tam *et al*, N Engl J Med. 2019 Jan 3;380(1):45-56.

# Tisagenlecleucel (CTL019; Novartis): JULIET Trials

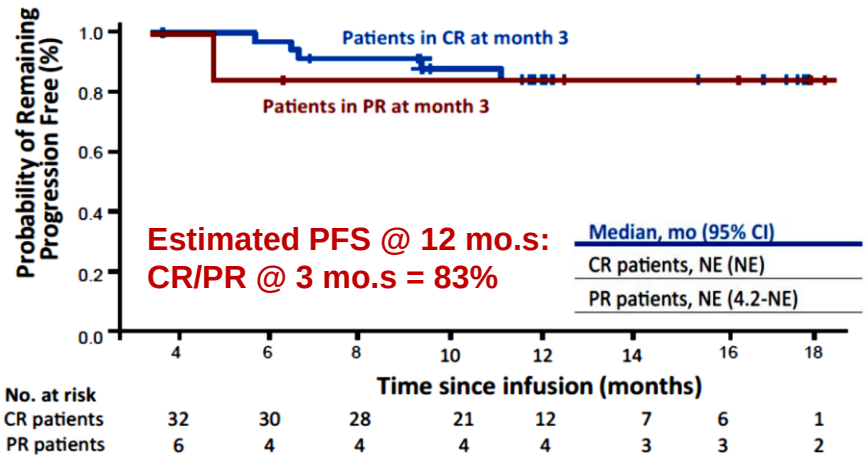
**1. @14 mo.s of FU median DOR not reached**



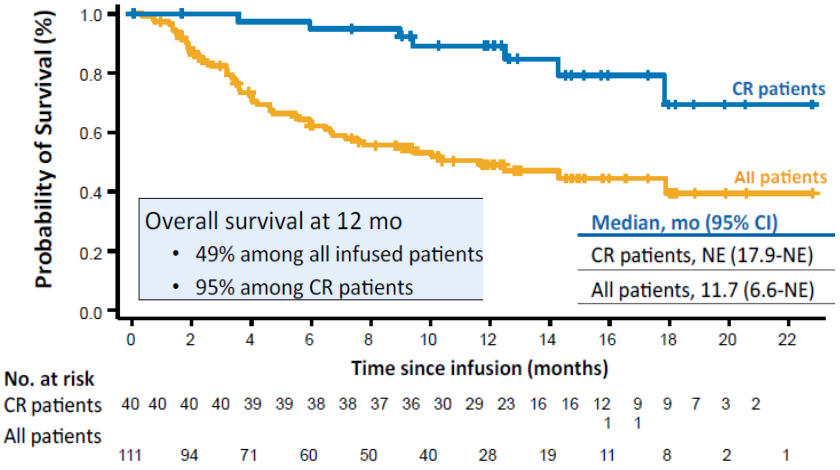

**2. Median PFS not reached in CR pts.**




**3. Response @ 3 mo.s may predict a durable benefit**




**4. 74% of pts. relapse-free @**



# Axicabtagene Ciloleucel (axi-cel; Kite-Gilead): ZUMA Trials

## ZUMA-1

**Phase 1**

**Refractory DLBCL/PMBCL/TFL** (cohort of n = 6)

**Phase 2**

- **Cohort 1** Refractory DLBCL
- **Cohort 2** Refractory PMBCL/TFL

### Eligibility criteria

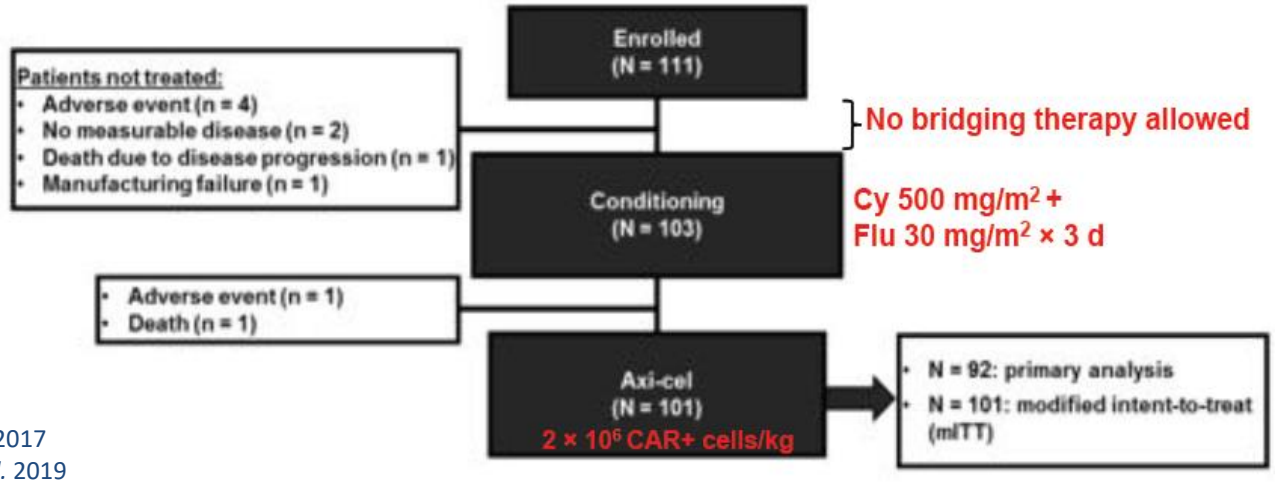
- Aggressive NHL: DLBCL, PMBCL, TFL
- Chemotherapy-refractory disease: no response to last chemotherapy or relapse ≤12 months post-ASCT
- Prior anti-CD20 mAb and anthracycline
- ECOG PS 0-1

### Primary end point

- Phase 2: Objective response rate (ORR)

### Key secondary end points

- DOR, OS, safety, levels of CAR T and cytokines

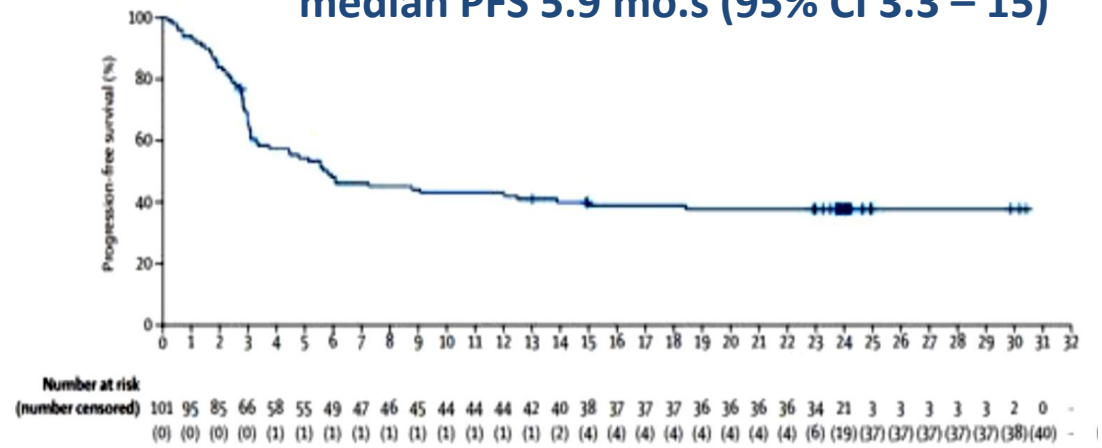



Neelapu *et al*. 2017
Abramson *et al.* 2019

# *Axicabtagene Ciloleucel (axi-cel; Kite-Gilead): ZUMA Trials*

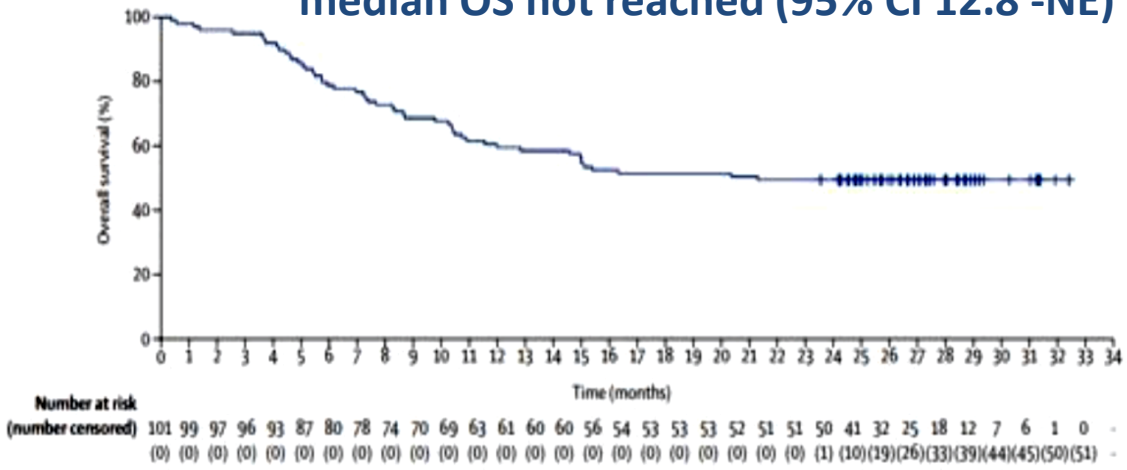
## Progression-Free Survival

**median PFS 5.9 mo.s (95% CI 3.3 – 15)**



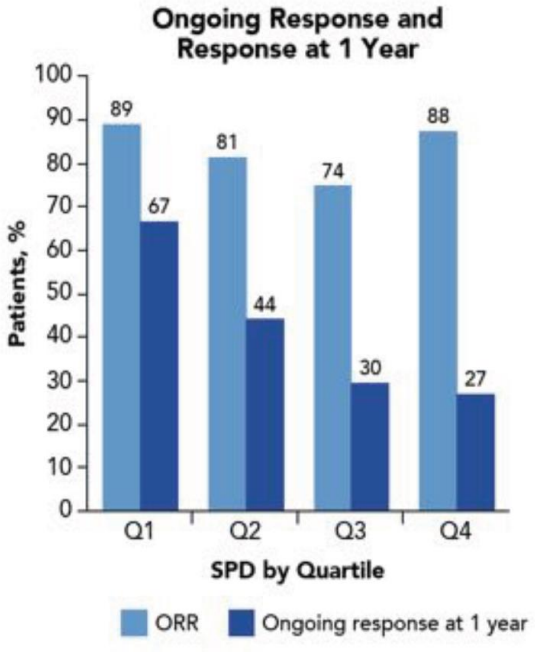

## Overall Survival

**median OS not reached (95% CI 12.8 -NE)**




## Zuma-1 Trial (Phase 2 part): @ median FU of 27 mo.s

- N = 101
- ORR = 82%; CR rate = 54%
- 52% @18 months




Locke et al. Lancet Oncol 2019

# *Axicabtagene Ciloleucel (axi-cel; Kite-Gilead): ZUMA Trials*

| Cytopenia | Any grade (%) | Grade 3-4 (%) |
|---|---|---|
| Any cytopenia | 93 | 86 |
| Neutropenia | 86 | 80 |
| Thrombocytopenia | 62 | 40 |
| Anemia | 68 | 45 |
| Cytopenia after month 3 | 34 | 17 |
| Neutropenia | 19 | 11 |
| Thrombocytopenia | 18 | 7 |
| Anemia | 18 | 3 |

ZUMA-1

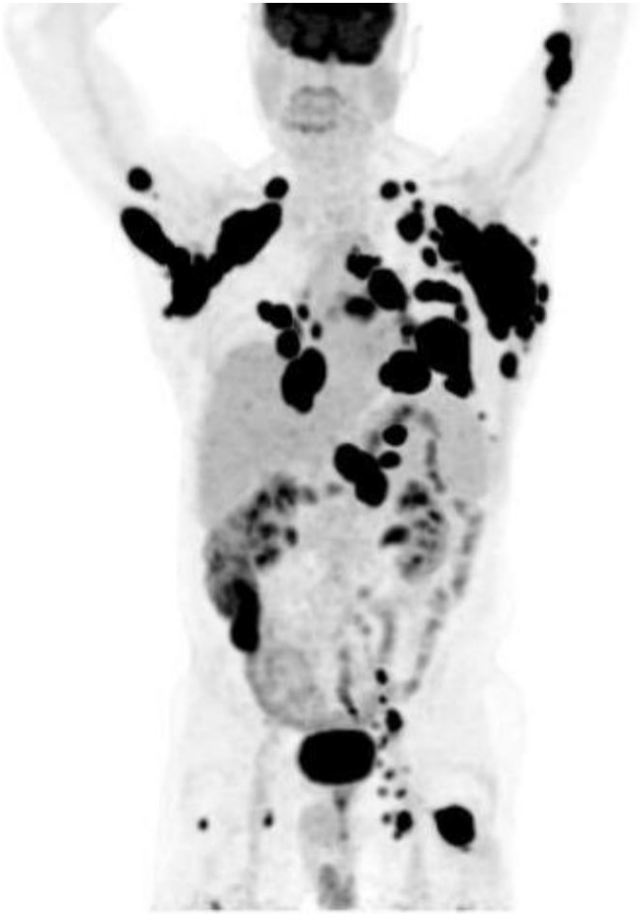
| n | 108 |
|---|---|
| Any CRS<br>Median time to onset | 93%<br>1 day |
| ≥ Gr 3 CRS (Lee criteria) | 13% |
| Any neurotoxicity | 64% |
| ≥ Gr 3 neurotoxicity | 28% |
| Tocilizumab | 43% |
| Steroids | 27% |

# CAR-T Cells-based treatments: opportunities and challenges

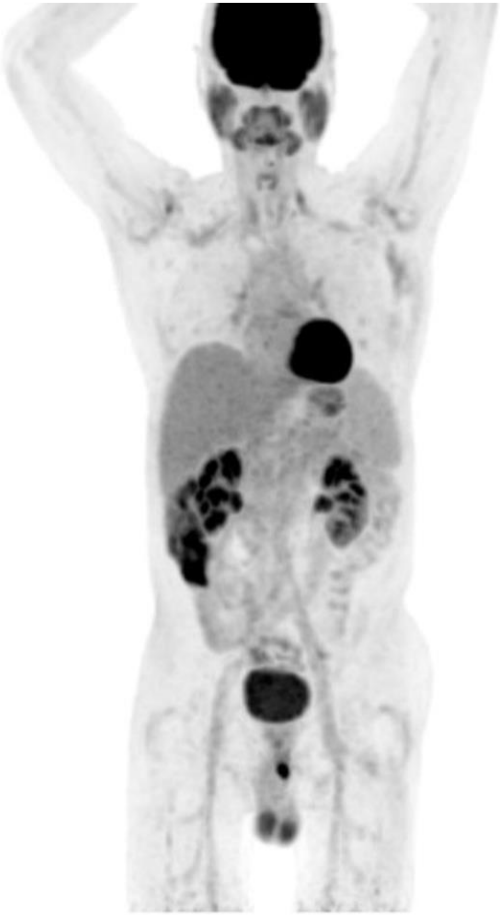
62 yo M with DLBCL
Prior therapies

- R-CHOP
- Radiation
- R-GDP
- Radiation
- R-ICE
- R-Revlimid

Baseline

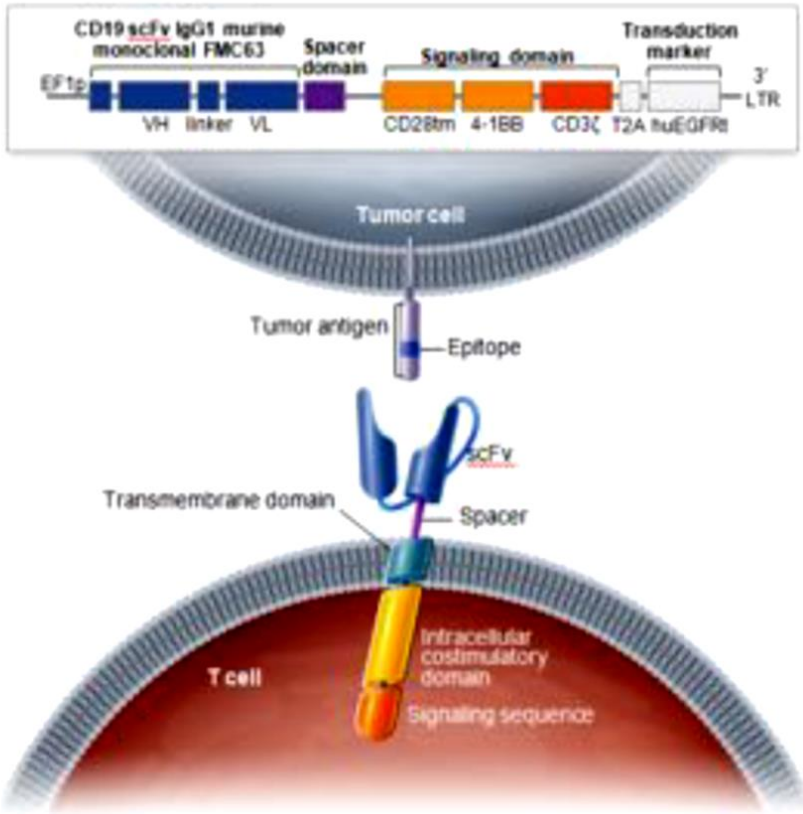


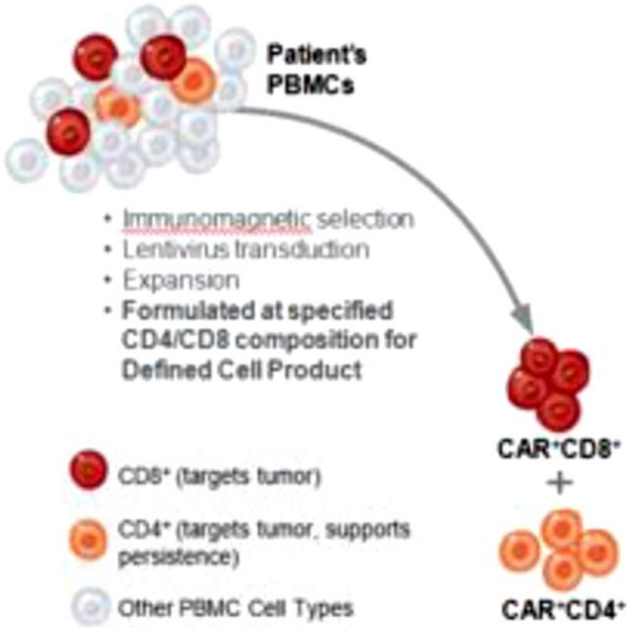
3 months

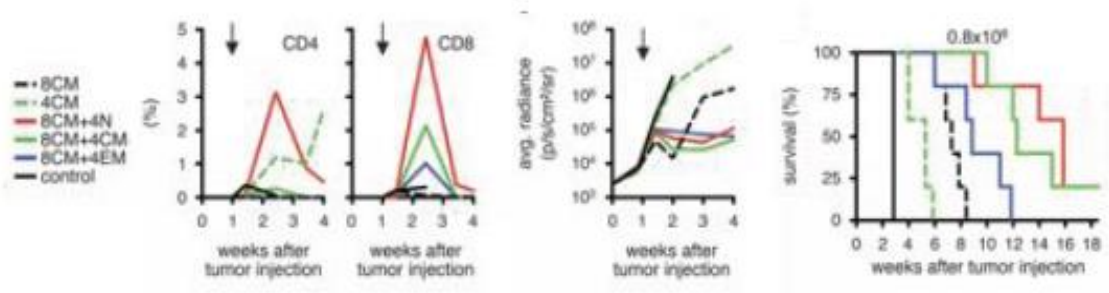


Remains in CR at 9 months following infusion of KTE-C19, ZUMA-1 trial.

# *Lisocabtagene maraleucel (liso-cel, JCAR017, Juno): TRASCEND Trials*

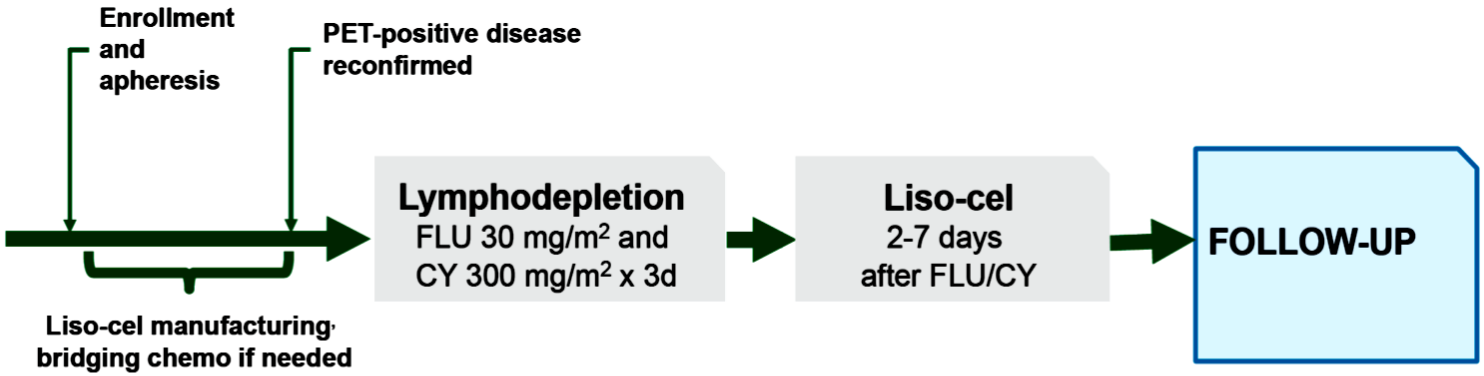



Abramson et al. EHA 2018

# *Lisocabtagene maraleucel (liso-cel, JCAR017, Juno): TRASCEND Trials*

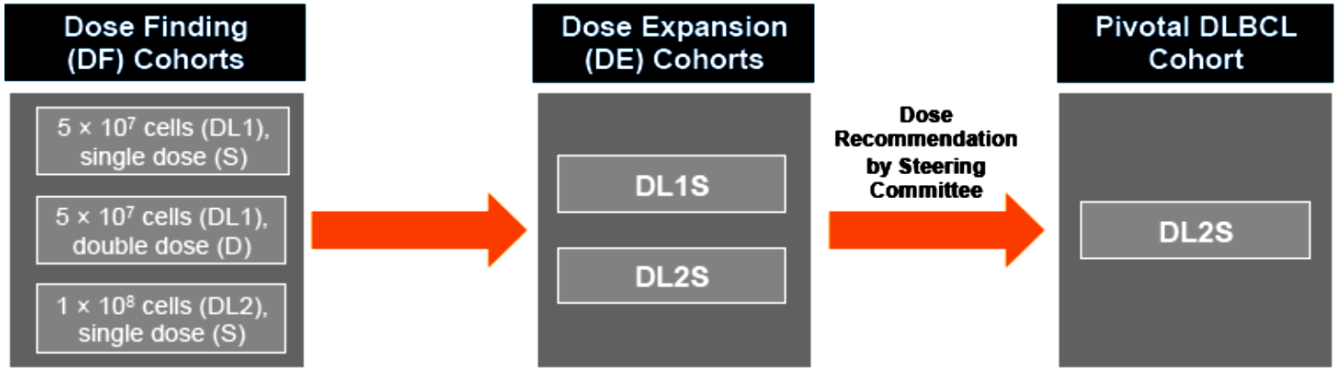



**ENROLLMENT COHORTS**

- DLBCL after 2 lines of therapy:
  - core:
    - DLBCL, NOS (de novo or transformed FL)
    - High grade B-cell lymphoma (double/triple hit)
    - DLBCL transformed from CLL or MZL
    - PMBCL
    - FL3B
- MCL after 1 line of therapy

**PATIENT ELIGIBILITY**

- Prior SCT allowed (core auto only)
- Secondary CNS involvement allowed
- ECOG PS 0-2 (core 0-1)
- No minimum absolute lymphocyte count requirement for apheresis




**Data presented from DF and DE DLBCL cohorts**

- 102 patients treated (**FULL**)
- 73 patients reflective of pivotal patient population (**CORE**)

Abramson et al. EHA 2018

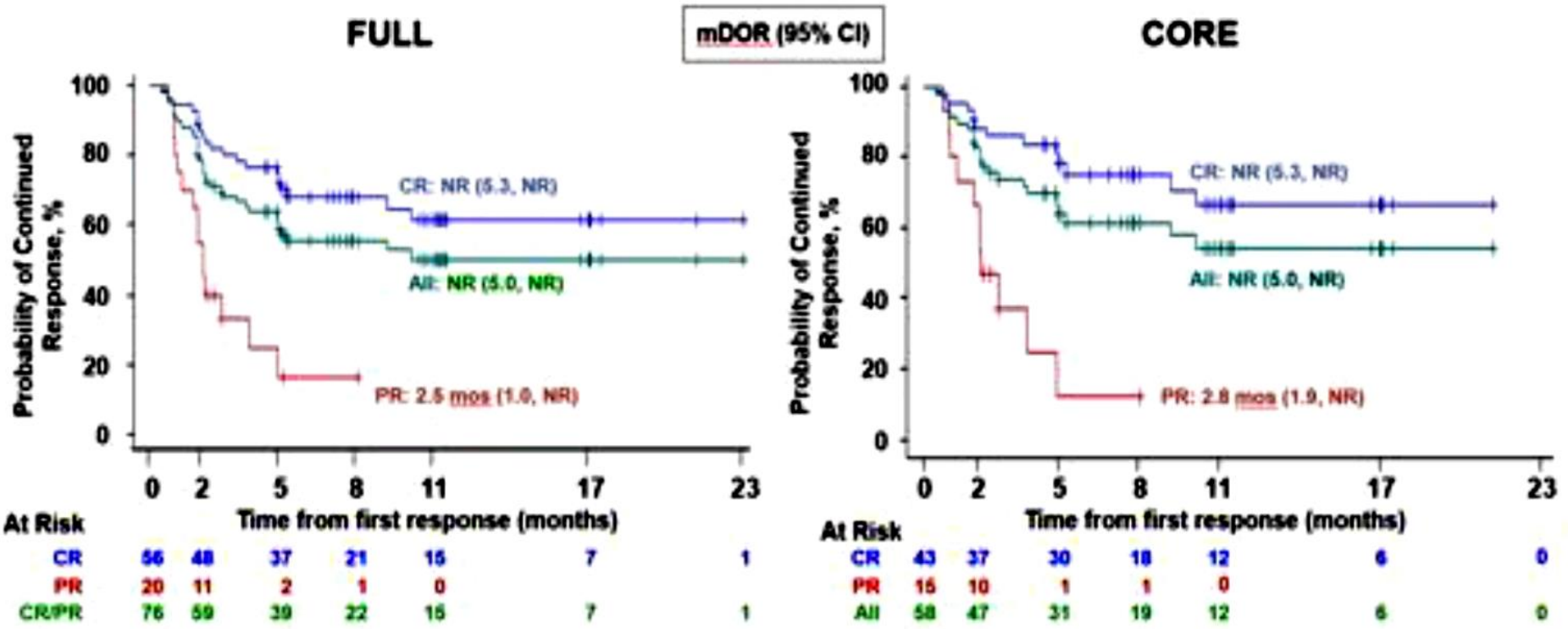
# *Lisocabtagene maraleucel (liso-cel, JCAR017, Juno): TRASCEND Trials*

| Characteristic | FULL N=102 | CORE N=73 |
|---|---|---|
| Median Age, years (range) | 61 (20-82) | 60 (20-82) |
| Double/triple hit | 19% | 22% |
| CNS involvement | 2% | 1% |
| Median prior lines (range) | 3 (1-8) | 3 (2-8) |
| Chemorefractory | 70% | 67% |
| Any HSCT | 40% | 38% |
| Allogeneic | 5% | 0 |
| Autologous | 37% | 38% |

| | All Subjects n=102 | CORE DL 1 SD n=33 | CORE DL 2 SD n=37 |
|---|---|---|---|
| **Best ORR** | 75% | 79% | 78% |
| **Best CRR** | 55% | 55% | 62% |
| **6 month ORR** | 40% | 42% | 49% |
| **6 month CRR** | 34% | 33% | 46% |

Abramson et al. EHA 2018

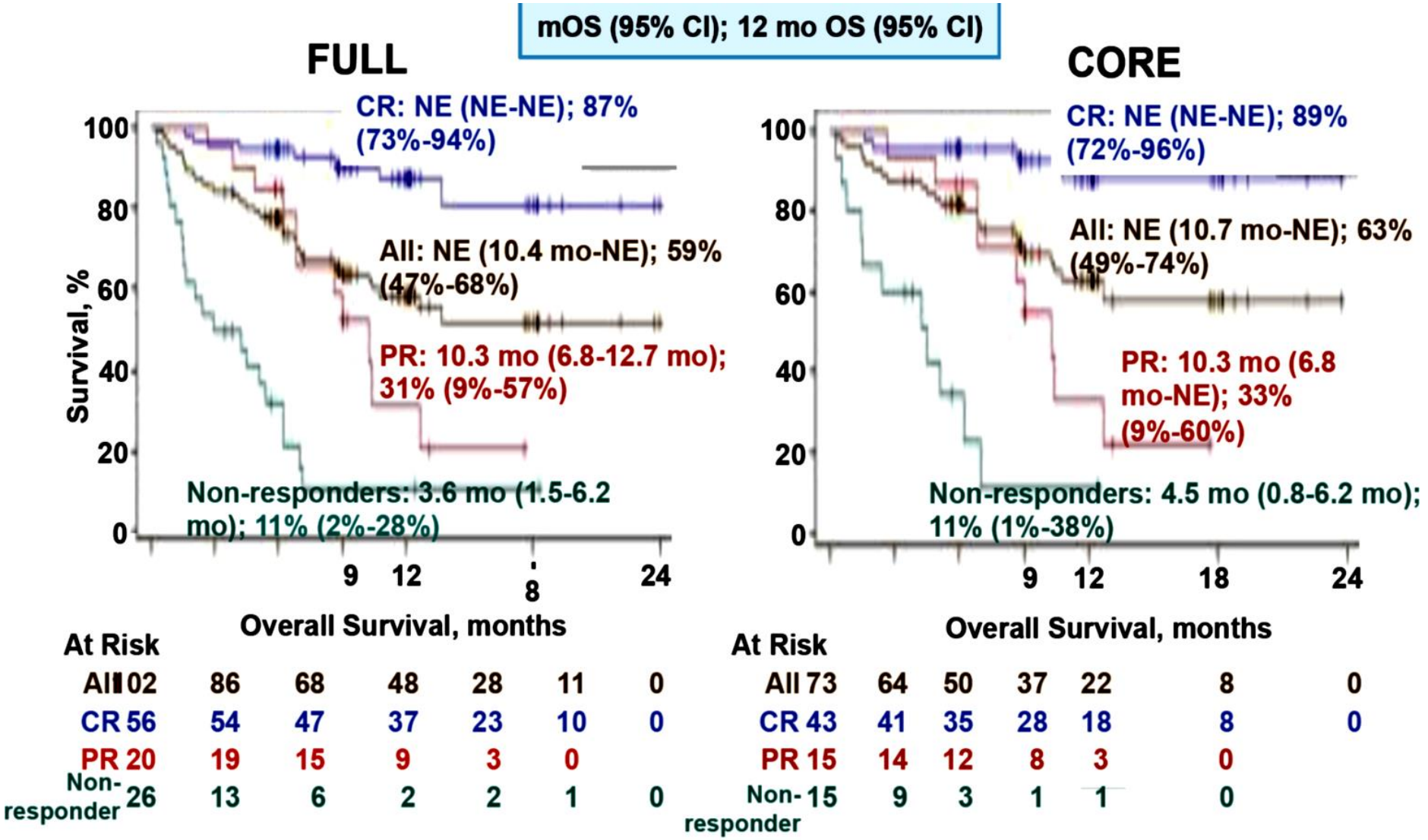
# *Lisocabtagene maraleucel (liso-cel, JCAR017, Juno): TRASCEND Trials*




**In CORE population, 88% of patients with CR at 3 months stay in CR at 6 months; 93% of patients in response at 6 months stay in response for a longer-term**

Median follow-up: 8 mos

# *Lisocabtagene maraleucel (liso-cel, JCAR017, Juno): TRASCEND Trials*




Median follow-up: 8 mos

# *New frontiers in the treatment of non-Hodgkin Lymphomas*

| Trial (name/ID) | DX | N | Construct | LDC | Cell Dose | ORR | CR | DOR | OS |
|---|---|---|---|---|---|---|---|---|---|
| KITE; KTE-C19 (axicabtagene ciloleucel) | | | | | | | | | |
| ZUMA-1[52] Phase 1 | DLCBL | 7 | CD3z/CD28 | Flu/Cy | $2 \times 10^6$/kg | 71% | 57% | – | – |
| ZUMA-1[23] Phase 2 | DLBCL, tFL, PMBCL | 101 | CD3z/CD28 | Flu/Cy | $2 \times 10^6$/kg | 82% | 54% | 11.1 mo | 52% @ 18 mo |
| NOVARTIS; CTL019 (tisagenlecleucel) | | | | | | | | | |
| JULIET[26] Phase 2 | DLCBL | 99 | CD3z/4-1BB | Flu/Cy Benda | $0.1\text{-}6 \times 10^8$ | 53% | 39.5% | NR | 64.5% @ 6 mo |
| JUNO; JCAR017 (lisocabtagene maraceucel) | | | | | | | | | |
| TRANSCEND[85] Phase 1 | DLBCL; tFL (CORE cohort) | 49 | CD3z/4-1BB | Flu/Cy | $1 \times 10^8$ | 84% | 61% | 9.2 mo | 88% @ 6 mo |
| TRANSCEND[85] Phase 1 | All DLBCL subtypes (FULL cohort) | 68 | CD3z/4-1BB | Flu/Cy | $0.5\text{-}1 \times 10^8$ | 74% | 52% | 5.0 mo | 75% @ 6 mo |

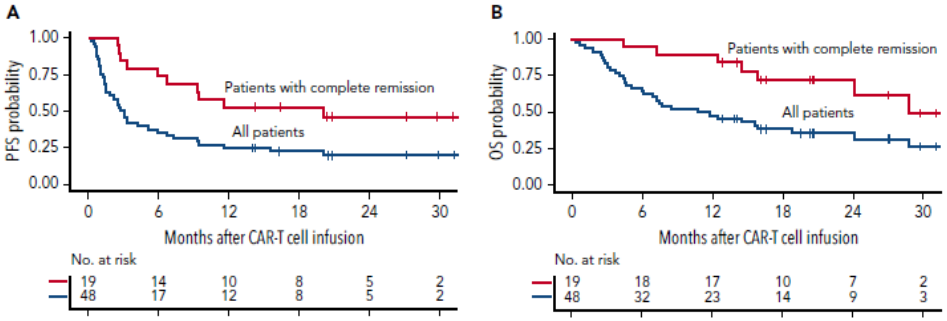
# New frontiers in the treatment of NHL: CAR-T Efficacy Predictors

## The response to lymphodepletion impacts PFS in patients with aggressive non-Hodgkin lymphoma treated with CD19 CAR T cells *

Alexandre V. Hirayama,[1] Jordan Gauthier,[1] Kevin A. Hay,[1,2] Jenna M. Voutsinas,[1] Qian Wu,[1] Ted Gooley,[1] Daniel Li,[3] Sindhu Cherian,[4] Xueyan Chen,[4] Barbara S. Pender,[1] Reed M. Hawkins,[1] Aesha Vakil,[1] Rachel N. Steinmetz,[1] Utkarsh H. Acharya,[1,5] Ryan D. Cassaday,[1,5] Aude G. Chapuis,[1,5] Tejaswini M. Dhawale,[5] Paul C. Hendrie,[5] Hans-Peter Kiem,[1,5] Ryan C. Lynch,[1,5] Jorge Ramos,[1,5] Mazyar Shadman,[1,5] Brian G. Till,[1,5] Stanley R. Riddell,[1,5] David G. Maloney,[1,5] and Cameron J. Turtle[1,5]

**KEY POINTS**

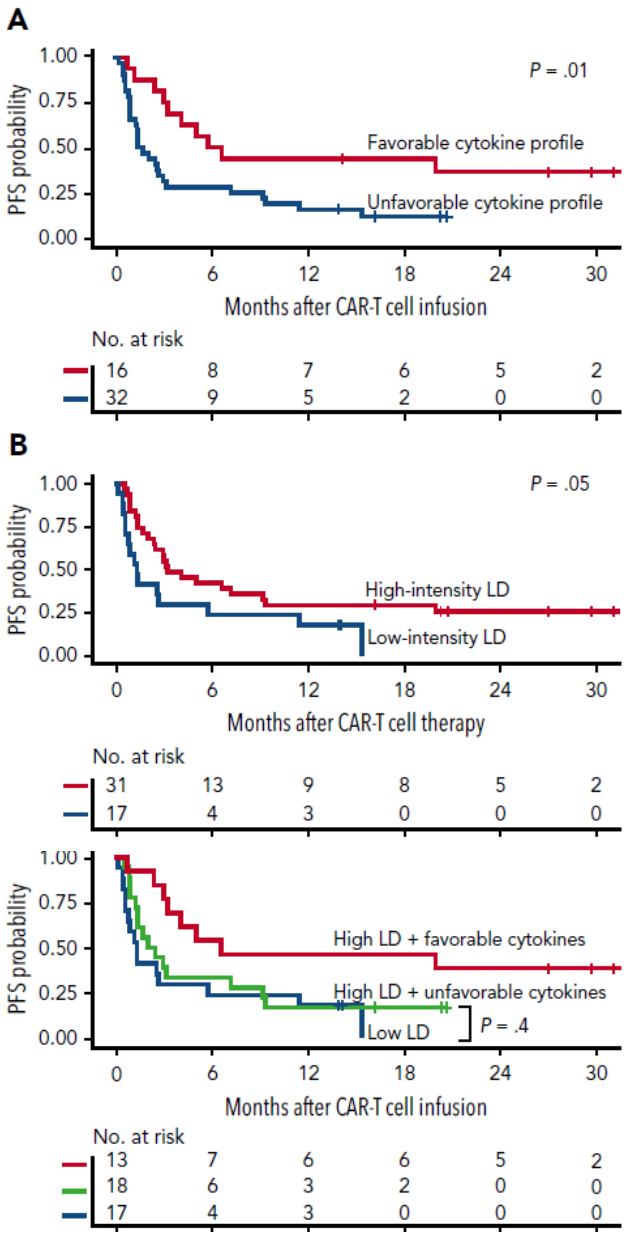
- LDH, MCP-1, and IL-7 are independently associated with PFS in patients with aggressive NHL after CD19 CAR T-cell immunotherapy.
- Higher intensity of cyclophosphamide and fludarabine lymphodepletion is associated with higher probability of a favorable cytokine profile.




| | Pre-LD | Day 0 (pre-CAR-T) | Day +4 (post-CAR-T) | PFS Probability |
|---|---|---|---|---|
| LDH | < median | | | Higher |
| Favorable Cytokine Profile* | | MCP-1 > median | Peak IL-7 > median | Higher |
| Hi-Intensity Lymphodepletion (LD) | | | | Higher |
| • With Favorable Cytokine Prolfile | | | | Higher |
| • With Unfavorable Cytokine Profile | | | | Lower |

Cy/Flu-based (Hi-intensity: Cy 60 mg/kg; Low-intensity Cy #1500 mg/m2 or 30 mg/kg total)

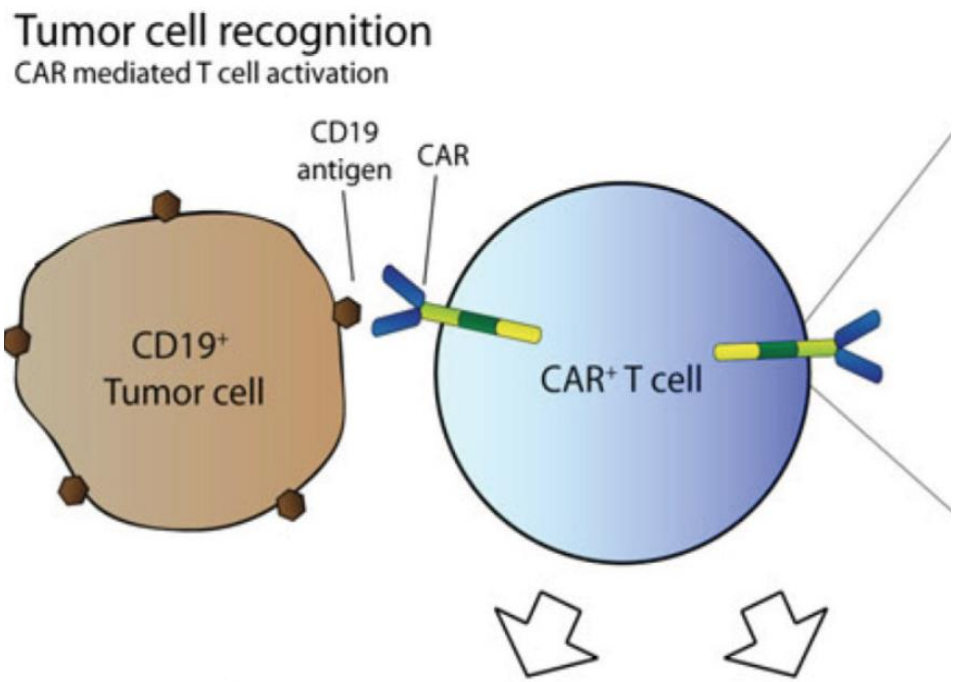
*Defined composition CD4+/CD8+ CD19 CAR T cells



# *CAR-T Cells-based treatments: opportunities and challenges*

- **The clinical *scenario***
  - *The unmet needs*
- **CAR-T cells as *a living drug***
  - The idea
  - How it works
  - The logistics
- **The Clinical Results**
  - Registered indications
- **The Toxicity & Safety Management**
  - CRS
  - ICAN (Immune effector Cells Associated Neurotoxicity)
  - Other
- **The Future (*tomorrow*)**

# CAR-T Cells-based treatments: opportunities and challenges




**ON TARGET EFFECTS** | **OFF TUMOR EFFECTS**

**CD19 on tumor cells** | **CD19 on non-tumor cells**

**"CLASS" EFFECTS**
Cytokine Release Syndrome
Neurologic Toxicity

| ON TARGET EFFECTS | OFF TUMOR EFFECTS |
|---|---|

## CRS

Post infusion
(15-50% G3/G4)

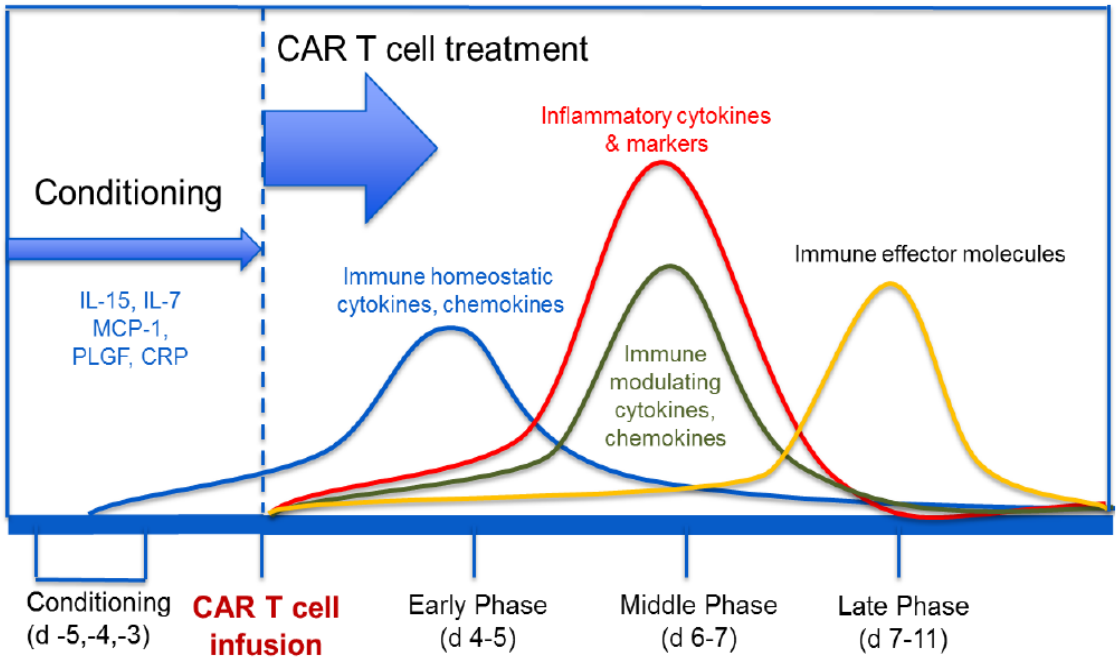
**ICAN/CRES**
(5-30%)
*Biphasic*

- ***Early*** (day 0 – 5) (associated to CRS)
- ***Delayed*** (day >5) (when CRS fades)

**B-cell aplasia**
**Cytopenias**
**Infections**
*Delayed*
Off-tumor
Lymphodepleting conditioning

# CAR-T Cells-based treatments: opportunities and challenges

## Cytokine pattern after CAR T infusion




**Symptoms**
Onset 1-14 days after infusion, duration 1-10 days
Fevers come first and get very high (105ºF/41ºC)
Myalgias, fatigue, anorexia, capillary leak, hypoxia, hypotension

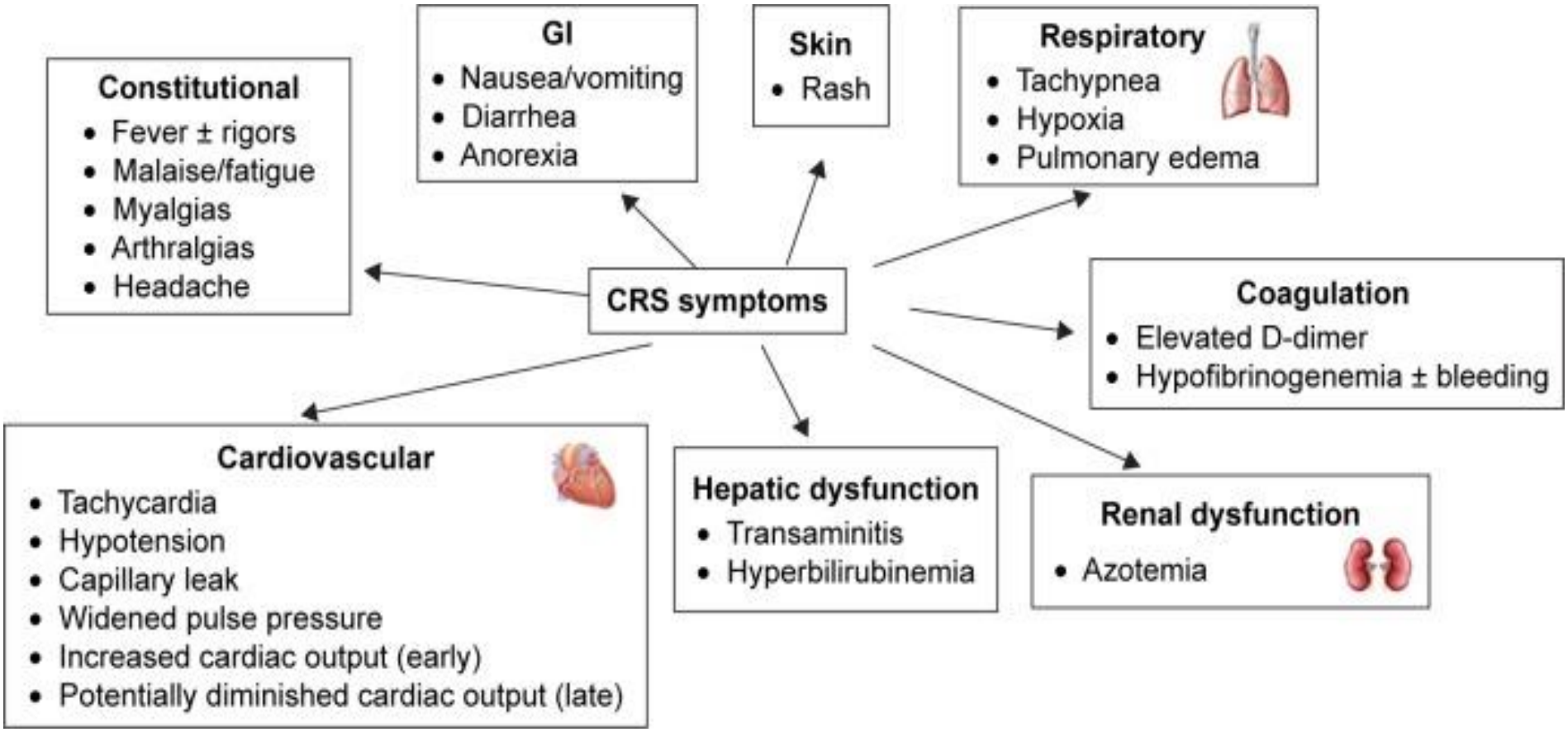
**Management**
Supportive care
Anti-cytokine interventions

| Immune homeostatic cytokines | Inflammatory cytokines and markers | Immune modulating cytokines | Chemokines | Other markers |
|---|---|---|---|---|
| IL-15 | IL-6 | IL-13 | IL-8 | PLGF |
| IL-7 | IL-1α | IL-4 | MCP-1 | sICAM-1 |
| IL-2 | IL-1β | IL-5 | MCP-4 | sVCAM-1 |
| | IL-17α | IL-10 | MIP-1α | VEGF |
| **Immune effectors** | TNFα | IFNγ | MIP-1β | VEGF-C |
| Granzyme A | TNFβ | IL-12p40 | IP-10 | VEGF-D |
| Granzyme B | GM-CSF | IL-12p70 | TARC | FGF-2 |
| sFASL | CRP | IL-16 | Eotaxin | |
| Perforin | SAA | | Eotaxin-3 | |
| | | | MDC | |

- IL6 levels correlate with degree of CRS.
- Tocilizumab, antibody binds to IL6 receptor.

*Perez, et al, ASH, 2015*

# CAR-T Cells-based treatments: opportunities and challenges



# TOCILIZUMAB

**CRS with high IL-6**

**TOCILIZUMAB for CRS[1]:**

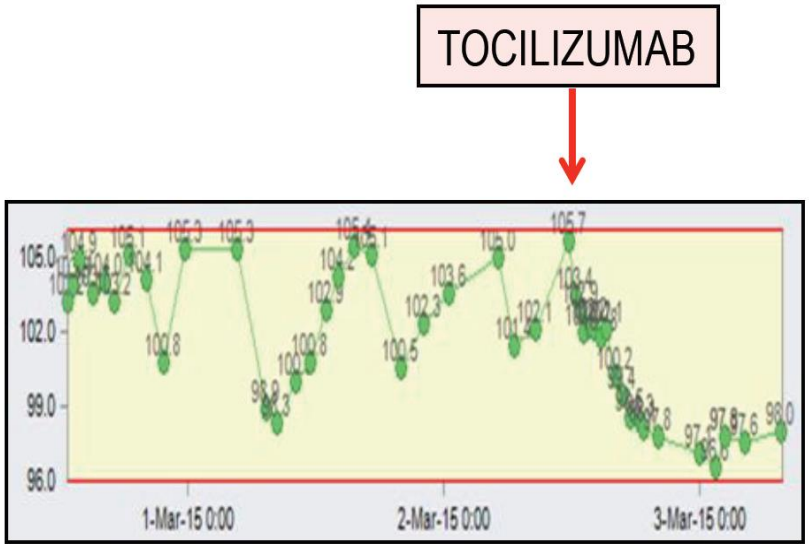
Humanized monoclonal antibody to IL6-R

Previously FDA approved adult RA, JIA

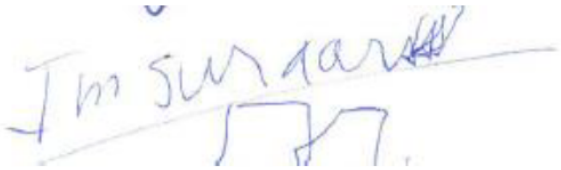
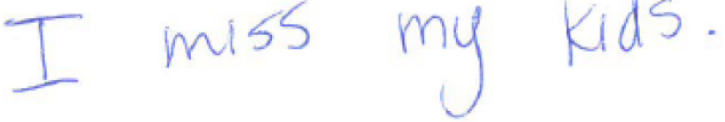
Effective for most patients

Does not appear to impact CAR T efficacy

Patient's fever curve during CRS



# CAR-T Cells-based treatments: opportunities and challenges

| | | MMSE score |
|---|---|---|
| Day 4<br>9 am |  | 29/30 |
| Day 5<br>01:30 PM<br>Toci 8 mg/kg |  | 27/30 |
| Day 5<br>03:30 PM |  | 27/30 |
| Day 6<br>9 am |  | 29/30 |

# NEUROLOGIC TOXICITY

**Mechanism**

T cell vs. cytokine mediated (endothelial activation)

CAR T cells are seen in the CSF[1-5]

**Symptoms**

Aphasia, delirium, encephalopathy, seizures

**Management**

No clear response to anti-cytokine treatment

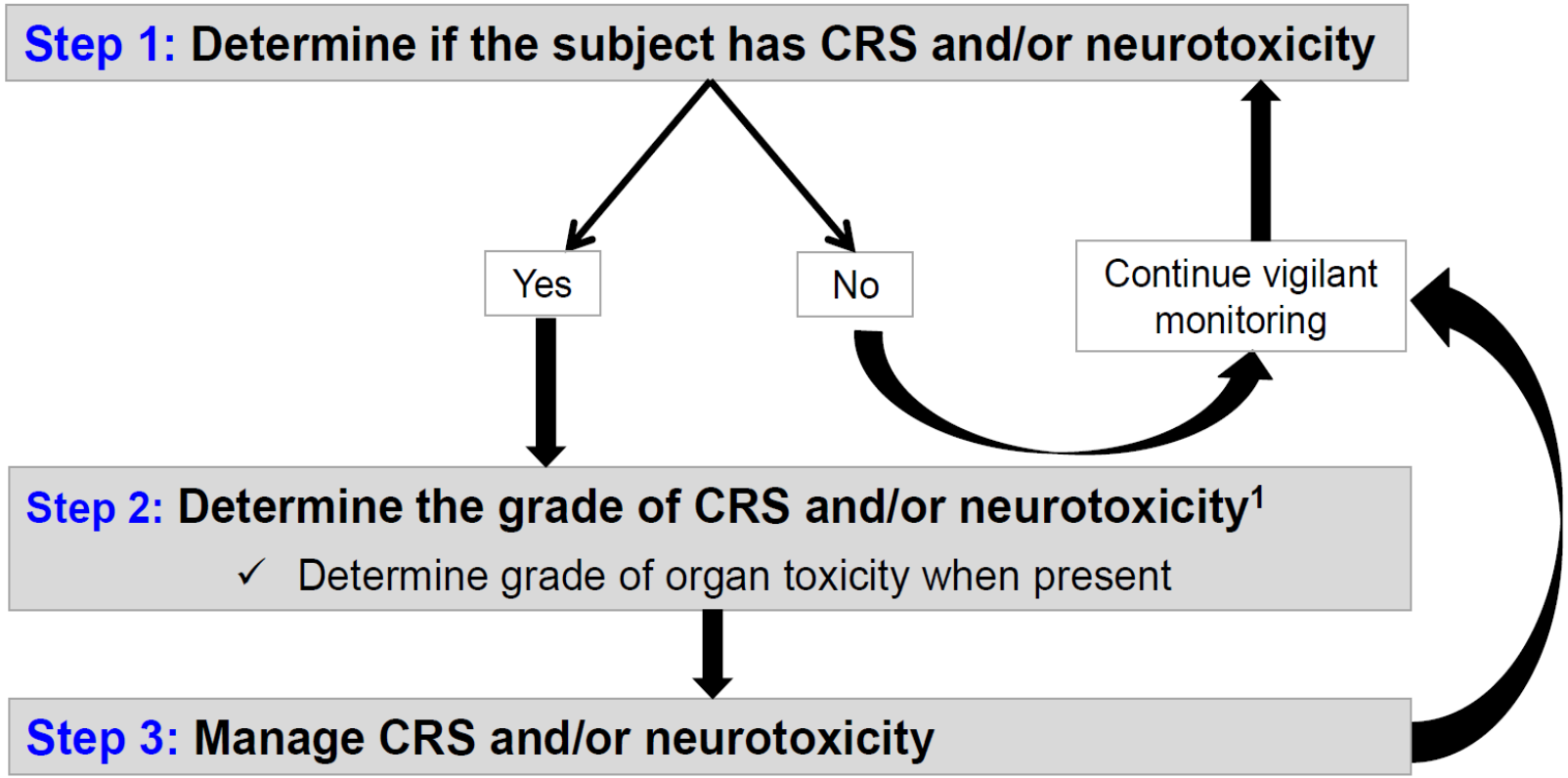
[1]Maude et al. NEJM 2014
[2]Davila et al. SciTranMed 2014
[3]Lee et al. The Lancet 2015
[4]Kochendorfer et al. JCO 2015
[5]Turtle et al. JCI 2016
[6]Gust et al. Cancer Discovery 2017

# CAR-T Cells-based treatments: opportunities and challenges

| Disease | Population | Lymphodepletion | CAR-T cell product | Costimulatory domain | CRS events (%) | Severe CRS[a] (%) | NT events (%) | Severe NT[b] (%) |
|---|---|---|---|---|---|---|---|---|
| ALL | *N* = 45/pediatric | Cy or Cy/Flu | Defined composition[c] | 4-1BB | 93 | 23 | 49 | 21[d] |
| ALL | *N* = 47/adult | Cy, Cy/E, or Cy/Flu | Defined composition[c] | 4-1BB | 74 | 17 | 53 | 30[e] |
| ALL | *N* = 75/pediatric, young adult | Physician's discretion | Tisagenlecleucel | 4-1BB | 77 | 47 | 40 | 13 |
| ALL | *N* = 53/adult | Cy or Cy/Flu | 19-28z | CD28 | 85 | 26 | 44 | 42[d] |
| NHL | *N* = 62/adult | Cy, Cy/E, or Cy/Flu | Defined composition[c] | 4-1BB | 61 | 8 | 32 | 13[e] |
| NHL | *N* = 28/adult | Physician's discretion | CTL019 (tisagenlecleucel) | 4-1BB | 57 | 18 | 39 | 11 |
| NHL | *N* = 101/adult | Cy/Flu | Axicabtagene ciloleucel | CD28 | 93 | 13 | 64 | 28[d] |
| NHL | *N* = 91/adult | Cy/Flu | Lisocabtagene maraleucel | 4-1BB | 35 | 1 | 19 | 12 |
| NHL | *N* = 111/adult | None, Cy/Flu, or bendamustine | Tisagenlecleucel | 4-1BB | 58 | 22 | 21 | 12 |
| CLL | *N* = 14/adult | Physician's discretion | Tisagenlecleucel | 4-1BB | 64 | 43 | 36 | 7 |
| CLL | *N* = 24/adult | Cy, Flu, or Cy/Flu | Defined composition[c] | 4-1BB | 83 | 8 | 33 | 25[e] |

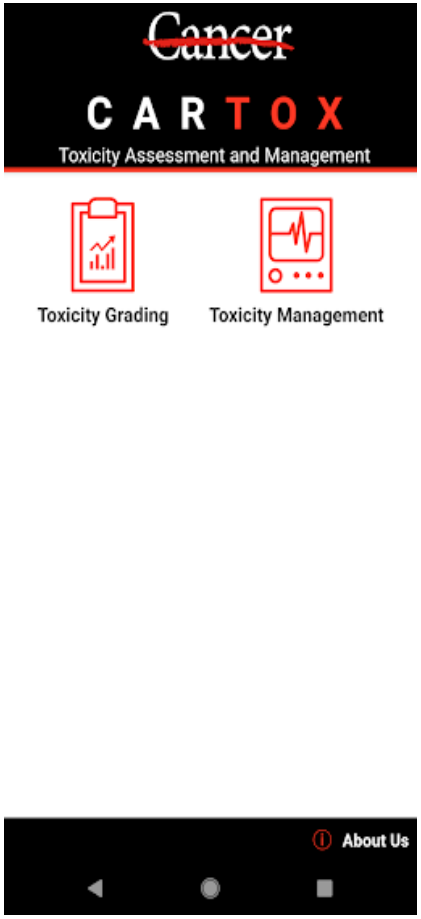
# MD Anderson CARTOX:
## CAR Cell Therapy Toxicity Assessment and Management

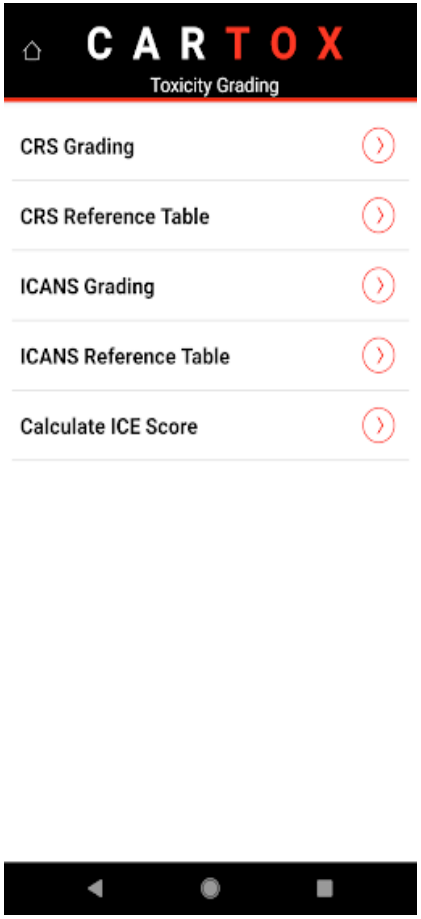
*Neelapu, Tummala, Kebriaei, Wierda, Loghin, Gutierrez, Shpall.*

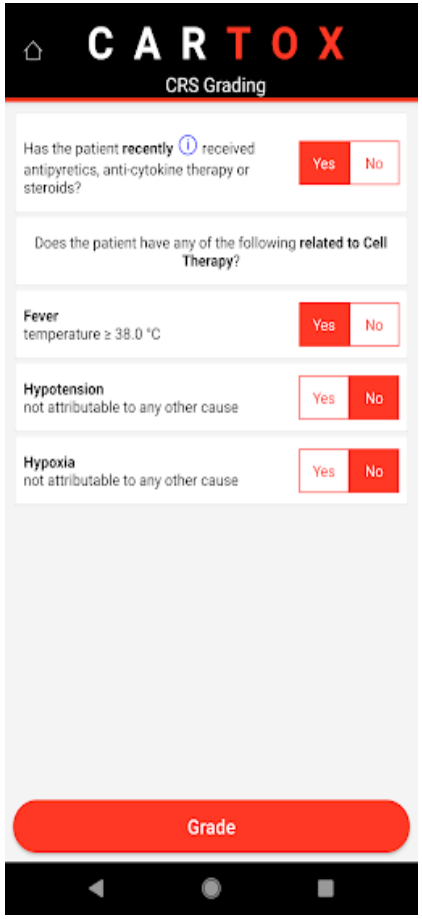


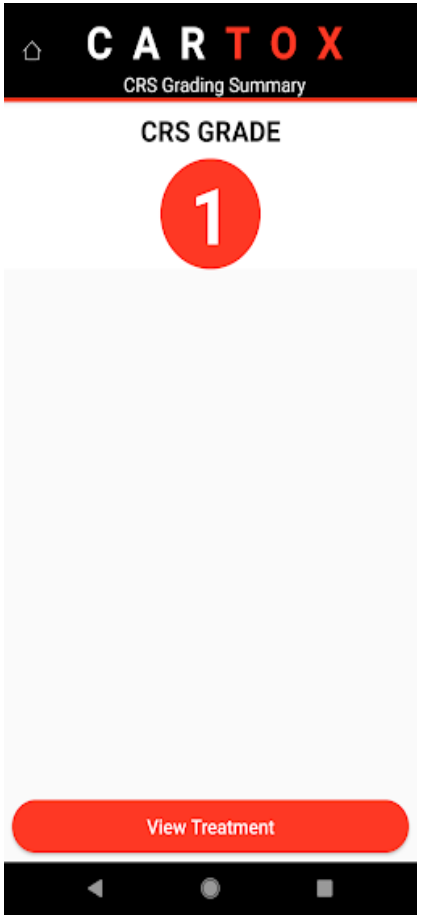

1. Adapted from Lee et al, Blood 2014;124:188-195

# CAR-T Cells-based treatments: opportunities and challenges



# *CAR-T Cells-based treatments: opportunities and challenges*

- **The clinical *scenario***
  - *The unmet needs*
- **CAR-T cells as *a living drug***
  - The idea
  - How it works
  - The logistics
- **The Clinical Results**
  - Registered indications
- **The Toxicity**
  - CRS
  - CRES (CAR related encephalopathy syndrome); ICAN
  - Other
- **The Future (*tomorrow*)**

# Refractory DLBCL

- **Primary Refractory** to R-CHOP and **progressing within 6 months** – CAR T-cell therapy

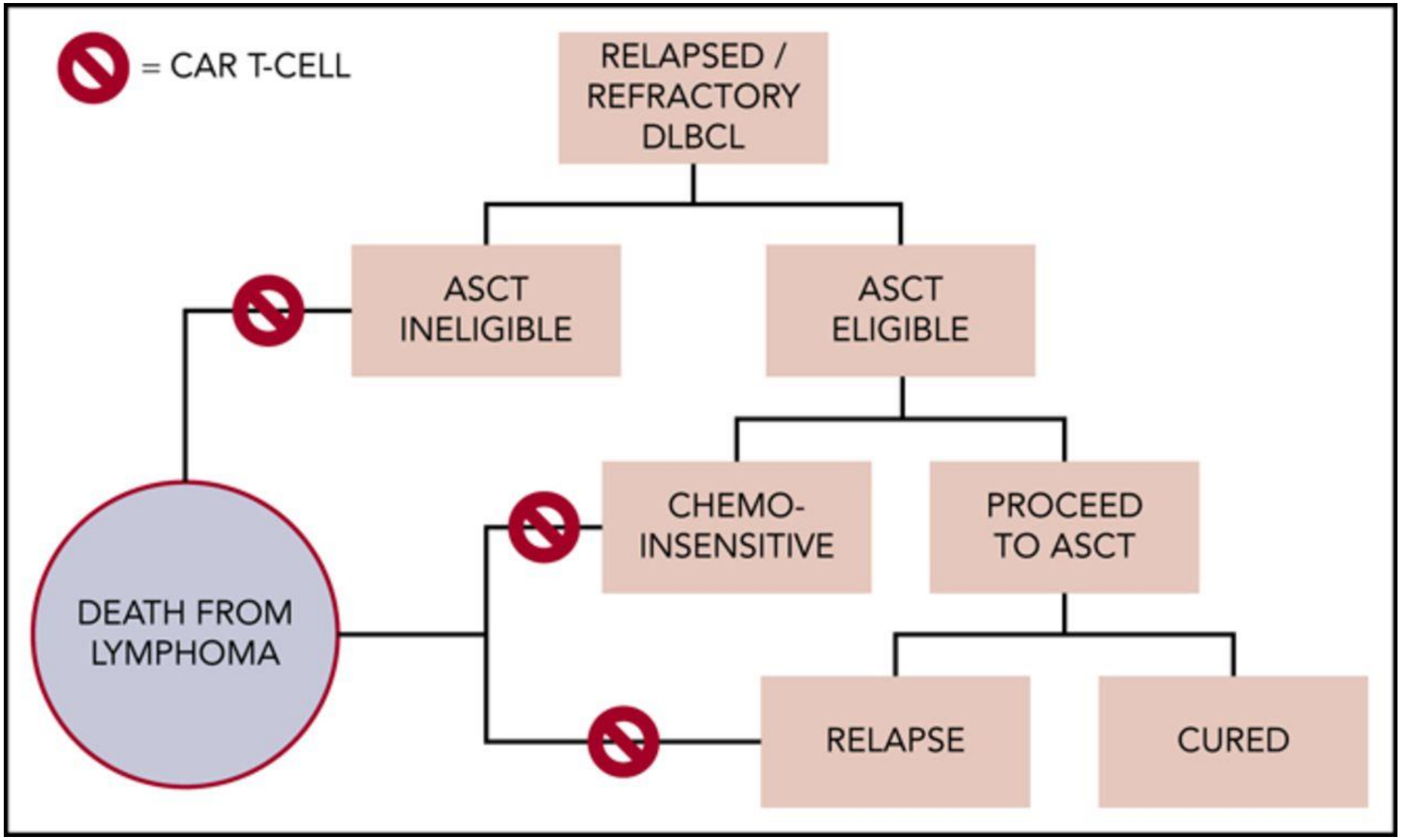
- **Progressing within 6-24 months** – Salvage chemotherapy
  - If PR or better – ASCT
  - If progresses or response less than a PR
  - CAR T-cell therapy

- **Progressing later than 24 months** – Salvage chemotherapy followed by ASCT

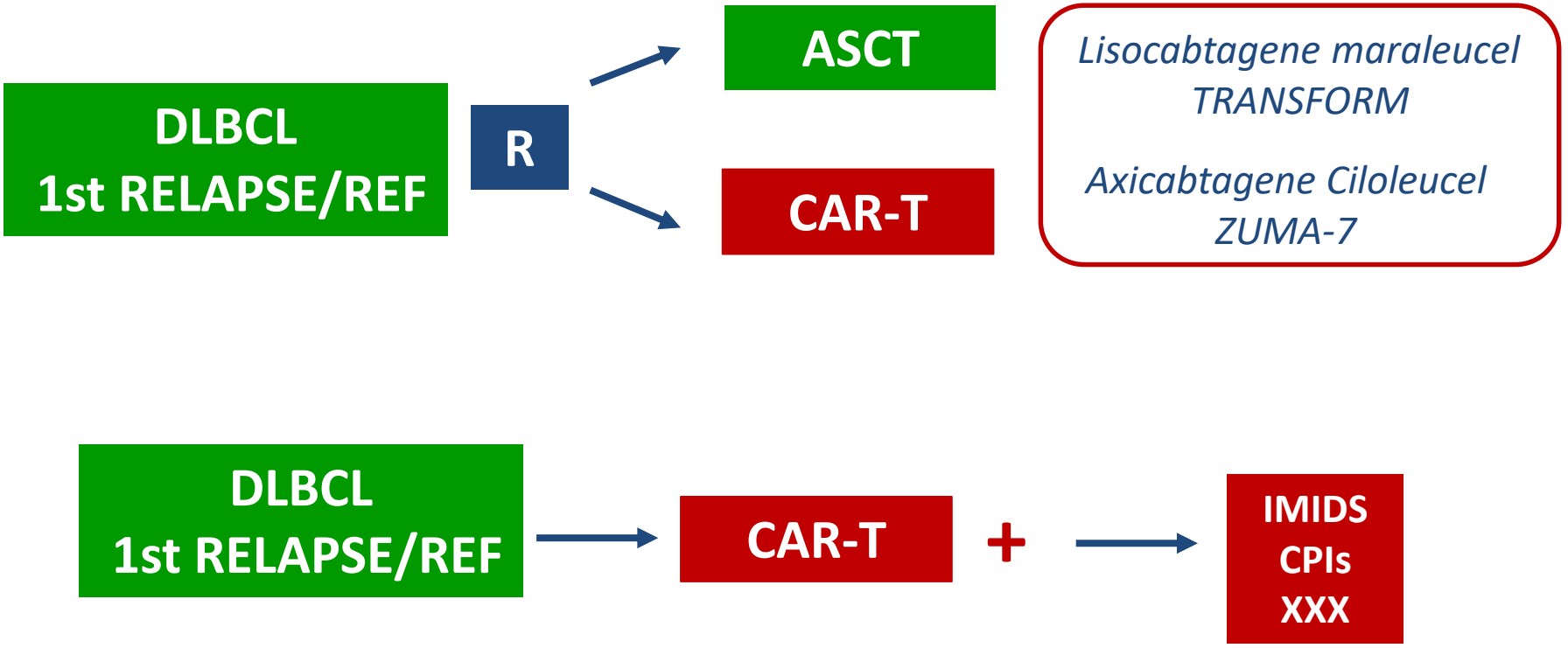
*S. Ansell, 2019*

blood

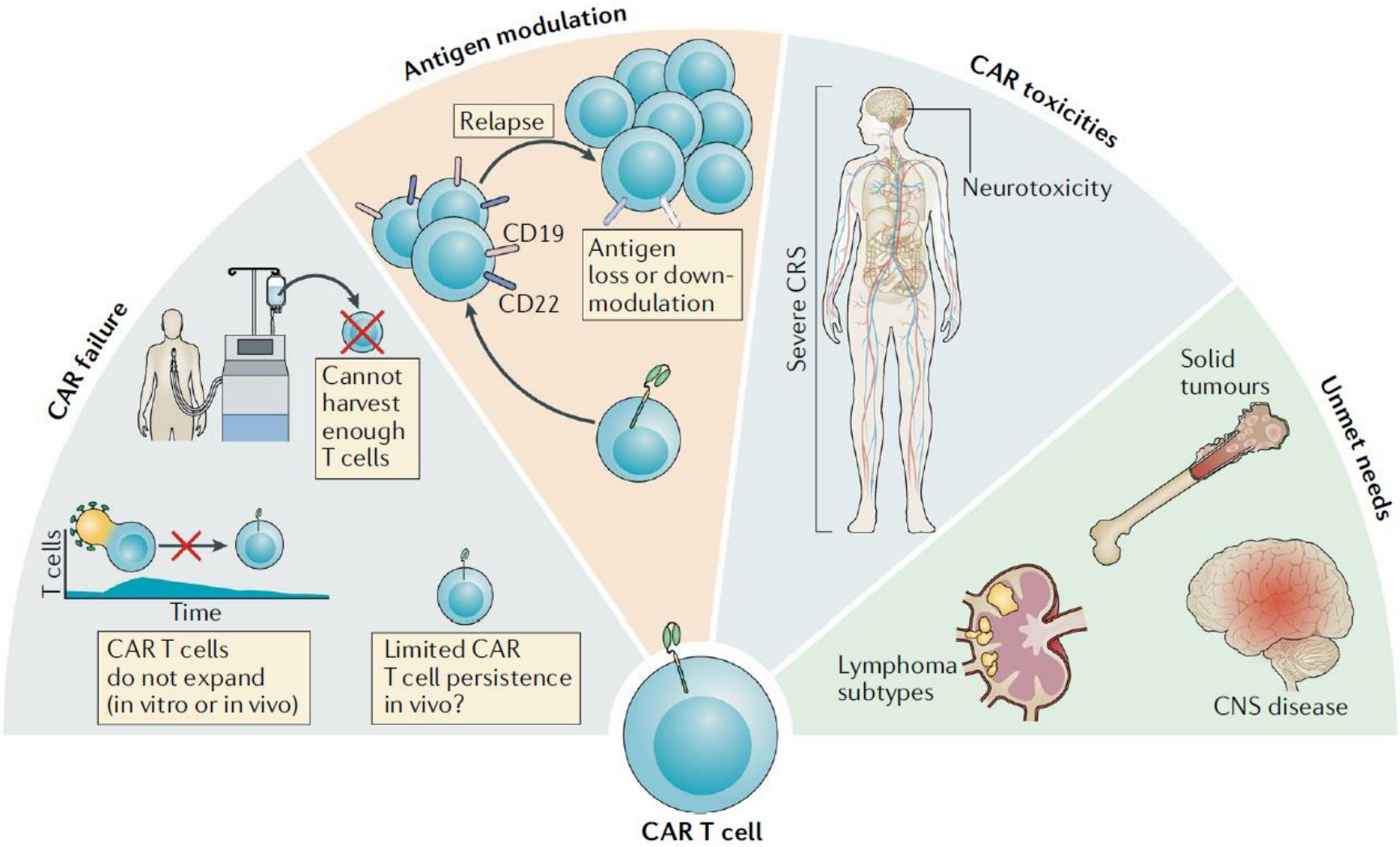
# Diffuse Large B-cell Lymphoma



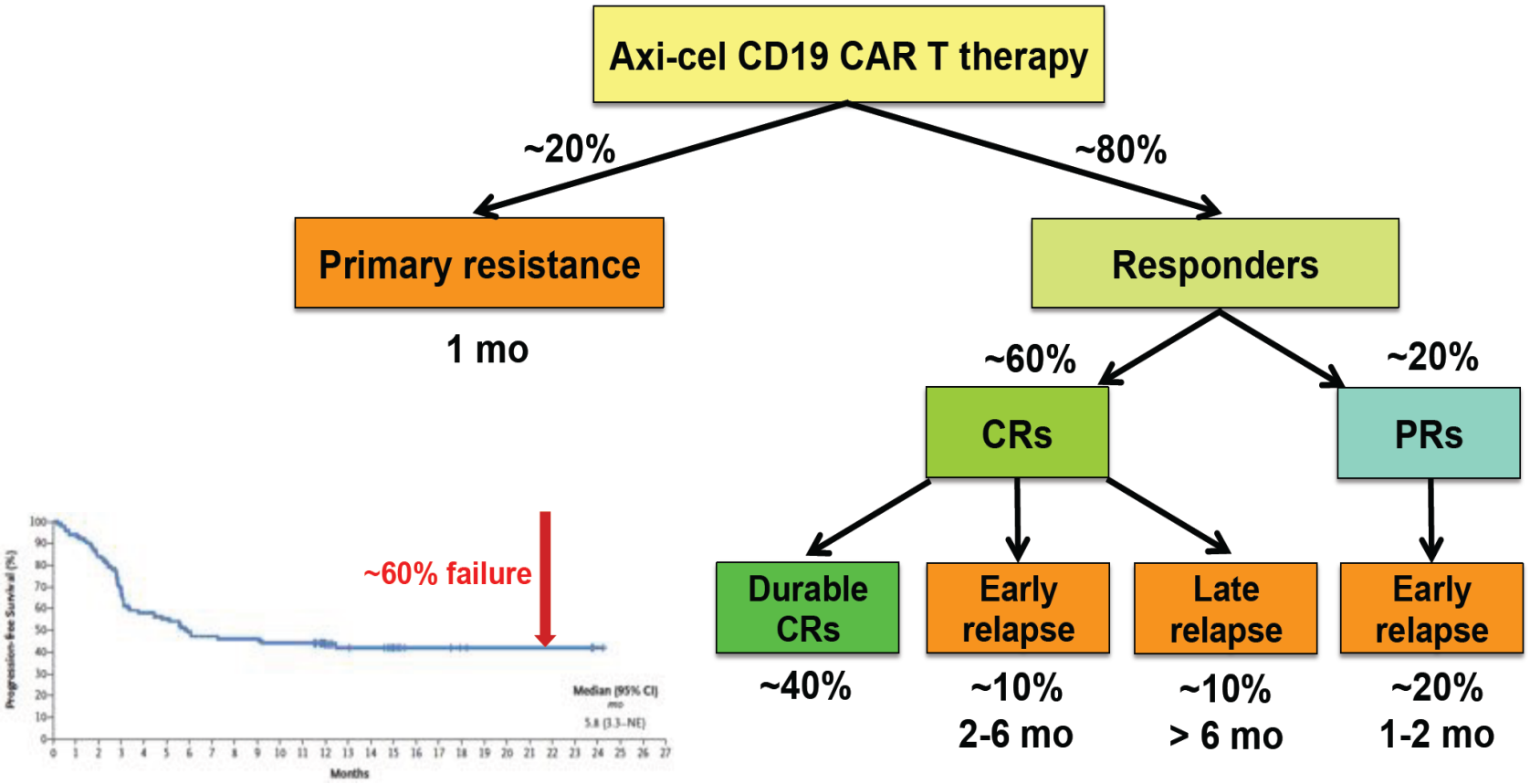
# *CAR-T Cells-based treatments: opportunities and challenges*



# CAR-T Cells-based treatments: opportunities and challenges



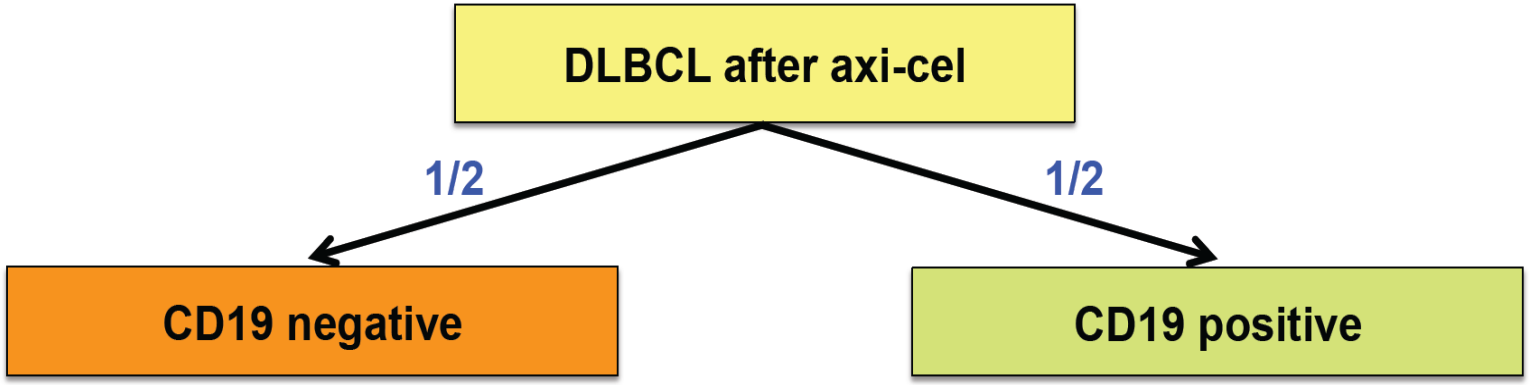
# Patterns of failure in DLBCL after axi-cel




Neelapu et al. *N Eng J Med* 2017

- All numbers are rounded off

# Mechanisms of resistance to axi-cel




- **Antigen escape**
  - CD19 alternative splicing
  - CD19 mutation
- **Impaired T-cell fitness**
  - Apheresis product
  - CAR T product
  - Host environment
  - Tumor microenvironment

# Diffuse Large B-cell Lymphoma

## Alternatively spliced variants of CD19 after CAR T therapy

- At relapse, 15/16 (94%) patients assessed had CD19 loss on ELIANA trial

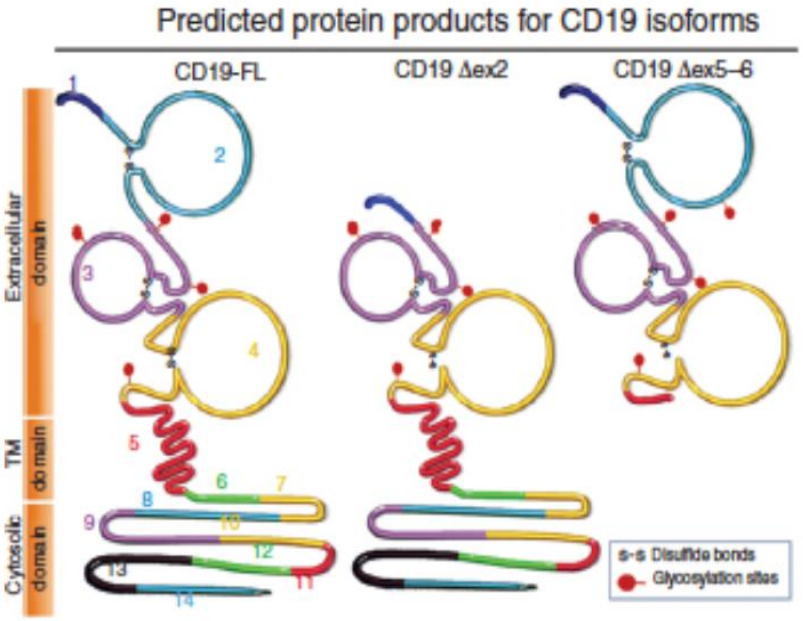
**Maude et al, N Eng J Med 2018**

**Cancer Discov 2015**

### Convergence of Acquired Mutations and Alternative Splicing of *CD19* Enables Resistance to CART-19 Immunotherapy

Elena Sotillo[1], David M. Barrett[2], Kathryn L. Black[1], Asen Bagashev[1], Derek Oldridge[2], Glendon Wu[1,3], Robyn Sussman[2], Claudia Lanauze[1,4], Marco Ruella[5], Matthew R. Gazzara[6,7], Nicole M. Martinez[7], Colleen T. Harrington[1,4], Elaine Y. Chung[1], Jessica Perazzelli[2], Ted J. Hofmann[2], Shannon L. Maude[2] Pichai Raman[1,2], Alejandro Barrera[6], Saar Gill[5,8], Simon F. Lacey[8], Jan J. Melenhorst[8], David Allman[9], Elad Jacoby[10], Terry Fry[10], Crystal Mackall[10], Yoseph Barash[5], Kristen W. Lynch[6], John M. Maris[2], Stephan A. Grupp[2], and Andrei Thomas-Tikhonenko[1,3,4,9]

**Loss of exon 2 or exons 5-6**



# CAR-T Cells-based treatments: opportunities and challenges

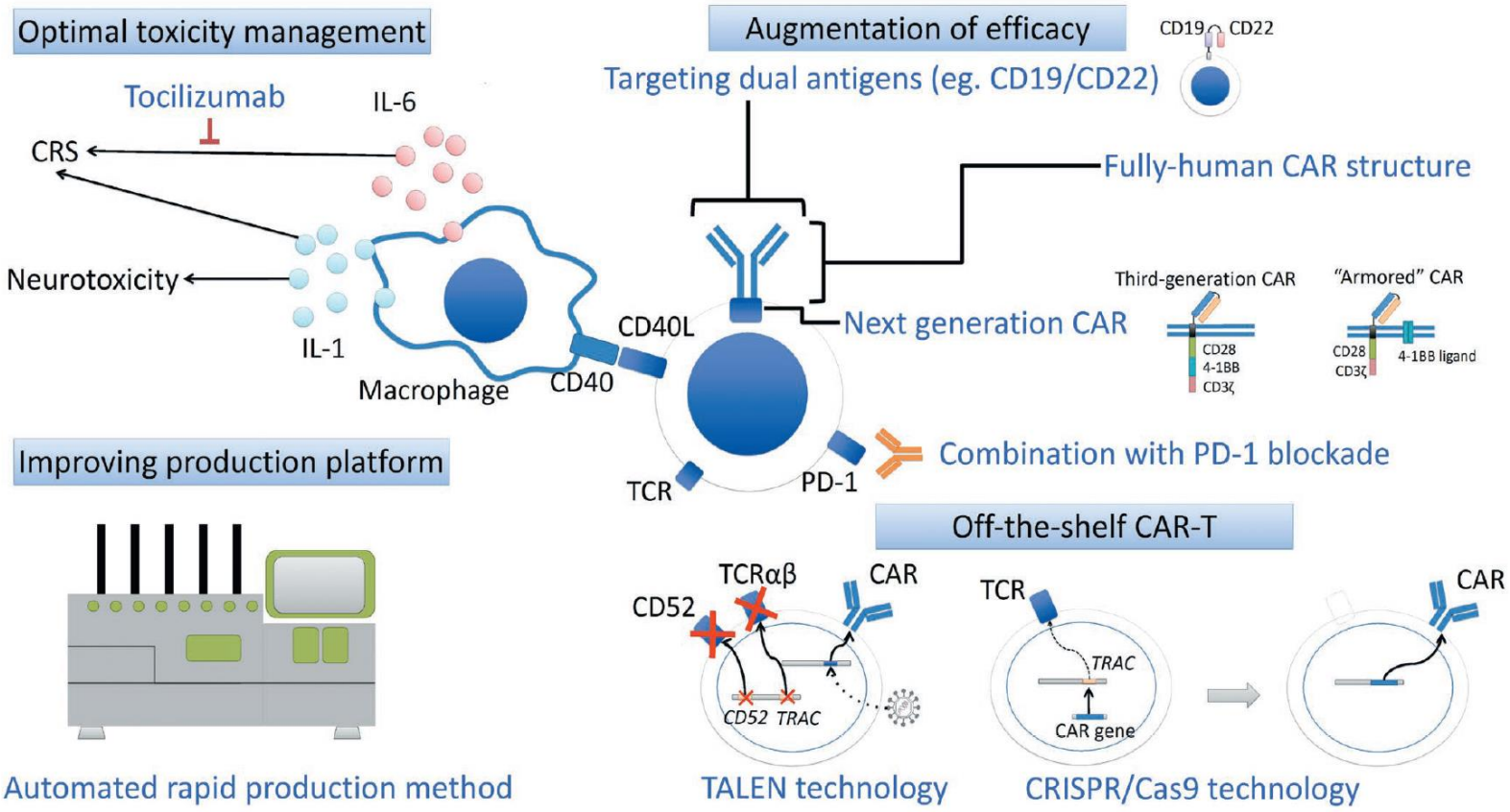
Table 1 | **Summary of antigen loss or modulation detected in published clinical trials of CAR T cell therapy**

| Target antigen | Publication | Number of patients treated | Number of CRs (%) | Number of patients with antigen modulation (%) | Median time to antigen loss or modulation (months) | Comments |
|---|---|---|---|---|---|---|
| CD19 | Lee et al.[7] | 21 | 14 (67) | 2 (14) | ~6 | 10 of 12 patients who were MRD-negative after CAR T cell therapy subsequently underwent HSCT |
| | Maude et al.[6] | 30 | 27 (90) | 4 (15) | ~3 | None |
| | Gardner et al.[8] | 43 | 40 (93) | 7 (18) | ~3 | 11 of 40 subsequently underwent HSCT |
| | Park et al.[9] | 53 | 44 (83) | 4 (9) | Unknown | None |
| | Maude et al.[10] | 75 | 61 (81) | 15 (25) | Unknown | None |
| CD22 | Fry et al.[15] | 21 | 12 (57) | 7 (58) | ~3 | None |

CAR, chimeric antigen receptor; CRs, complete responses; HSCT, haematopoietic stem cell transplantation; MRD, minimal residual disease.

# CAR-T Cells-based treatments: opportunities and challenges

**Figure 4. Possible way to overcome the problems in the treatment of second-generation anti-CD19 CAR T-cell therapy.**




There are several controversial issues and problems awaiting solutions, including optimal management of toxicities, overcoming relapsed/refractory disease after CAR T-cell therapy, and improving CAR-T manufacturing platform. The ways to overcome these problems are currently investigated.

CAR, chimeric antigen receptor; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; CRS, cytokine-release syndrome; PD-1, programmed death 1; TALEN, transcription activator-like effector nuclease; TCR, T-cell receptor.

# CAR-T Cells-based treatments: opportunities and challenges

Table 2 | **Active clinical trials of multi-antigen CAR T cells in the USA and UK**

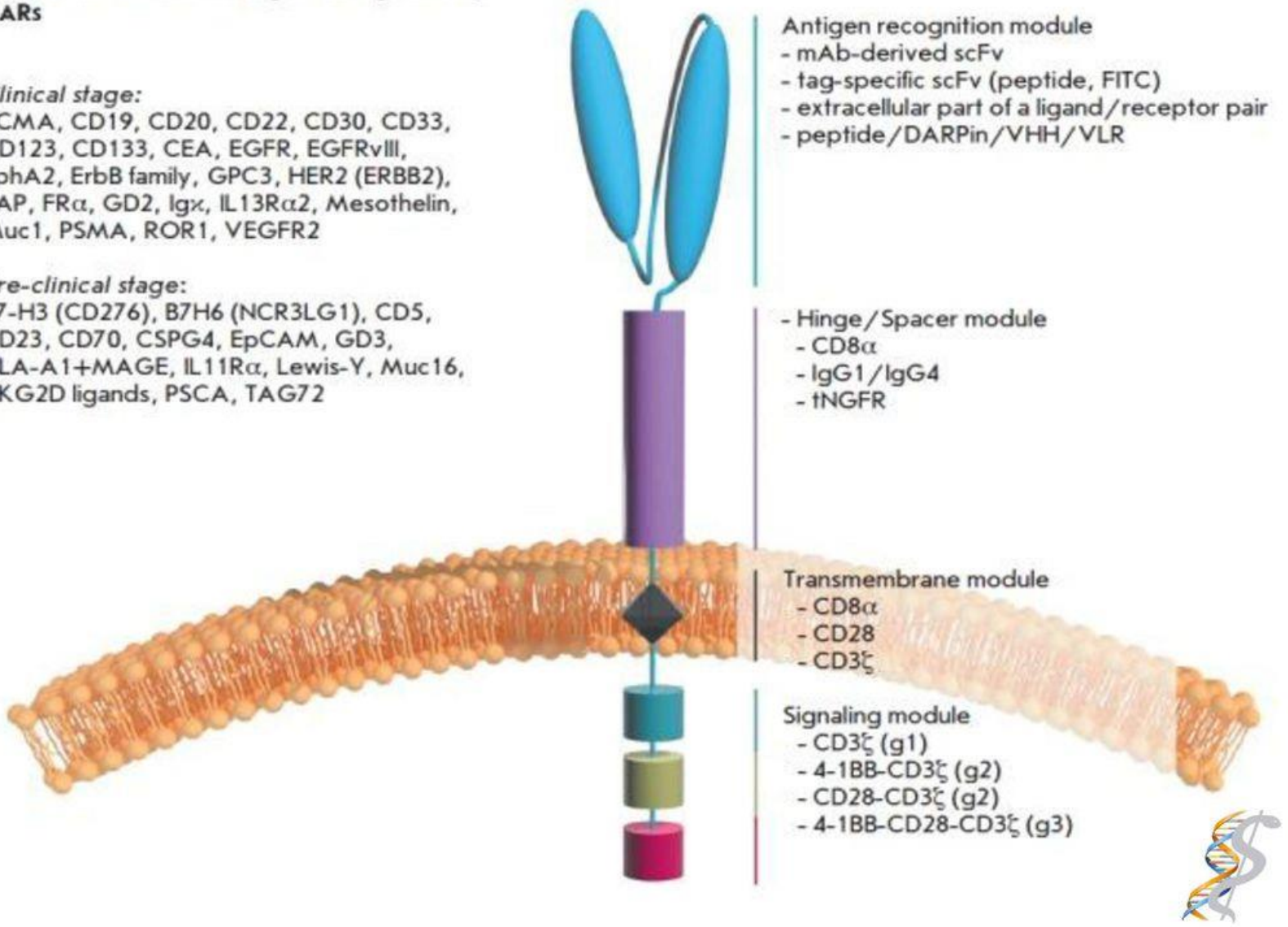
| Target antigens | Disease | Age group (years) | CAR construct signalling domains | Treatment centre | ClinicalTrials.gov reference number |
|---|---|---|---|---|---|
| CD19 and CD22 | ALL and NHL | 1–26 | CD3ζ–4-1BB (combinatorial approach with anti-CD19 CAR T cells, anti-CD22 CAR T cells and co-transduced anti-CD19 and anti-CD22 CAR T cells) | Seattle Children's Hospital (Seattle, WA, USA) | NCT03330691 (PLAT-05) |
| | ALL | 1–30 | CD3ζ–4-1BB | Lucile Packard Children's Hospital, Stanford University (Palo Alto, CA, USA) | NCT03241940 |
| | ALL and DLBCL | ≥18 | CD3ζ–4-1BB | Stanford University (Palo Alto, CA, USA) | NCT03233854 |
| | ALL and NHL | 3–30 | CD3ζ–4-1BB | National Cancer Institute (Rockville, MD, USA) | NCT03448393 |
| | ALL | 1–24 | CD3ζ–OX40 (CD19) and CD3ζ–4-1BB (CD22) | Great Ormond Street Hospital (London, UK) | NCT03289455 |
| CD19 and CD20 | NHL and CLL | 18–70 | CD3ζ–4-1BB | Medical College of Wisconsin (Milwaukee, WI, USA) | NCT03019055 |

# CAR-T Cells-based treatments: opportunities and challenges

**Cancer cell-associated targets recognized by CARs**

*Clinical stage:*
BCMA, CD19, CD20, CD22, CD30, CD33, CD123, CD133, CEA, EGFR, EGFRvIII, EphA2, ErbB family, GPC3, HER2 (ERBB2), FAP, FRα, GD2, Igκ, IL13Rα2, Mesothelin, Muc1, PSMA, ROR1, VEGFR2

*Pre-clinical stage*:
B7-H3 (CD276), B7H6 (NCR3LG1), CD5, CD23, CD70, CSPG4, EpCAM, GD3, HLA-A1+MAGE, IL11Rα, Lewis-Y, Muc16, NKG2D ligands, PSCA, TAG72



# Specialità Farmaceutiche a base di CAR-T cells (Kymriah; Yescarta) approvate dall'EMA

| PRODOTTO | PRODUTTORE / DISTRIBUTORE | INDICAZIONE EMA |
|---|---|---|
| Tisagenlecleucel | Novartis Pharma   | 1. Pazienti pediatrici e giovani adulti fino a 25 anni di età con leucemia linfoblastica acuta (LLA) a cellule B che è refrattaria, in recidiva post-trapianto o in seconda o ulteriore recidiva.<br>2. Pazienti adulti con linfoma diffuso a grandi cellule B (DLBCL) in recidiva o refrattario dopo due o più linee di terapia sistemica. |
| Axicabtagene ciloleucel | Kite Pharma Gilead   | Pazienti adulti con linfoma diffuso a grandi cellule B refrattario o recidivante (diffuse large B-cell lymphoma, DLBCL) e linfoma primitivo del mediastino a grandi cellule B (primary mediastinal large B-cell lymphoma, PMBCL), dopo due o più linee di terapia sistemica. |

# *CAR-T Cells-based treatments: opportunities and challenges*

# CAR-T Cells-based treatments: opportunities and challenges

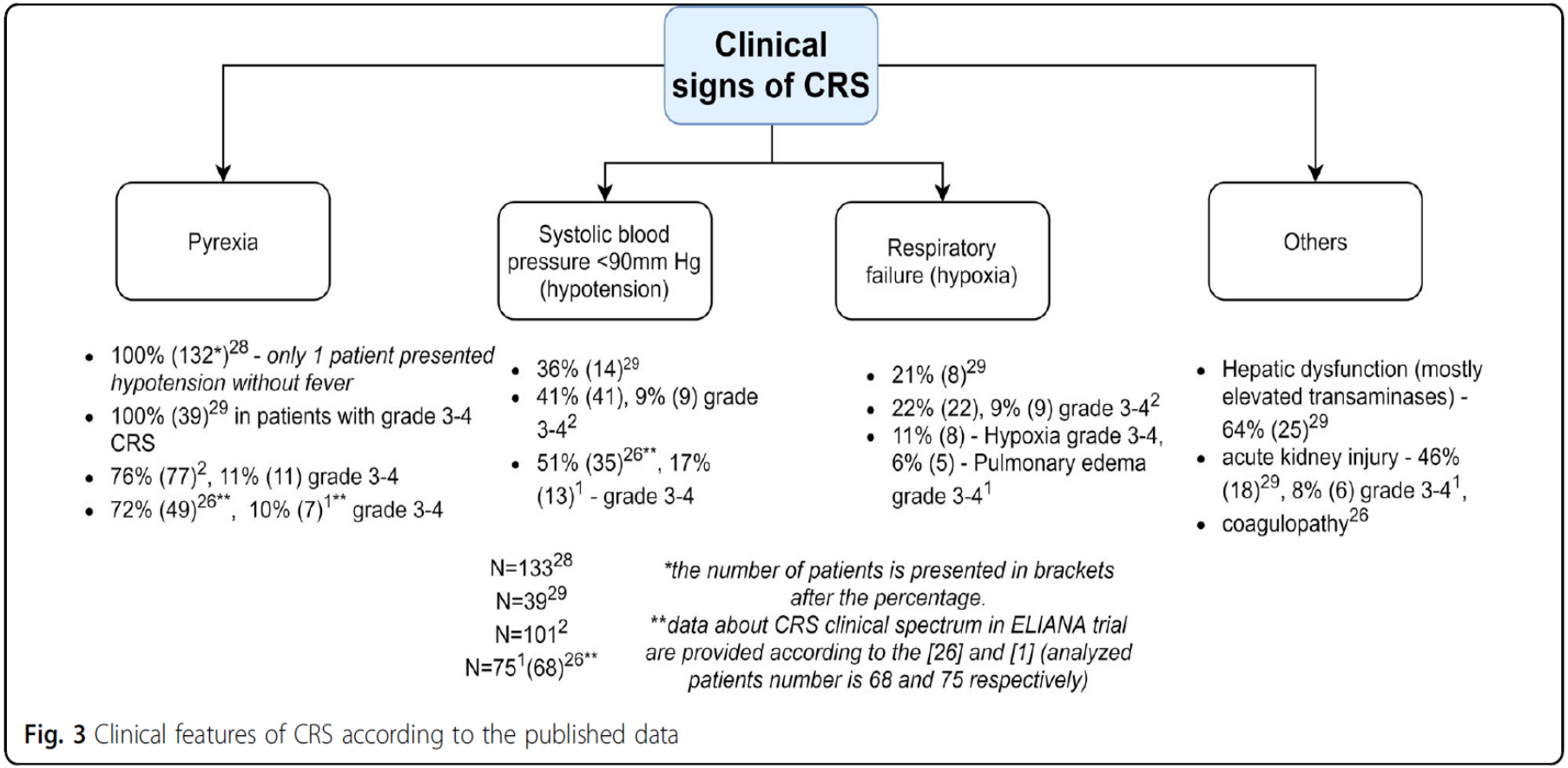
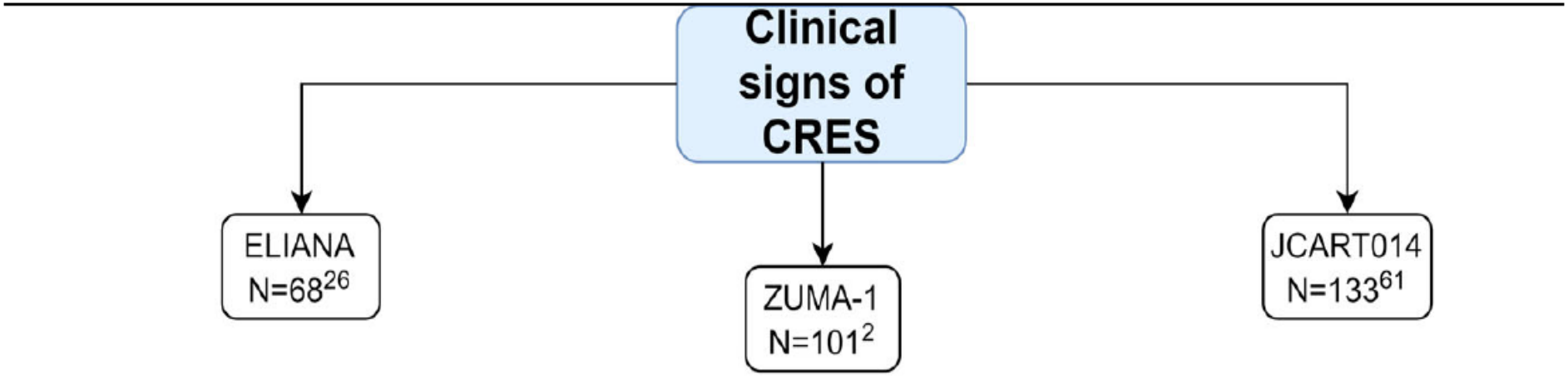



**Fig. 3** Clinical features of CRS according to the published data

# CAR-T Cells-based treatments: opportunities and challenges




**44% (30*)**
- in 6 patients CRES occurred after CRS resolution
- serious neurotoxicity (encephalopathy, delirium, seizures, focal deficits) analyzed only

**the number of patients is presented in brackets after the percentage*

**64% (65), grade≥3 28% (28)**

Encephalopathy 34% (34)
Confusional state 29% (29)
Aphasia 18% (18)
Somnolence 15% (15)
Tremor 29% (29)

**40% (53), grade≥3 21% (28)**

delirium with preserved alertness 26% (35)
Headache 21% (29)
Decreased consciousness 10% (13)
Language disturbance 13% (18)
Seizures 3% (4)
Intracranial hemorrhage >1% (1)

# Refractory DLBCL

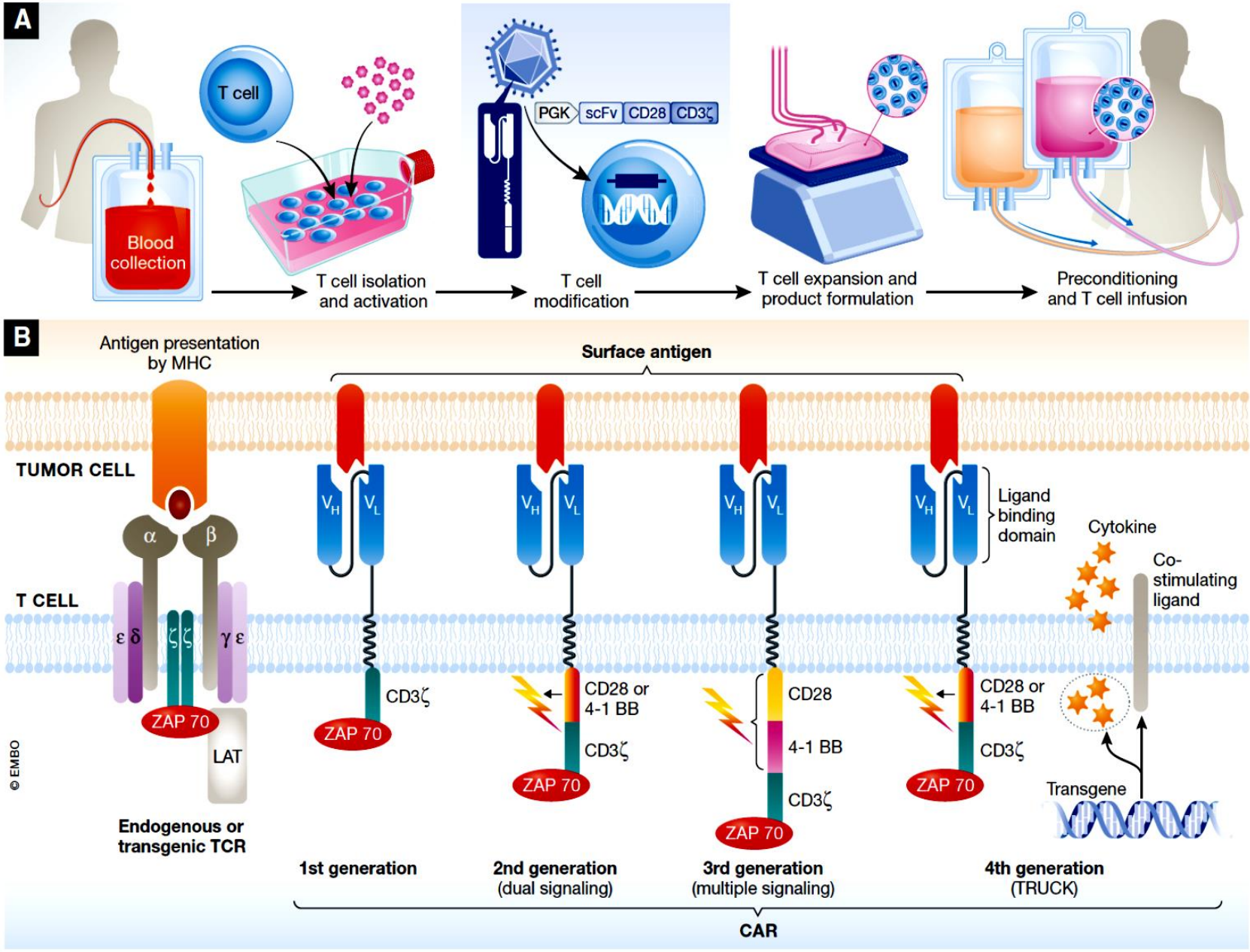
- **Primary Refractory** to R-CHOP and **progressing within 6 months:**
  - CAR T-cell therapy

- **Progressing within 6-24 months:**
  Salvage chemotherapy
  - If PR or better – ASCT
  - If progresses or response less than a PR
  - CAR T-cell therapy

- **Progressing later than 24 months:**
  Salvage chemotherapy followed by ASCT

*S. Ansell, 2019*

# New frontiers in the treatment of NHL: CAR-T cells




Hartmann et al. EMBO Mol Med (2017) 9: 1183–1197

# *New frontiers in the treatment of non-Hodgkin Lymphomas*

| Trial (name/ID) | DX | N | CRS ≥gr3 | CRES ≥gr3 | Notes |
|---|---|---|---|---|---|
| KITE; KTE-C19 (axicabtagene ciloleucel) | | | | | |
| ZUMA-1[52] Phase 1 | DLCBL | 7 | 14% | 57% | 3 pts w/ ongoing CR at 12+ mo |
| ZUMA-1[23] Phase 2 | DLBCL, tFL, PMBCL | 101 | 13% | 28% | 40% CR @ 1 y 3 deaths from SAE |
| NOVARTIS; CTL019 (tisagenlecleucel) | | | | | |
| JULIET[26] Phase 2 | DLCBL | 99 | 23% | 12% | 30% CR @ 6 mo |
| JUNO; JCAR017 (lisocabtagene maraceucel) | | | | | |
| TRANSCEND[85] Phase 1 | DLBCL; tFL (CORE cohort) | 49 | – | – | 52% CR @ 6 mo |
| TRANSCEND[85] Phase 1 | All DLBCL subtypes (FULL cohort) | 68 | 1% | 14% | Higher 3 mo ORR/ CR seen w/ DL2 |

## Erogazione delle terapie a base di CAR-T cells. Activity plan e competenze

| ACTIVITY PLAN | FIGURE PROFESSIONALI | STRUTTURE COINVOLTE |
|---|---|---|
| **Valutazione candidabilità del paziente alla terapia con CAR-T** | - Medico Ematologo con competenza specifica nelle patologie in indicazione e nella gestione delle terapie CAR-T<br>- Study Manager/centro erogatore | Ematologia Oncologica, Unità clinica |
| **Fase di aferesi (generazione del prodotto cellulare)** | - Medico Trasfusionista- Team infermieristico di aferesi | Medicina Trasfusionale, Unità aferetica |
| **Fase di spedizione del prodotto cellulare alle Officine Farmaceutiche delle Ditte titolari dell'AIC** | - Medico trasfusionista- Biologo & Team Laboratorio GMP | Medicina Trasfusionale, Unità di Manipolazione e Criopreservazione Cellulare |
| **Fase di ponte «Bridging» tra aferesi e reinfusione CAR-T (terapia linfodepletiva)** | - Medico Ematologo con competenza specifica nelle patologie in indicazione e nella gestione delle terapie CAR-T<br>- Team infermieristico dedicato | Ematologia Oncologica, Unità clinica |
| **Fase di ricezione/stoccaggio del prodotto farmaceutico a base di CAR-T (AIC)** | - Farmacista ospedaliero dedicato<br>- Medico Trasfusionista- Team Laboratorio GMP | Farmacia, Unità GMP<br>Medicina Trasfusionale, Unità di Manipolazione e Criopreservazione |
| **Fase di infusione** | - Medico Ematologo con competenza nella gestione delle terapie CAR-T- Biologo & Team Laboratorio GMP<br>- Team infermieristico dedicato | Ematologia Oncologica, Unità clinica<br>Medicina Trasfusionale, Unità di Manipolazione e Criopreservazione Cellulare |
| **Fase precoce post reinfusione (dal giorno 0 al giorno +30)** | - Medico Ematologo con competenza nella gestione delle terapie CAR-T- Medico intensivista/rianimatore<br>- Neurologo- Team infermieristico dedicato<br>- Farmacista ospedaliero dedicato | Ematologia Oncologica, Unità clinica<br>Anestesia/rianimazione, Unità clinica dedicata<br>Farmacia |
| **Fase tardiva post reinfusione (dal giorno +30 e durante il follow up lungo termine)** | - Medico Ematologo con competenza nella gestione delle terapie CAR-T- Team infermieristico dedicato (outpatient)- Medico Radiologo, Medico Nucleare | Ematologia Oncologica, Unità clinica e ambulatorio/DH<br>Radiologia<br>Medicina Nucleare |