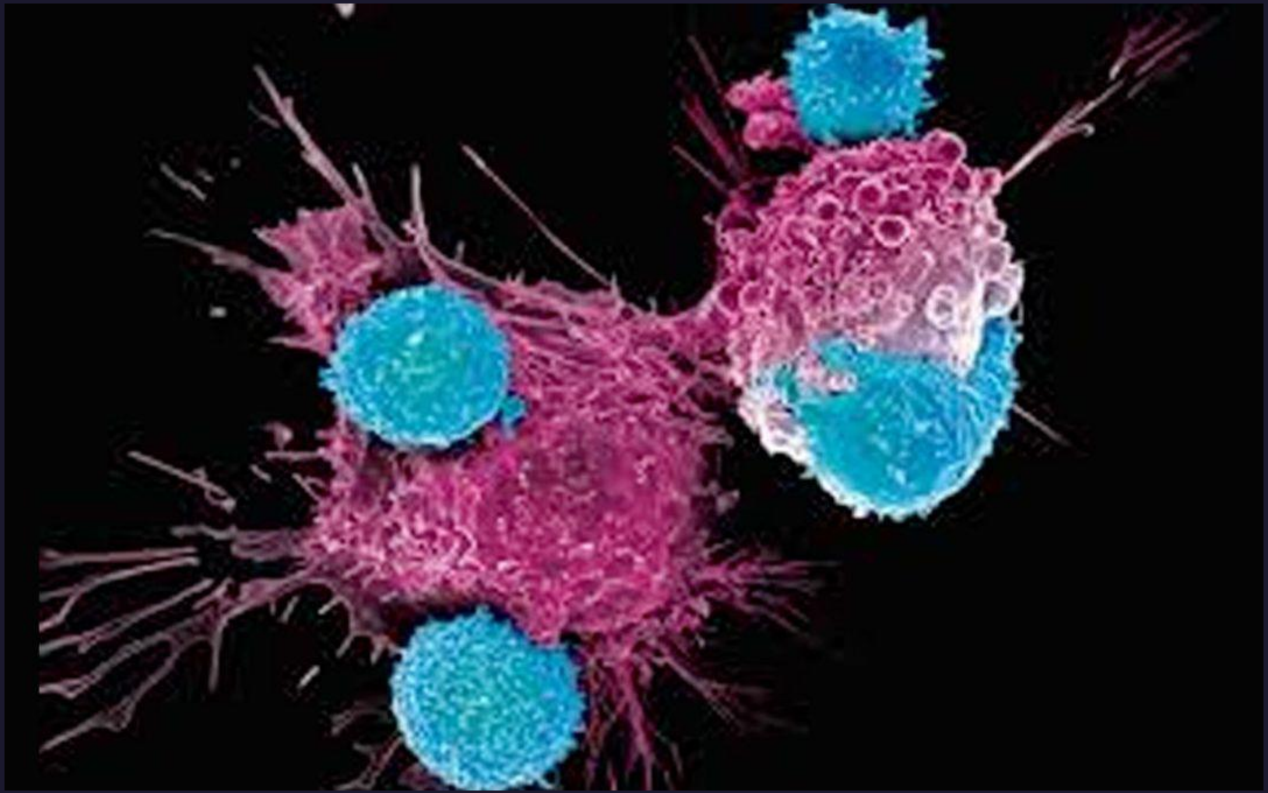
The Car-T cells Therapy in Hemattology

Dr. Simona Bernardi

31st May 2024

Agenda

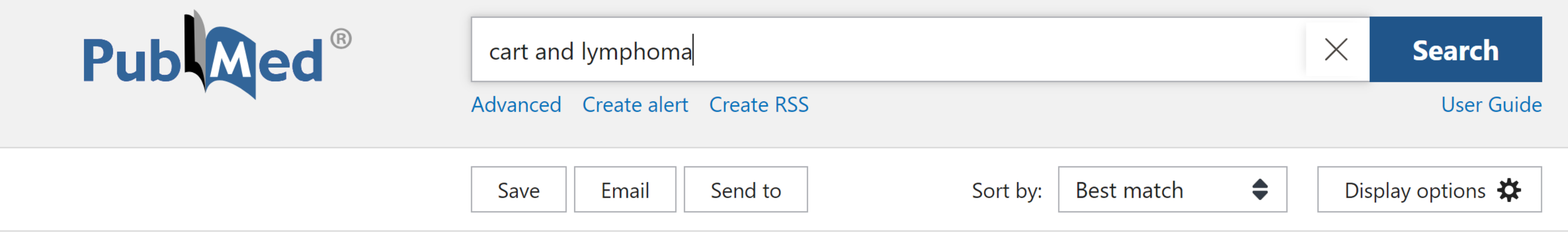
1. What Car-T cells are and how they are produced
2. The Car-T cells efficacy and toxicity
3. The related biological studies
4. Future perspectives



Definition

The CAR-T cells are an innovative therapy for hematological malignancies. The therapy is based on T lymphocites that are isolated, engineered to express the Car, and reinfused to the patient. The final aim is to make them competent against the malignant cells population.

An hot topic!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cart and lymphoma

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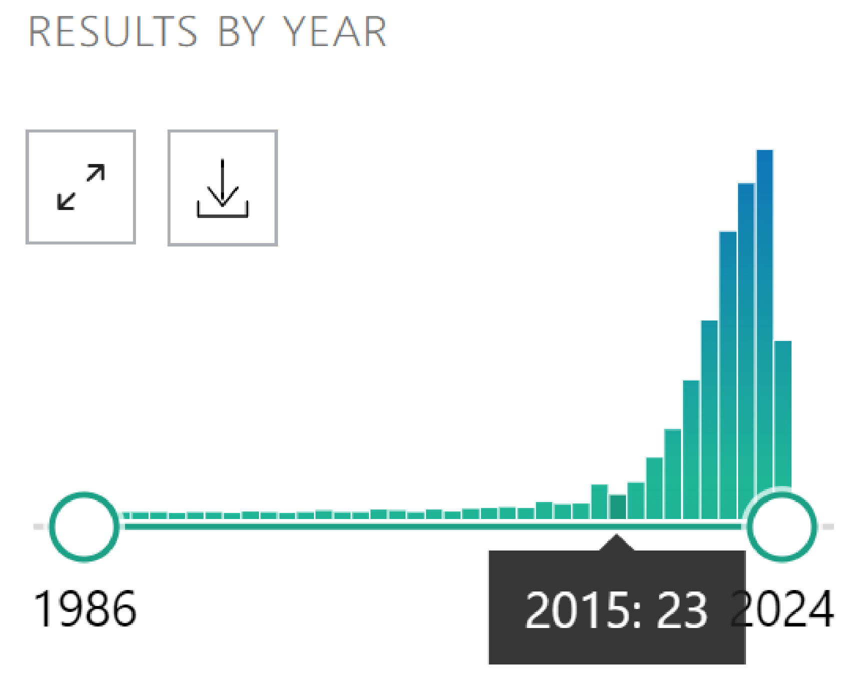


29 may 2024

Therapeutic indication in hematology

APPROVED

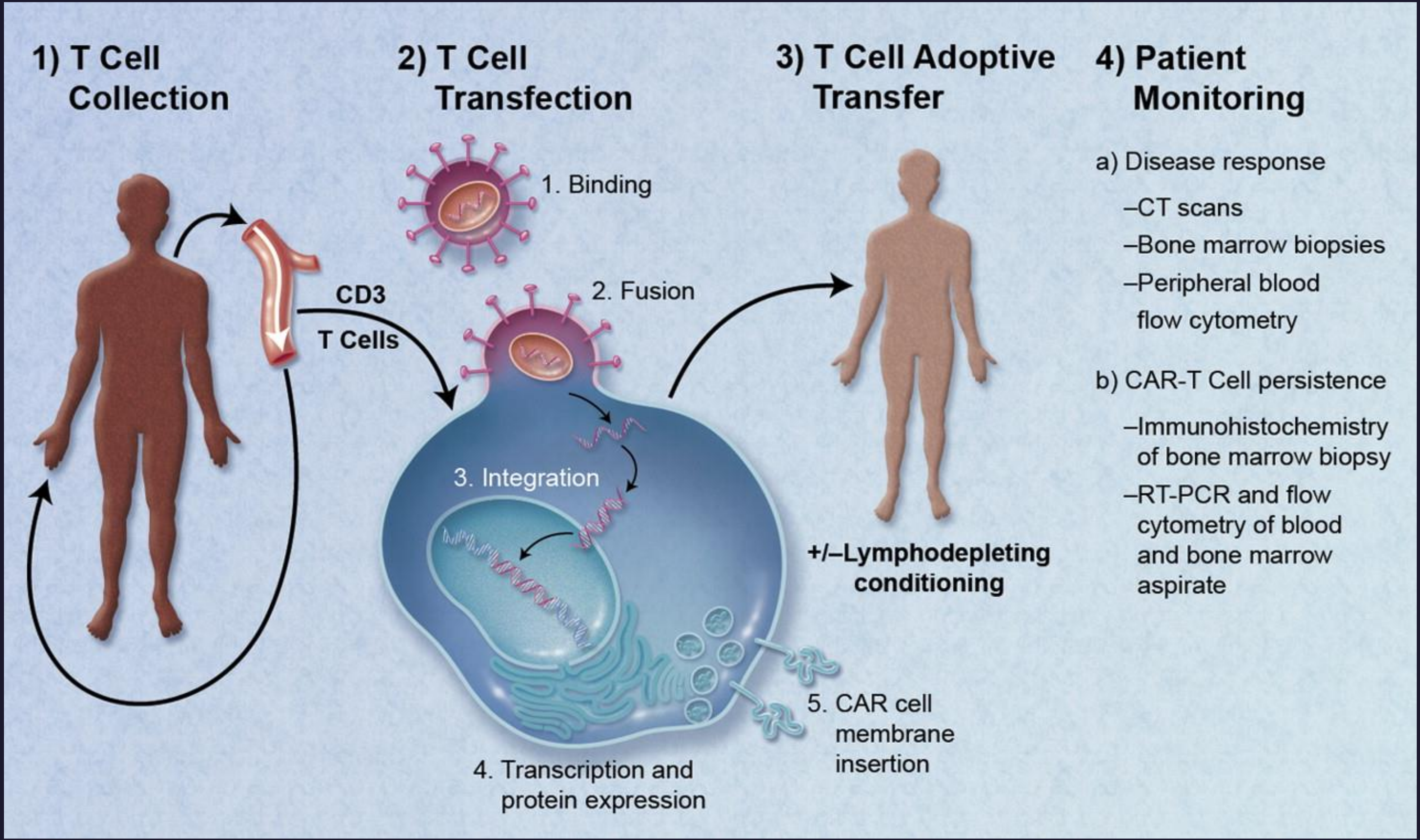
- Diffuse large B cell lymphoma
- Primitive mediastinal lymphoma
- Mantle lymphoma
- Follicular lymphoma
- Refractory ALL

SOON

- Multiple myeloma
- AML

IN DEVELOPMENT

- Allogeneic Car-T



1) T Cell Collection

CD3 T Cells

2) T Cell Transfection

1. Binding
2. Fusion
3. Integration
4. Transcription and protein expression
5. CAR cell membrane insertion

3) T Cell Adoptive Transfer

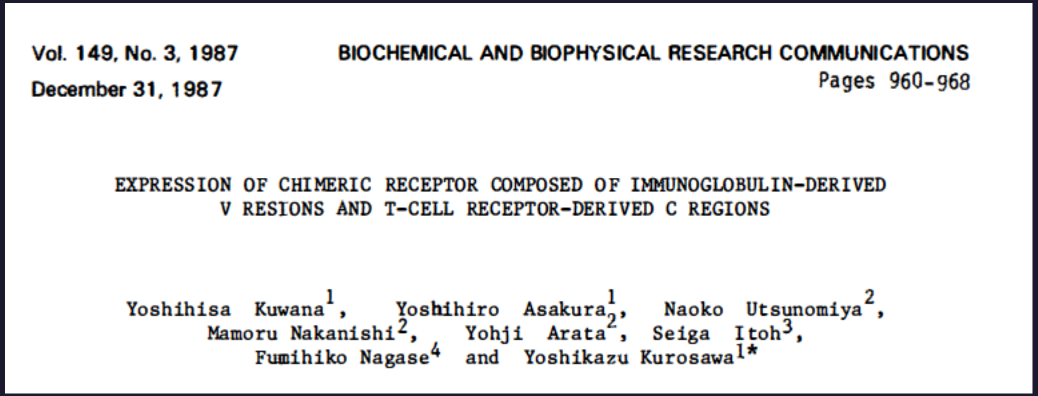
+/–Lymphodepleting conditioning

4) Patient Monitoring

a) Disease response
  - –CT scans
  - –Bone marrow biopsies
  - –Peripheral blood flow cytometry

b) CAR-T Cell persistence
  - –Immunohistochemistry of bone marrow biopsy
  - –RT-PCR and flow cytometry of blood and bone marrow aspirate

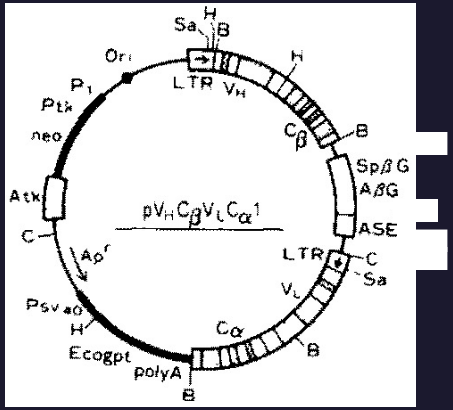
Different CAR generation



Vol. 149, No. 3, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 31, 1987 Pages 960-968

EXPRESSION OF CHIMERIC RECEPTOR COMPOSED OF IMMUNOGLOBULIN-DERIVED
V RESIONS AND T-CELL RECEPTOR-DERIVED C REGIONS

Yoshihisa Kuwana[1], Yoshihiro Asakura[1], Naoko Utsunomiya[2],
Mamoru Nakanishi[2], Yohji Arata[2], Seiga Itoh[3],
Fumihiko Nagase[4] and Yoshikazu Kurosawa[1]*


Sa
H
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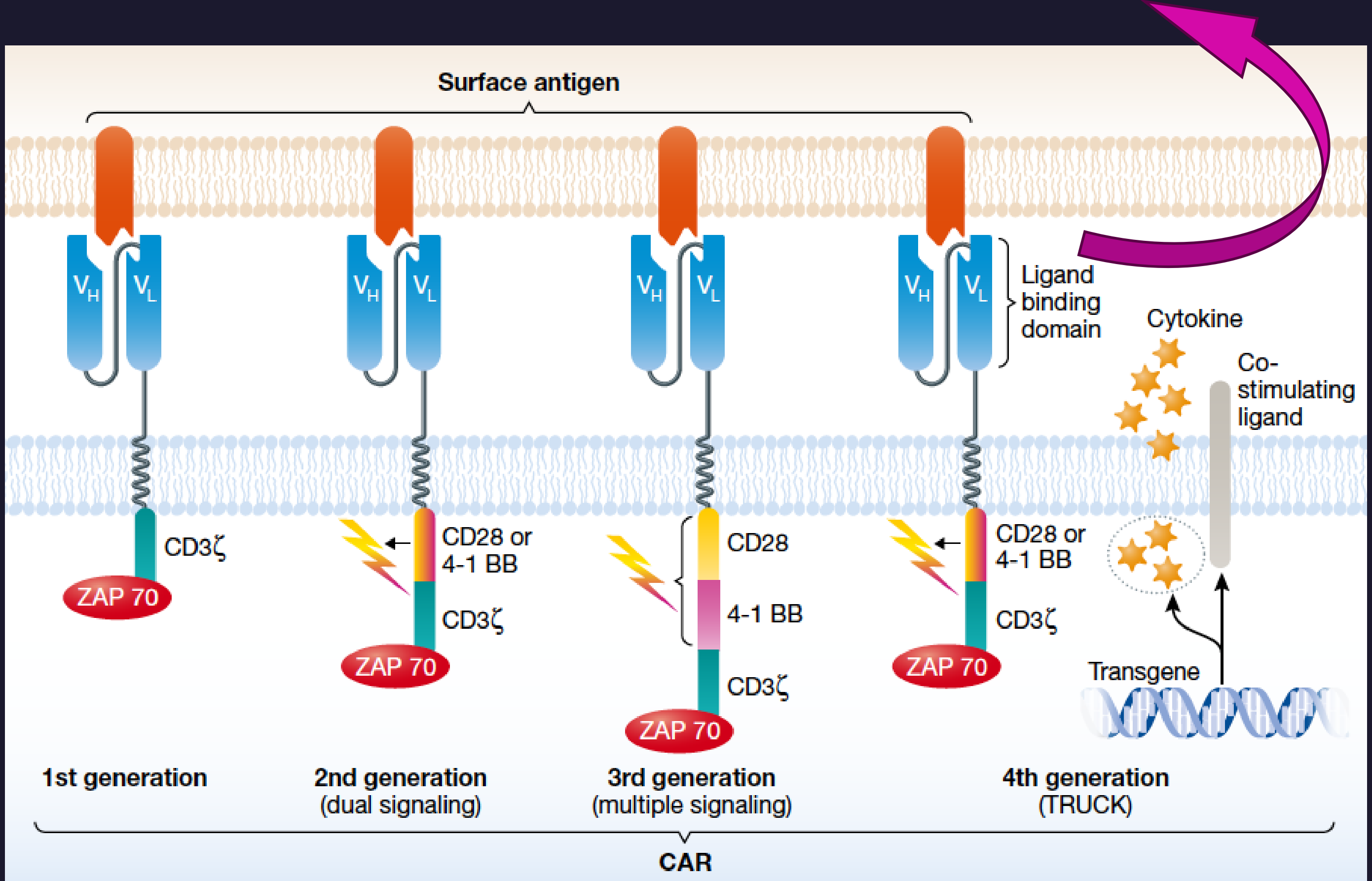
Cβ
B
SpβG
AβG
Atk
pVHCβVLCα1
ASE
C
LTR
C
Apr
Sa
VL
Psv40
H
Cα
B
Ecogpt
polyA
B

Anti-CD19

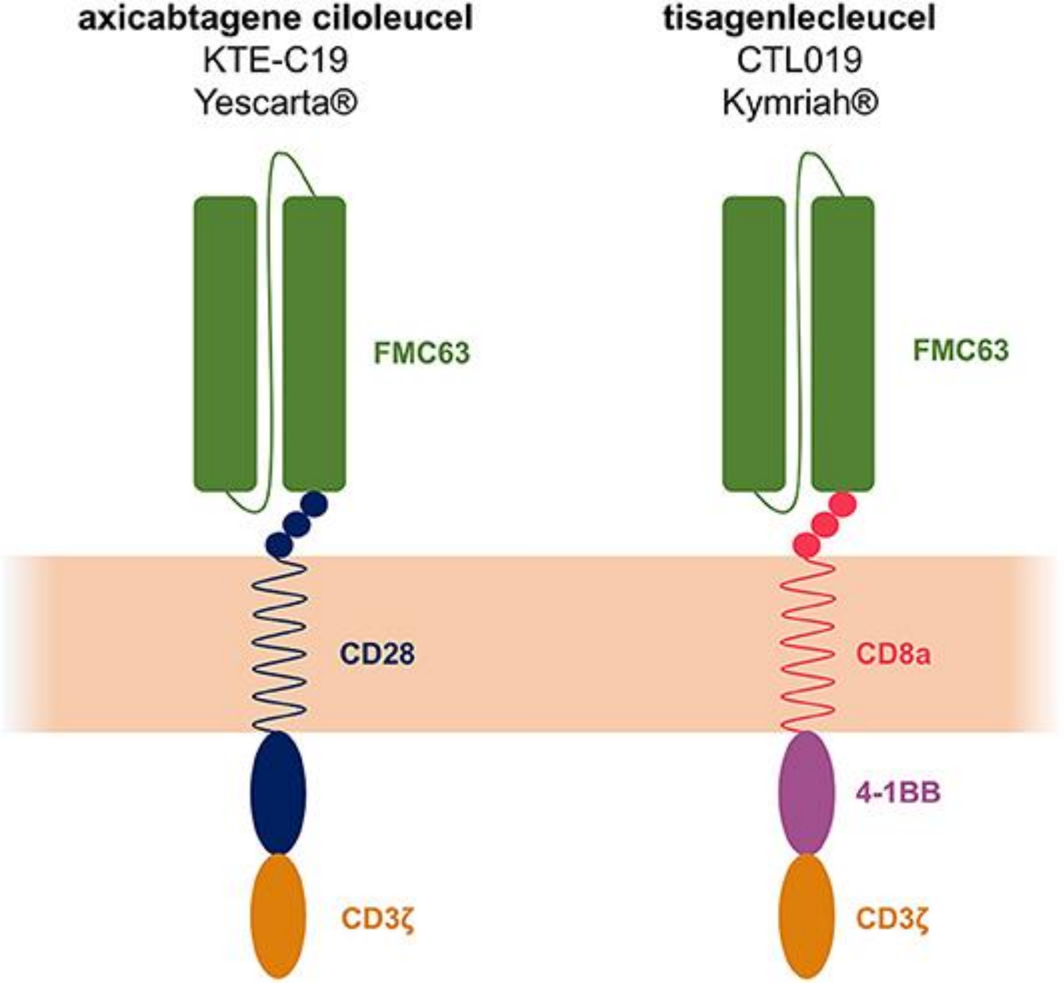

Surface antigen
VH
VL
Ligand binding domain
Cytokine
Co-stimulating ligand
CD3ζ
ZAP 70
CD28 or 4-1 BB
CD3ζ
ZAP 70
CD28
4-1 BB
CD3ζ
ZAP 70
CD28 or 4-1 BB
CD3ζ
ZAP 70
Transgene
1st generation
2nd generation (dual signaling)
3rd generation (multiple signaling)
4th generation (TRUCK)
CAR

Kuwana et al. Biochem Biophys Res Commun 1987; Gross et al. Transplant Proc 1989; Hartmann et al., EMBO Mol Med. 2017

Commercial solutions approved in Italy

AXI-CEL

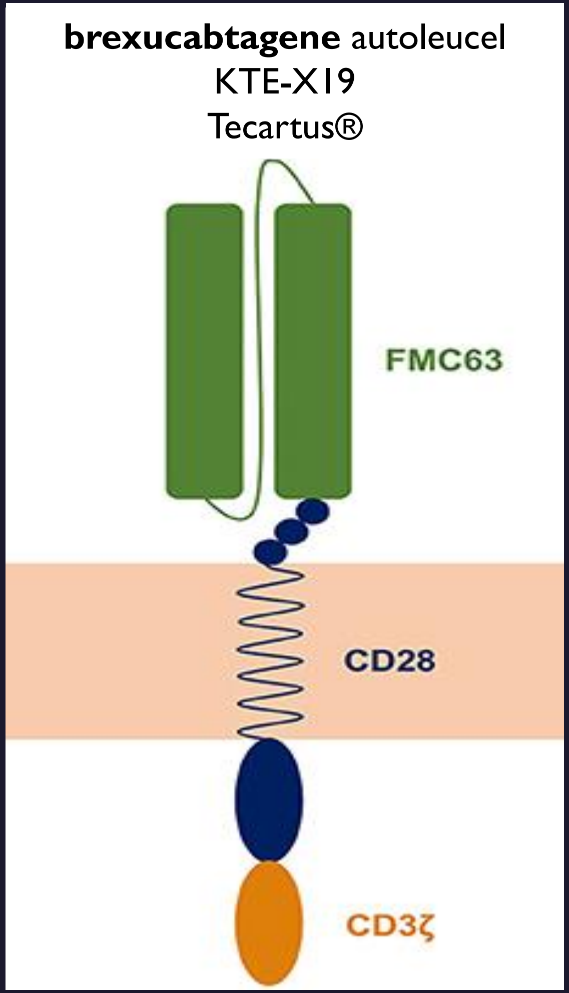
TISA-CEL


axicabtagene ciloleucel
KTE-C19
Yescarta®
FMC63
CD28
CD3ζ
tisagenlecleucel
CTL019
Kymriah®
FMC63
CD8a
4-1BB
CD3ζ

GILEAD

NOVARTIS

BREXU-CEL


brexucabtagene autoleucel
KTE-X19
Tecartus®
FMC63
CD28
CD3ζ

GILEAD

2° generation CAR

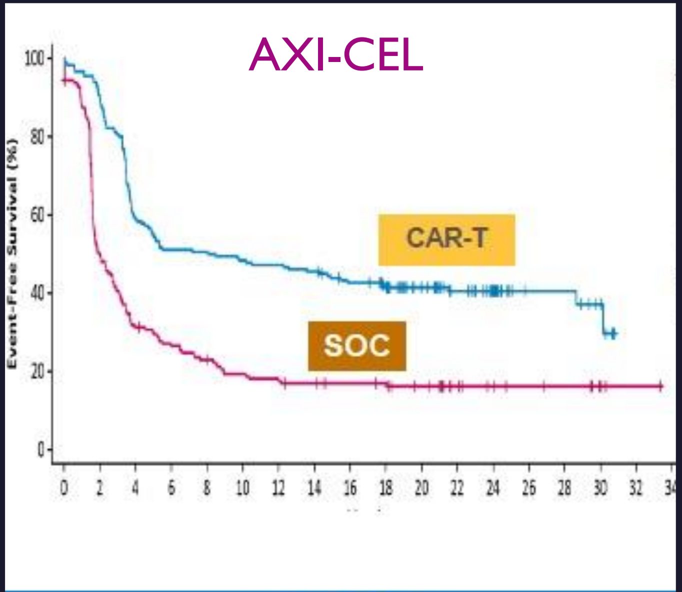
→ CD19 anti-body

→ Hinge

→ Costimulatory domain

→ Activation domain

Randomized Ph3 Studies (CAR-T vs. SOC ASCT): EFS

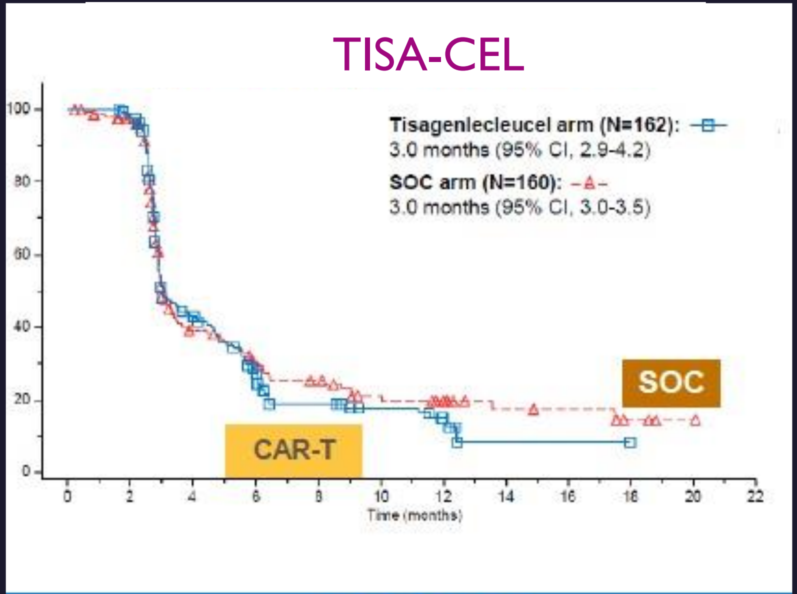


ZUMA-7

Median EFS = 8.3 vs. 2 mons

1. Progression or death
2. New treatment
3. No CR/PR by 150 days

ORR: 83% vs. 50%
CR: 65% vs. 32%



BELINDA

Median EFS = 3 vs. 3 mons

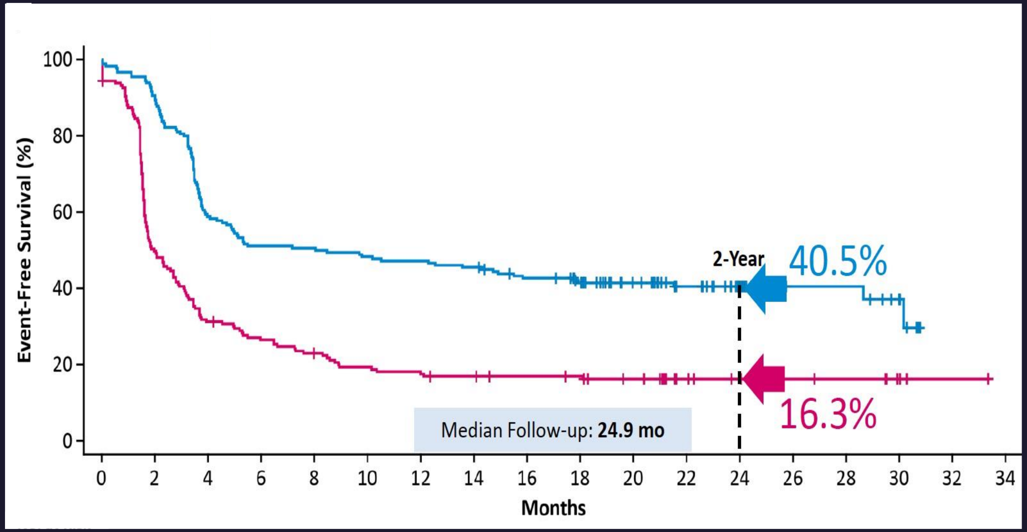
1. Progression or death
2. SD/PD @/after 12wks

ORR: 46.3% vs. 42.5%
CR: 28.4% vs. 27.5%

Locke et al., NEJM 2022, Kamdar et al., Lancet 2022; Bishop et al., NEJM 2022

Primary EFS endpoint: Axi-cel is superior to SOC

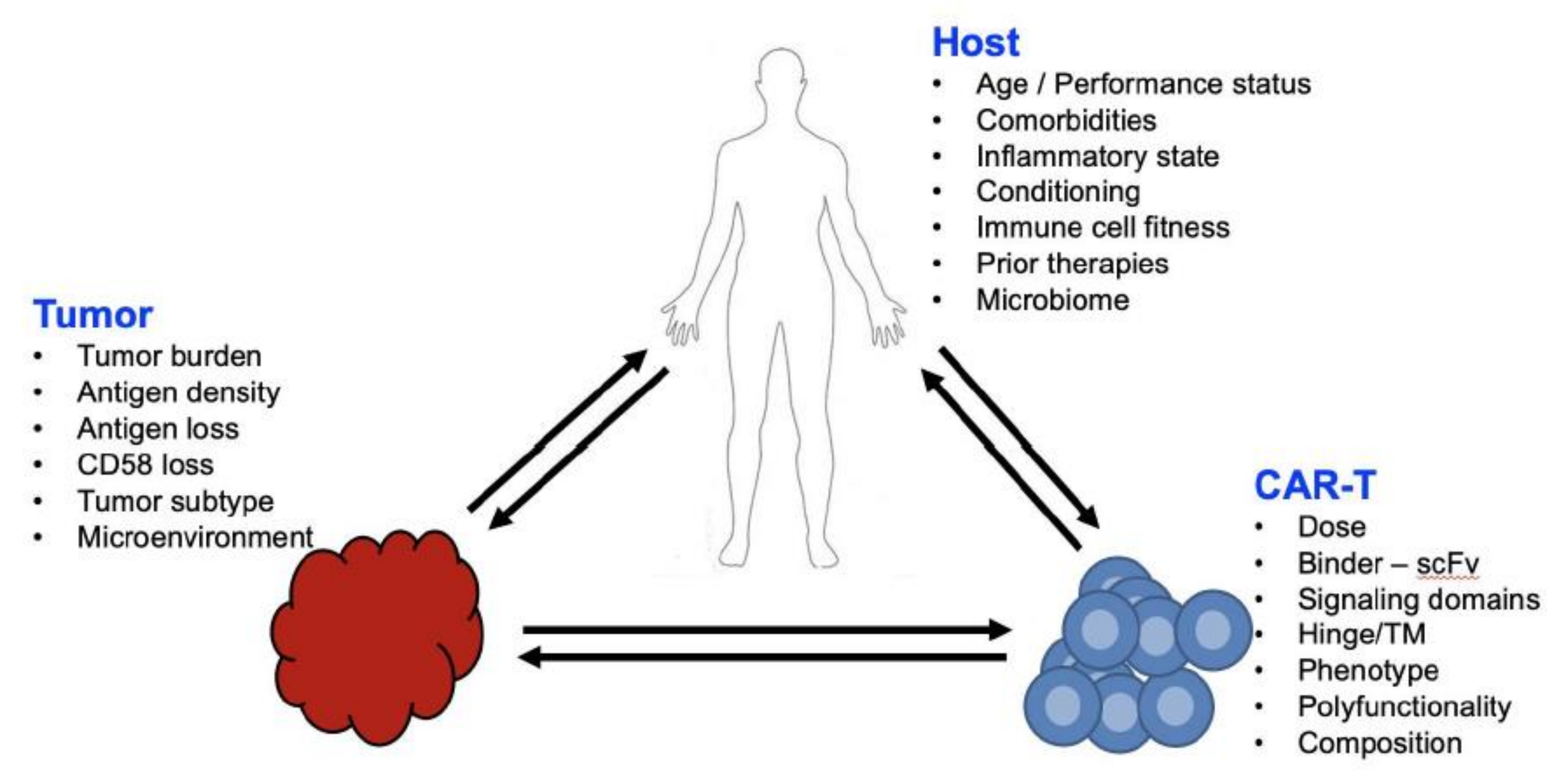
Median FU: 24.9 months



HR 0.398 (95% CI, 0.308-0.514), *P*<0.0001

	Median EFS (95% CI), mo	24-mo EFS Rate (95% CI), %
Axi-cel (N=180)	8.3 (4.5-15.8)	40.5% (33.2-47.7)
SOC (N=179)	2.0 (1.6-2.8)	16.3% (11.1-22.2)

Factors affecting CAR-T efficacy



Host

- Age / Performance status
- Comorbidities
- Inflammatory state
- Conditioning
- Immune cell fitness
- Prior therapies
- Microbiome

Tumor

- Tumor burden
- Antigen density
- Antigen loss
- CD58 loss
- Tumor subtype
- Microenvironment

CAR-T

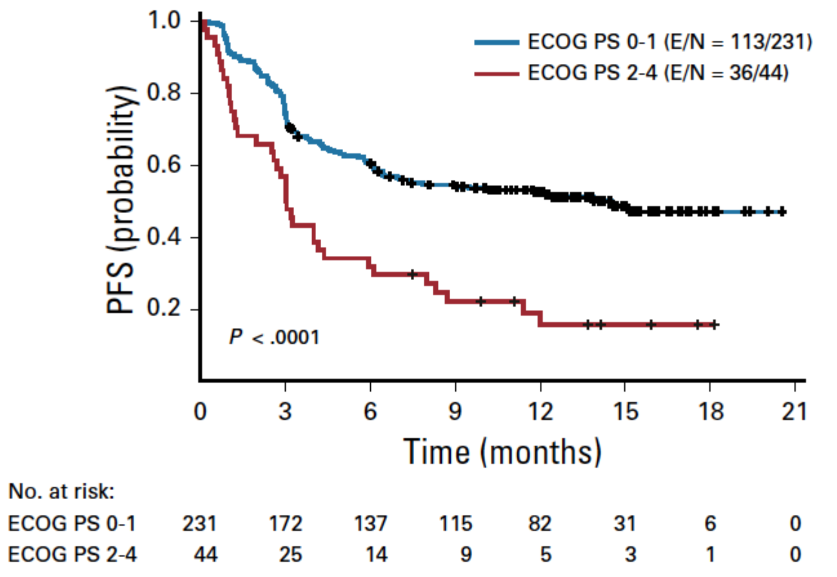
- Dose
- Binder – scFv
- Signaling domains
- Hinge/TM
- Phenotype
- Polyfunctionality
- Composition

Outcome based on patient's characteristics

Axi-cel in the real-world setting
Progression Free Survival

ECOG PS

N = 275

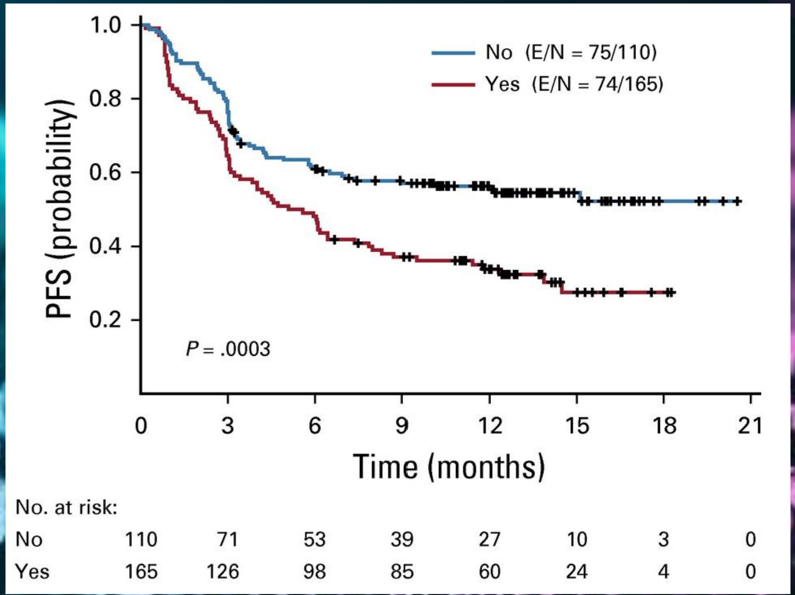


No. at risk:

ECOG PS 0-1	231	172	137	115	82	31	6	0
ECOG PS 2-4	44	25	14	9	5	3	1	0

Comorbidities

N = 275



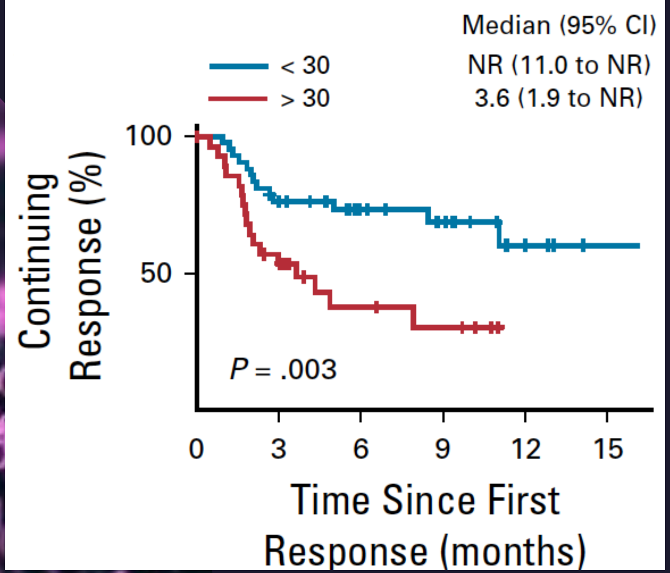
No. at risk:

No	110	71	53	39	27	10	3	0
Yes	165	126	98	85	60	24	4	0

Axi-cel in the real-world setting

CRP at Day 0

N = 119



Nastoupil et al., JCO 2020; Jacobson et al., JCO 2020

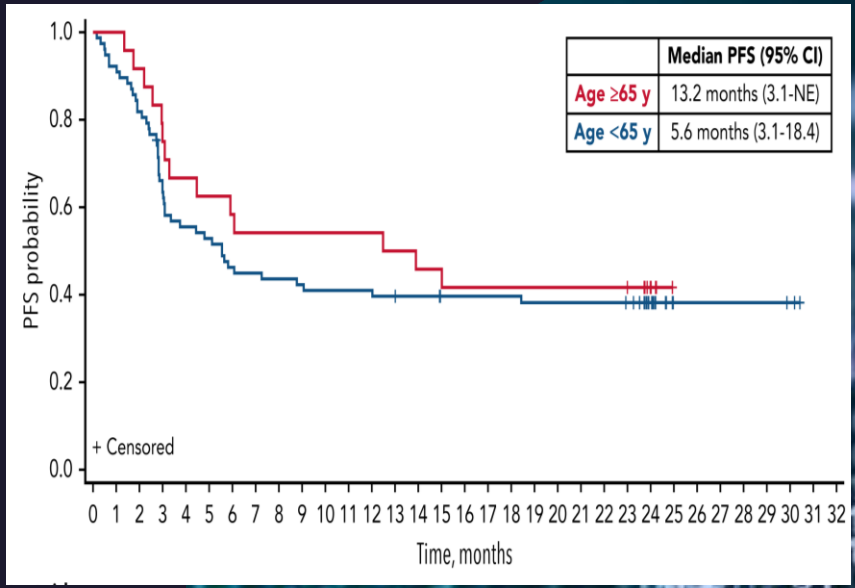
Outcome based on patient's characteristics

Age

Progression Free Survival

ZUMA-1 trial (Axi-cel)

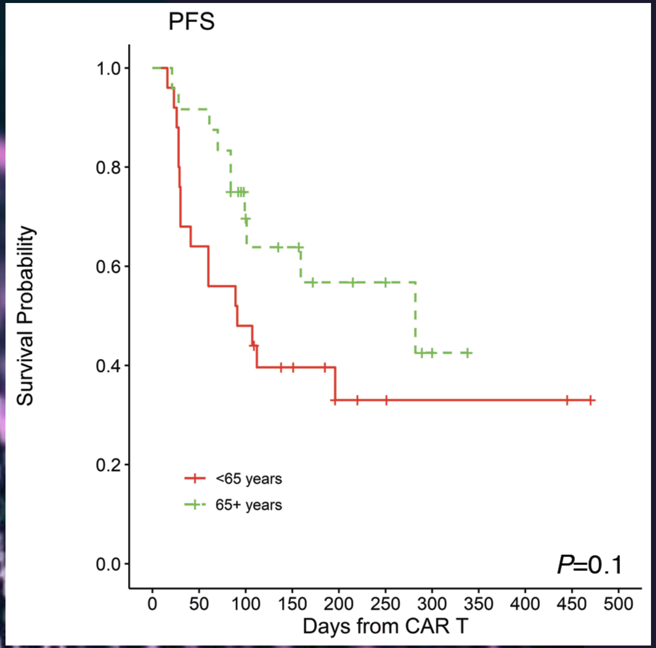
N = 101



	Median PFS (95% CI)
Age ≥65 y	13.2 months (3.1-NE)
Age <65 y	5.6 months (3.1-18.4)

MSK experience (Axi-cel;Tisa-cel)

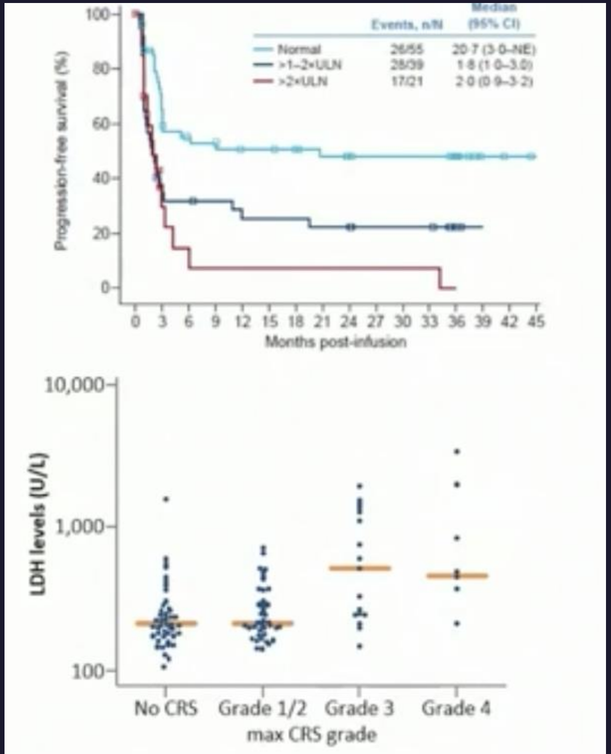
N = 49



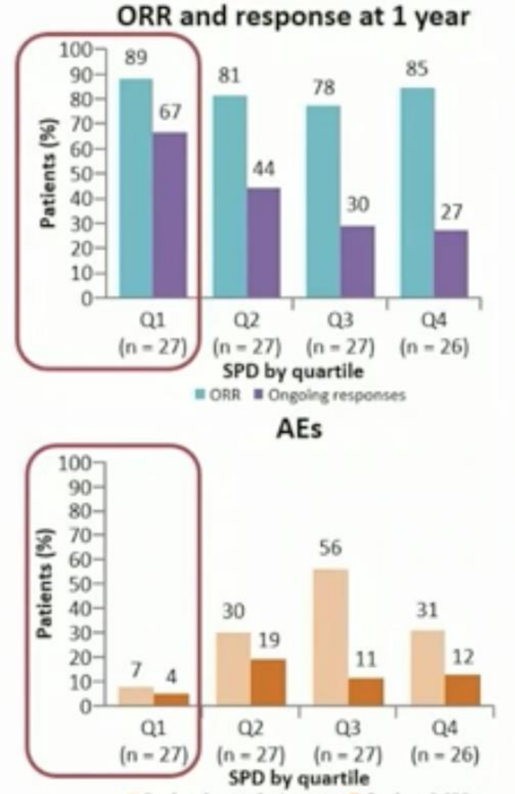
1. Neelapu et al., Blood 2020; Lin et al., Haematologica 2021

Outcome based on disease characteristics: Tumor burden

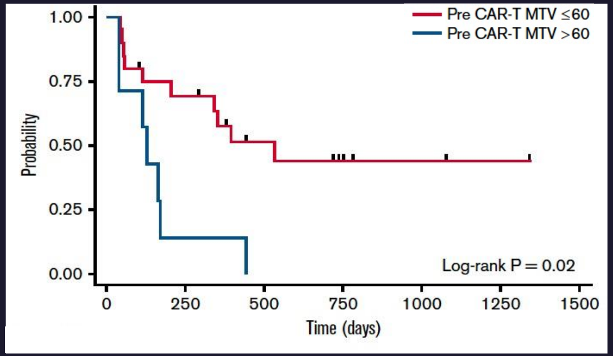
LDH
JULIET (Tisa-cel)[1]

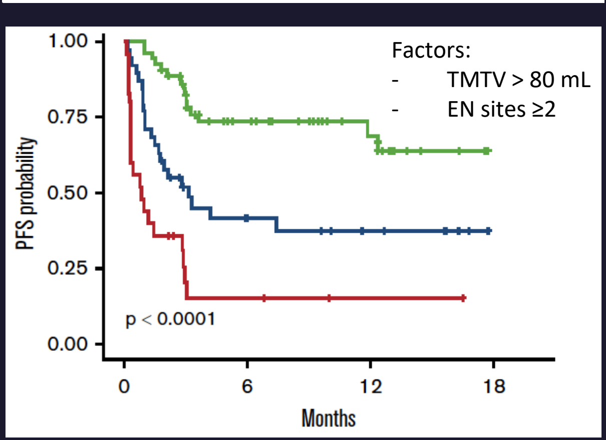


SPD (image)
ZUMA-1 (Axi-cel)[2]



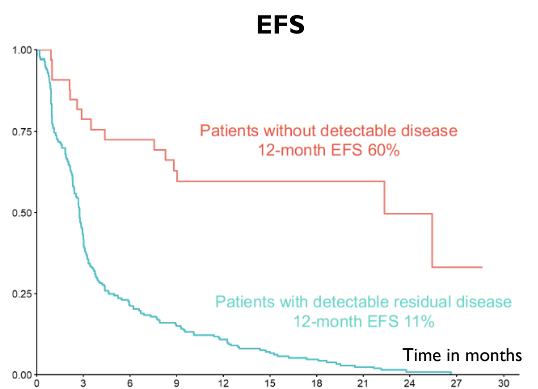
Metabolic TV
Real World data[3]





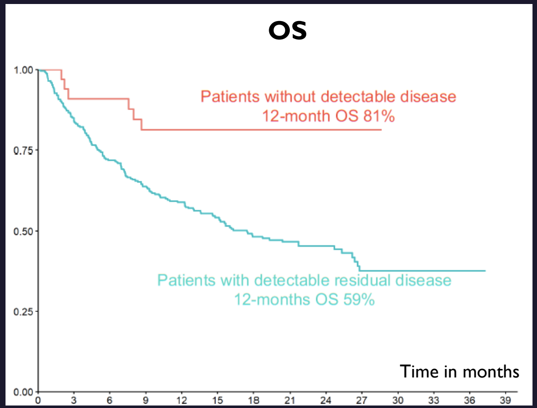
Factors:
- TMTV > 80 mL
- EN sites ≥2

PET neg
Real World data[4]



Patients without detectable disease
12-month EFS 60%

Patients with detectable residual disease
12-month EFS 11%



Patients without detectable disease
12-month OS 81%

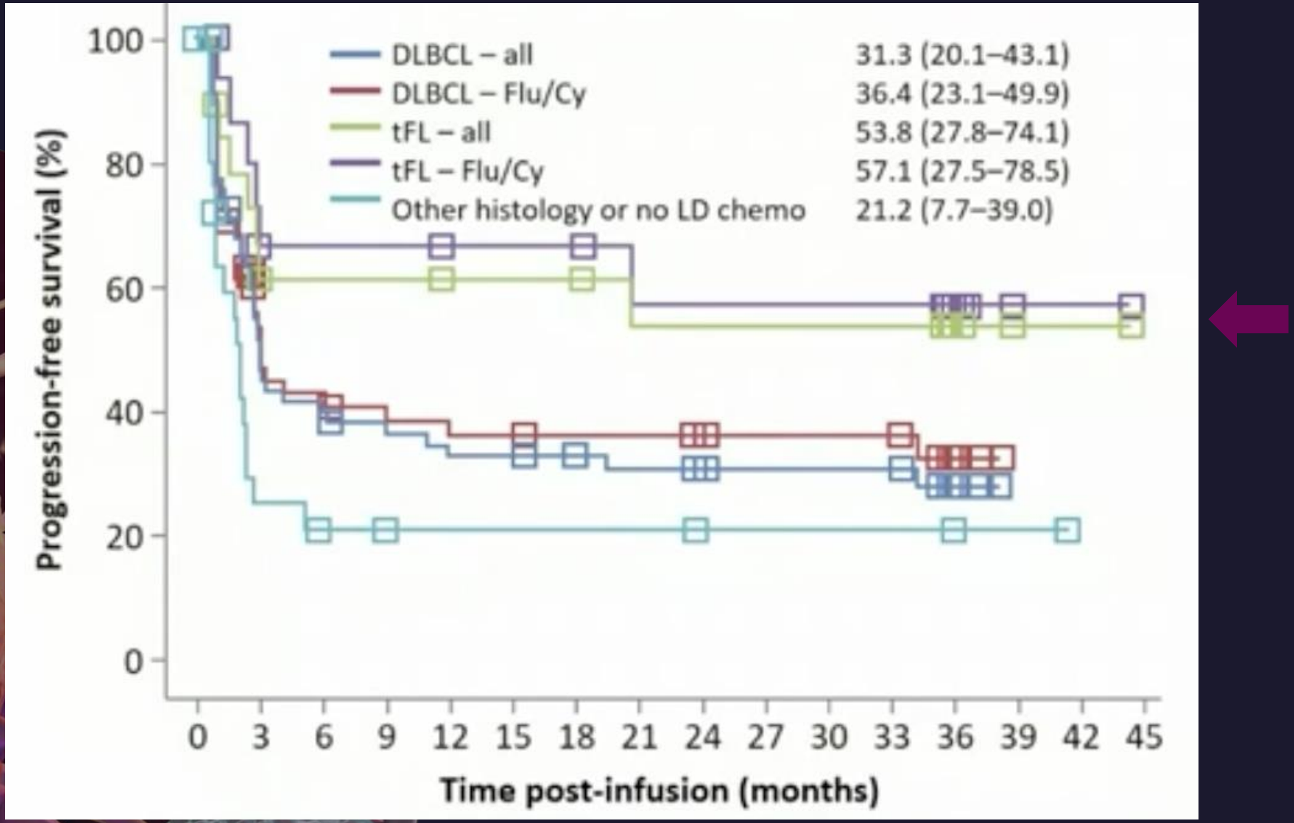
Patients with detectable residual disease
12-months OS 59%

1. Schuster et al., Lanc Onc 2020; 2. Locke et al., JCO 2018; 3. Voorhees et al., Blood Adv 2021; Vercellino et al., Blood Adv. 2020; 4. Wudhikarn et al., Blood Adv. 2022

Outcome based on disease characteristics: Histology

JULIET (Tisa-cel)

tFL > DLBCL

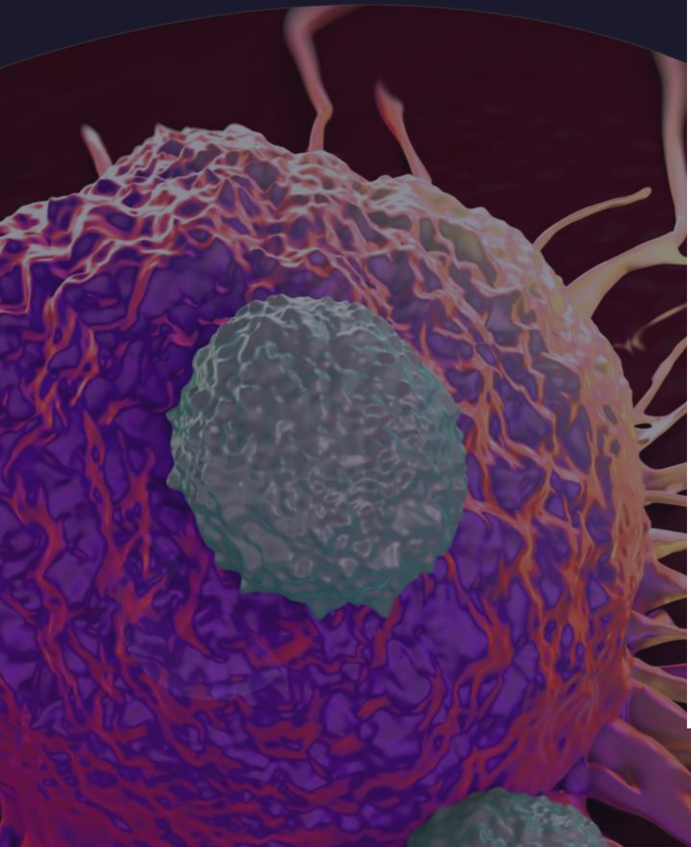


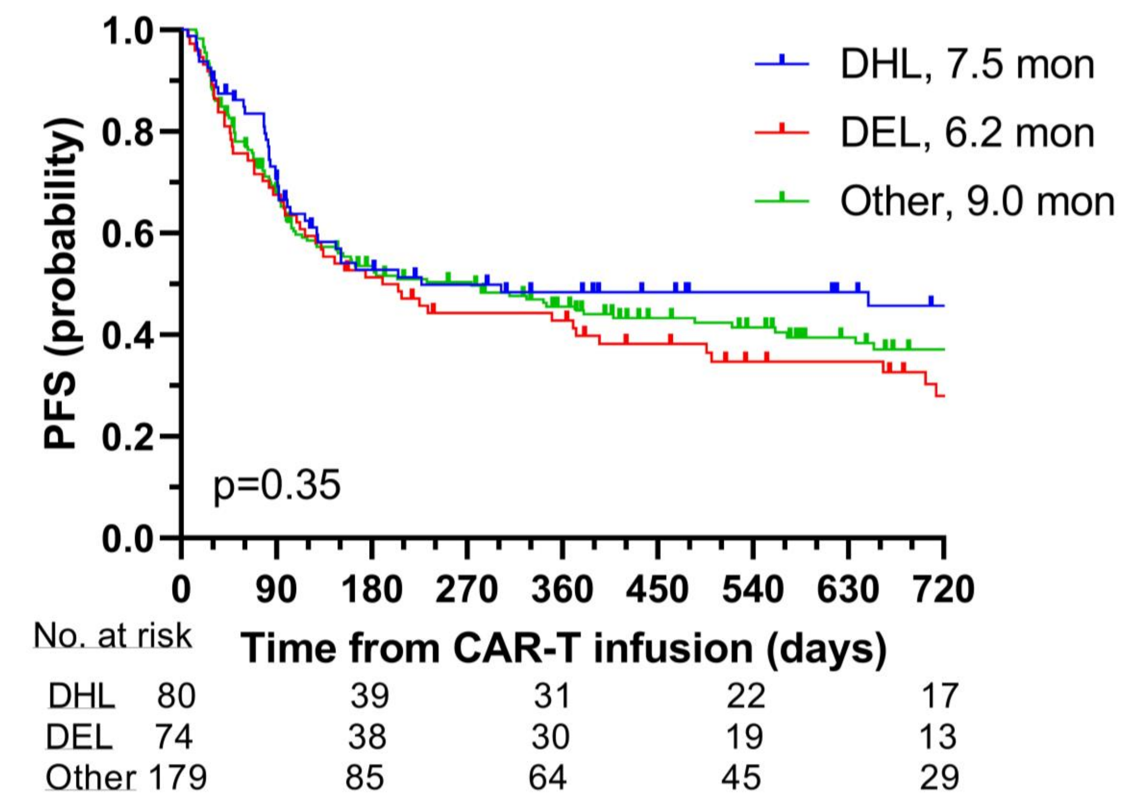
1. Schuster et al., Lancet Oncol 2021

Outcome based on disease characteristics: biological HR

MYC and BCL2 and/or BCL6 rearrangements & DEL

Progression Free Survival





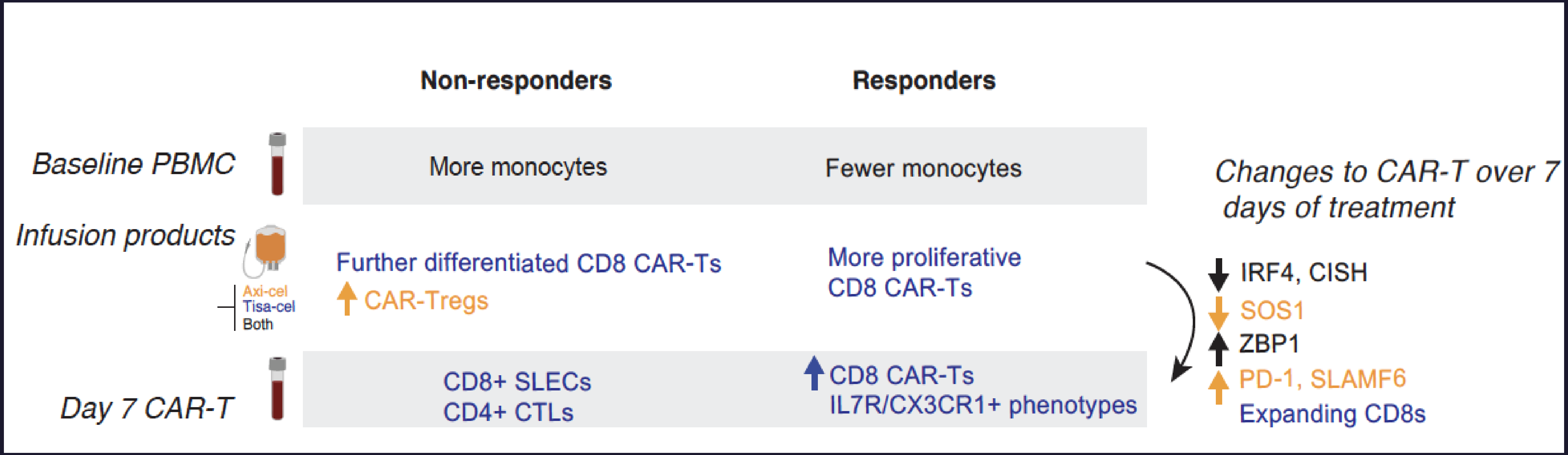
No. at risk					
DHL	80	39	31	22	17
DEL	74	38	30	19	13
Other	179	85	64	45	29

333 patients

Zurko et al., ASH 2022, Abstract 154

Outcome based on CAR-T cells characteristics

Key Findings from scRNAseq data on Tisa-cel and Axi-cel



	Non-responders	Responders
Baseline PBMC	More monocytes	Fewer monocytes
Infusion products	Further differentiated CD8 CAR-Ts ↑ CAR-Tregs	More proliferative CD8 CAR-Ts
Day 7 CAR-T	CD8+ SLECs CD4+ CTLs	↑ CD8 CAR-Ts IL7R/CX3CR1+ phenotypes

Changes to CAR-T over 7 days of treatment

↓ IRF4, CISH
↓ SOS1
↑ ZBP1
↑ PD-1, SLAMF6
Expanding CD8s

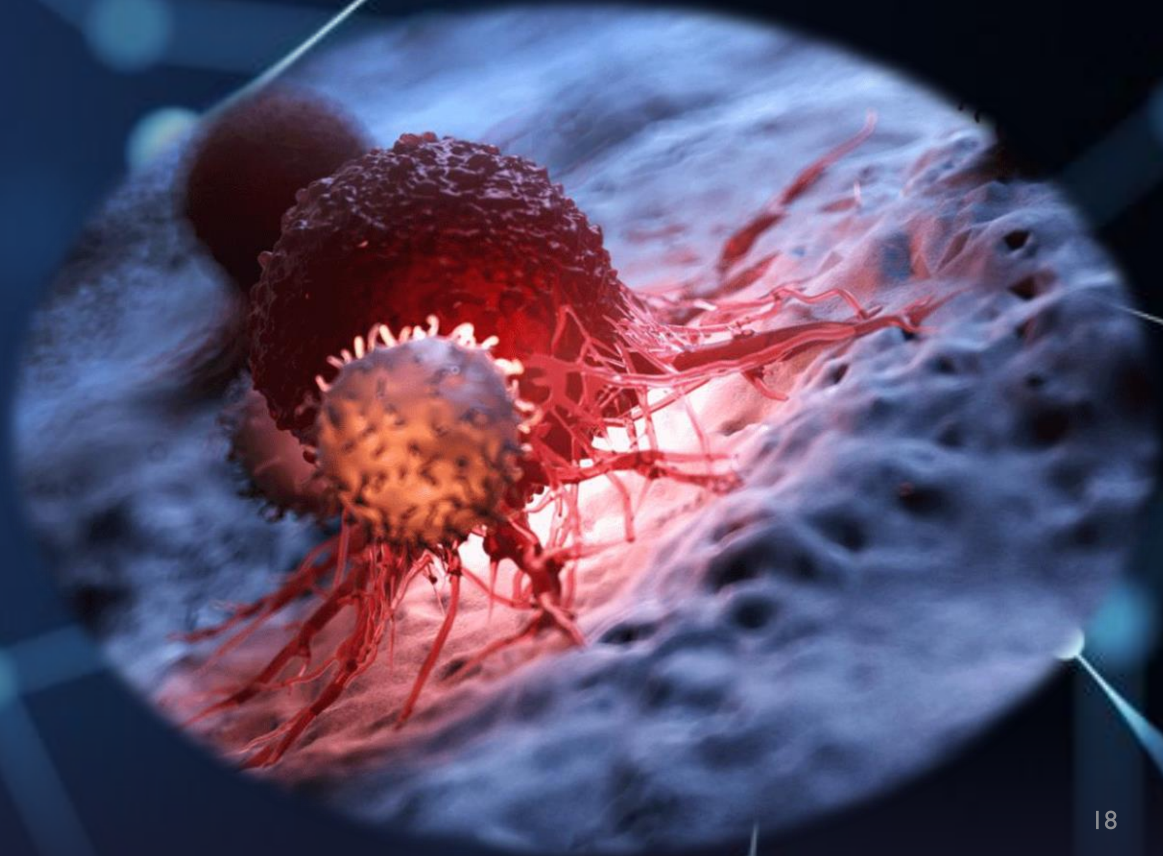
Haradhvala et al., Nature Med 2022

Early toxicity post CD19 Car-T

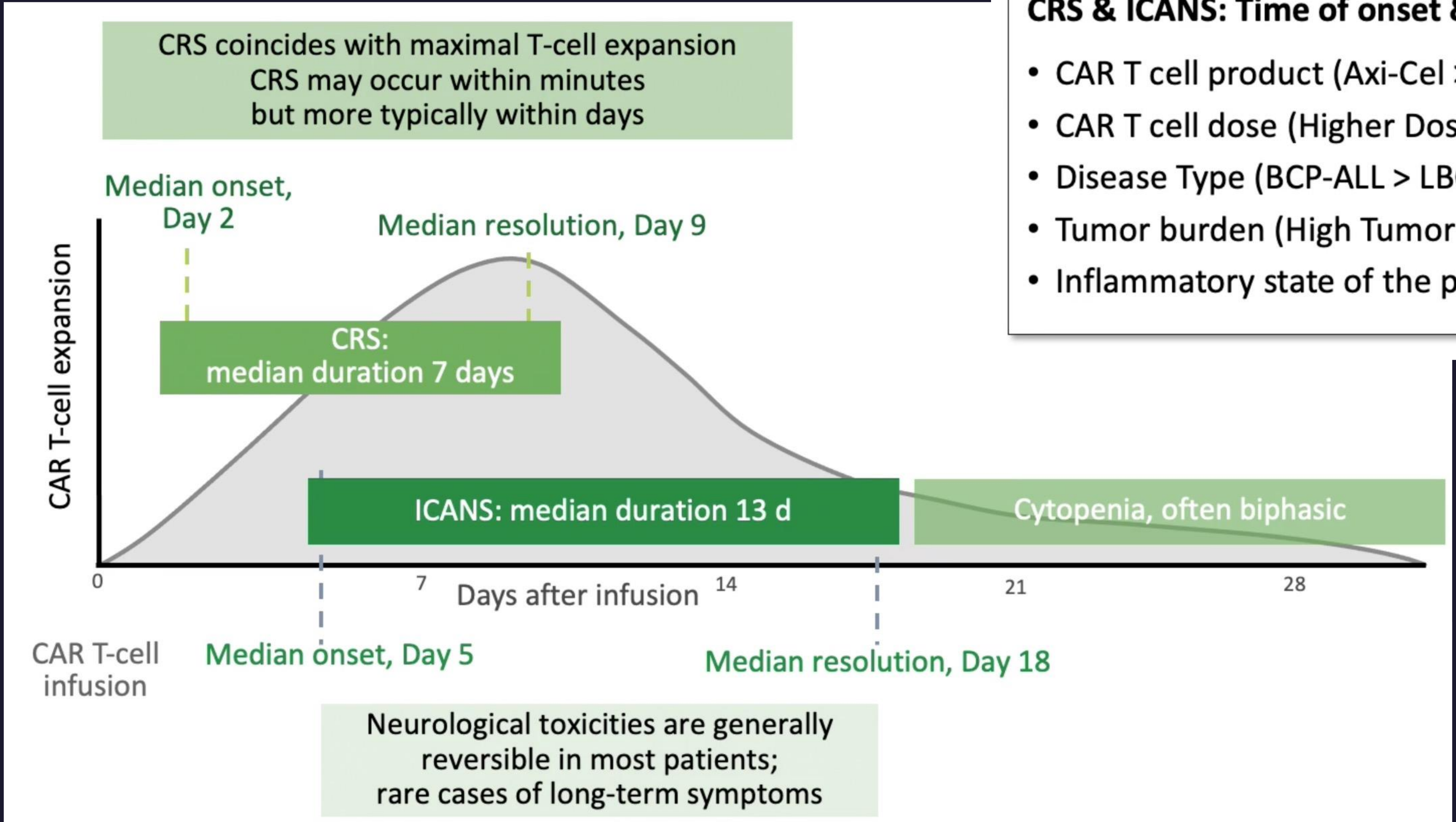
CRS

ICANS

Cytopenia



Early Toxicity post CD19 CAR-T



CRS coincides with maximal T-cell expansion
CRS may occur within minutes
but more typically within days

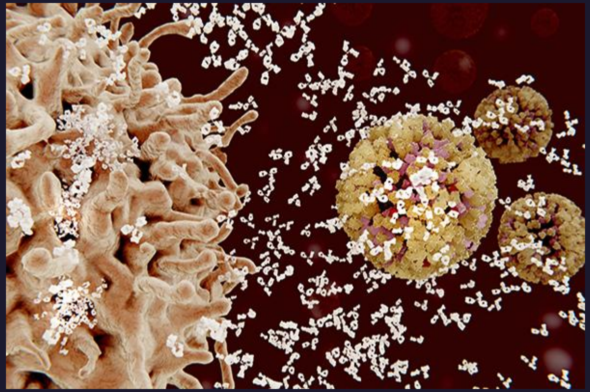
Neurological toxicities are generally
reversible in most patients;
rare cases of long-term symptoms

CRS & ICANS: Time of onset & duration depends on

- CAR T cell product (Axi-Cel > Tisa-Cel)
- CAR T cell dose (Higher Dose)
- Disease Type (BCP-ALL > LBCL)
- Tumor burden (High Tumor Volumne)
- Inflammatory state of the patient (Ferritin, CRP)

Hayden et al., Ann Oncol 2022; Rejeski et al., Blood 2021;
Hill and Seo, Blood 2020; Jain et al., Biol Blood Marrow Transplant 2019

Clinical manifestations



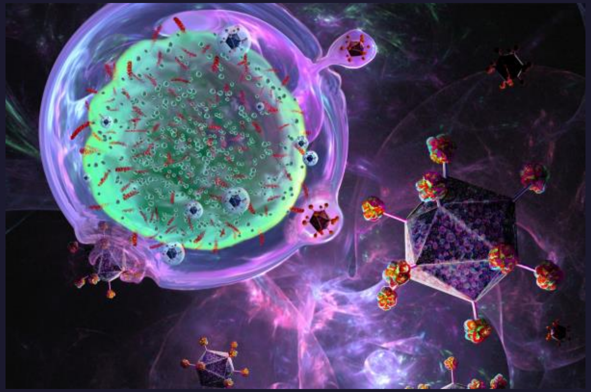
CRS: Cytokine Release Syndrome

The most common

Fever, hypotension, hypoxia

Rapidly fatal

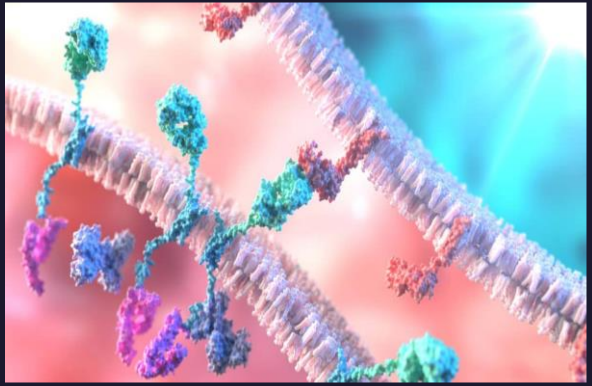
Based on endogenous or infused T cells over-activation



ICANS: Immune effector cell-associated neurotoxicity syndrome

The second most common

Involves the CNS and results in the activation or engagement of endogenous or infused T cells and/or other immune effector cells.



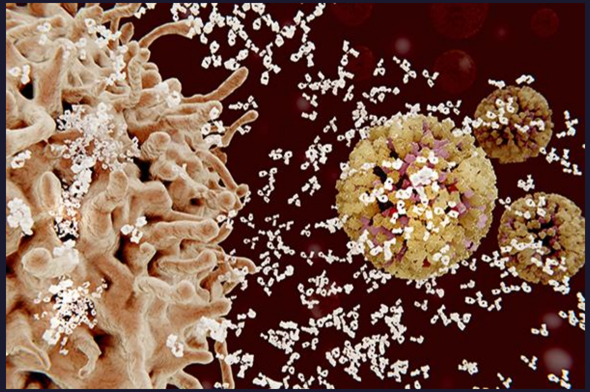
Cytopenia

"on-target, off-tumor" effect

B-cell aplasia, which results in cytopenias and hypogammaglobulinemia.

Typically occurs within the first 30 days after cell infusion, but can take months to resolve

…from the lab point of view



CRS: Cytokine Release Syndrome

Transaminitis (within 2/3 w)

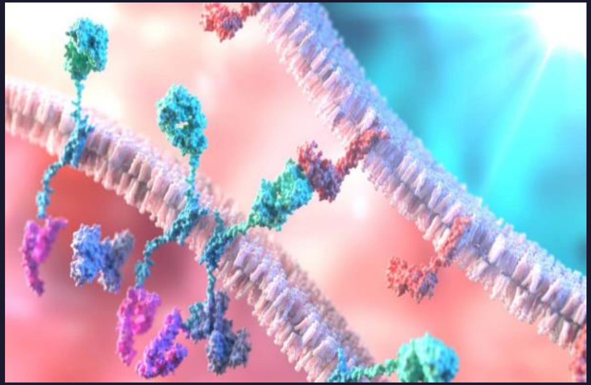
Coagulopathy

Ferritin and CRP ⬆

NO hyperphosphatemia, hyperuricemia, hypocalcemia, and hyperkalemia

ICANS: Immune effector cell-associated neurotoxicity syndrome

Microbiological investigation and coagulation assessment in order to differentially diagnose infections and stroke



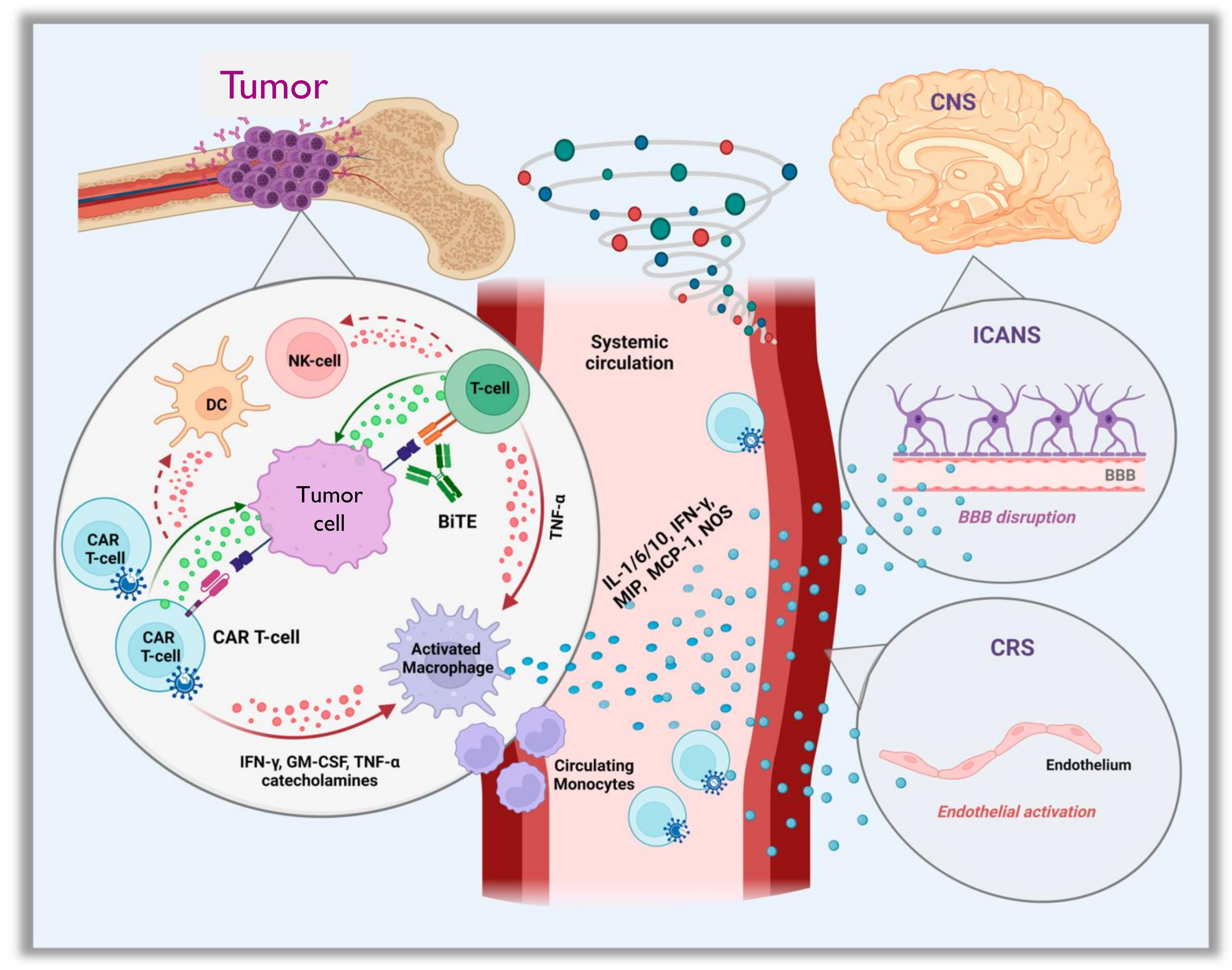
Cytopenia

Closely monitoring of blood cells count

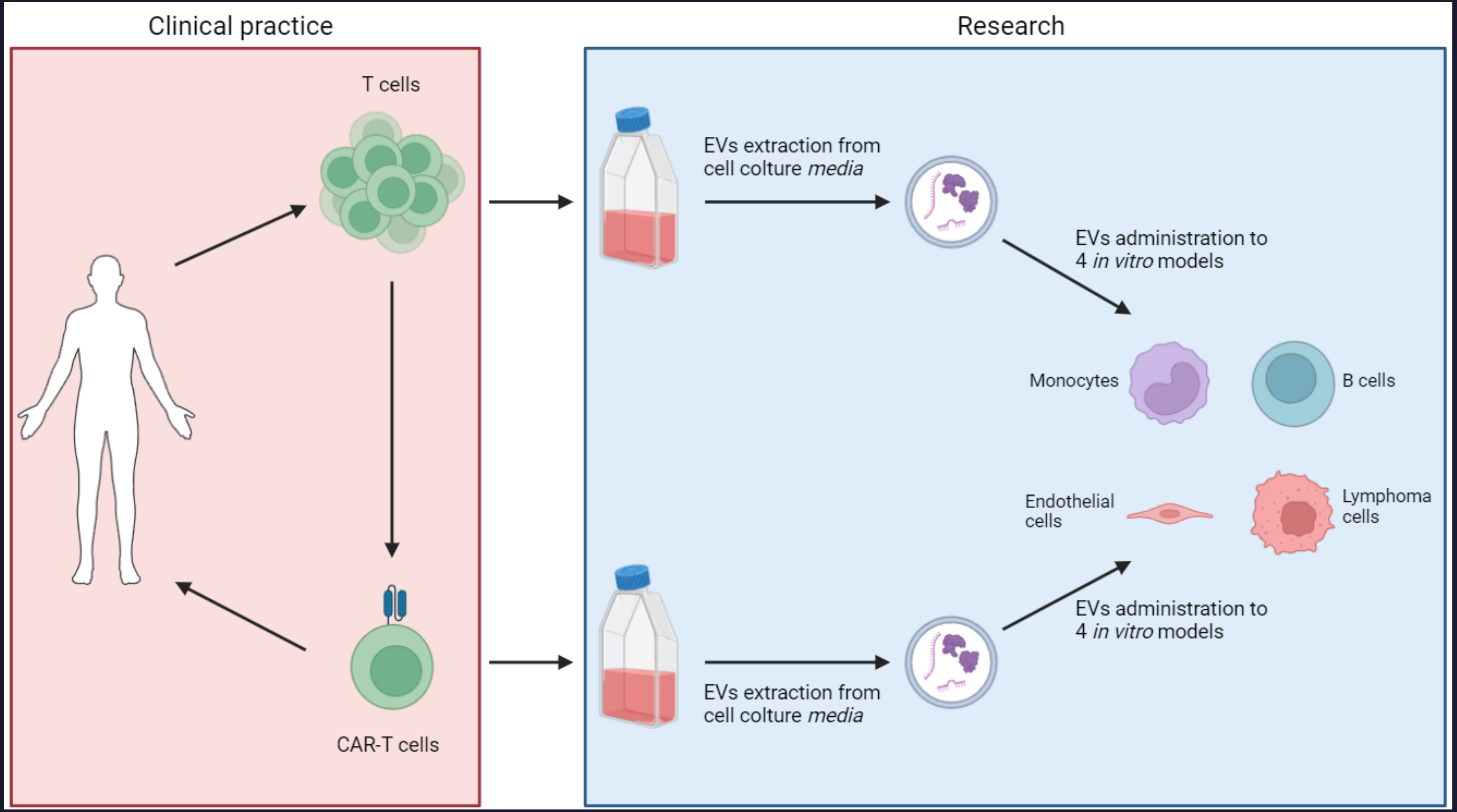
Proteinemia

Sievers et al., Front Onc 2020

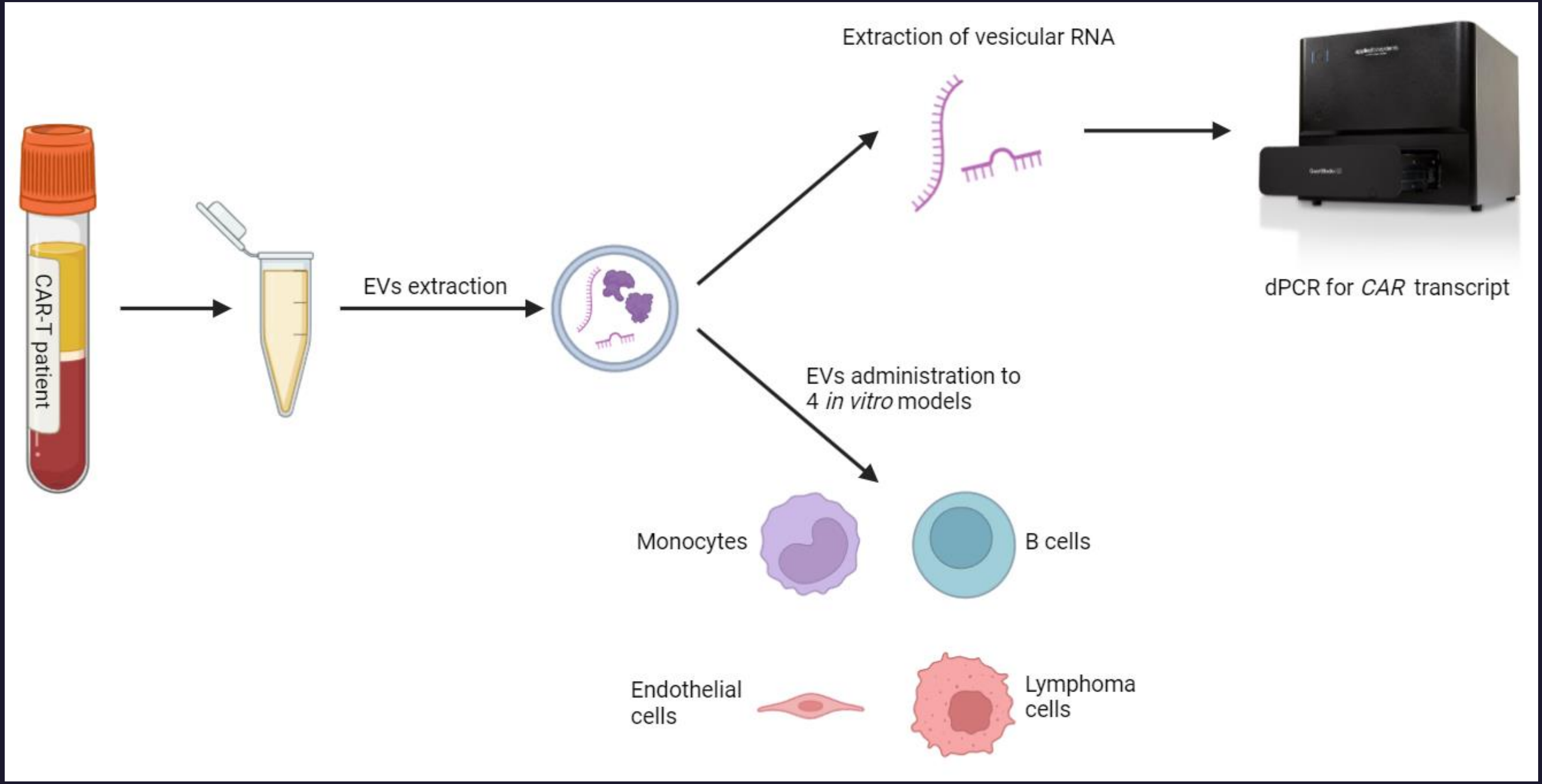
…from the pathogenic point of view


Tumor
CNS
Systemic circulation
NK-cell
DC
T-cell
Tumor cell
BiTE
TNF-α
CAR T-cell
CAR T-cell
CAR T-cell
Activated Macrophage
IFN-γ, GM-CSF, TNF-α catecholamines
IL-1/6/10, IFN-γ, MIP, MCP-1, NOS
Circulating Monocytes
ICANS
BBB
BBB disruption
CRS
Endothelium
Endothelial activation

EVASIET Project


Clinical practice
Research
T cells
CAR-T cells
EVs extraction from
cell colture *media*
EVs administration to
4 *in vitro* models
Monocytes
B cells
Endothelial
cells
Lymphoma
cells
EVs administration to
4 *in vitro* models
EVs extraction from
cell colture *media*

EVASIET Project


CAR-T patient
EVs extraction
Extraction of vesicular RNA
dPCR for *CAR* transcript
EVs administration to 4 *in vitro* models
Monocytes
B cells
Endothelial cells
Lymphoma cells

CAR-T toxicities at different times

SHORT-TERM:

- CRS
- ICANS
- Infections
- Cytopenias
- HLH/MAS
- …

MIDDLE-TERM:

- Chronic neutropenia
- Hypogammaglobulinemia
- B-cell aplasia
- Infections
- CD19- relapse

LONG-TERM:

- Secondary malignancies?
- Cognitive defects?
- Transmission to the offspring

frontiers | Frontiers in Oncology

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SPECIALTY SECTION
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High risk-myelodysplastic syndrome following CAR T-cell therapy in a patient with relapsed diffuse large B cell lymphoma: A case report and literature review

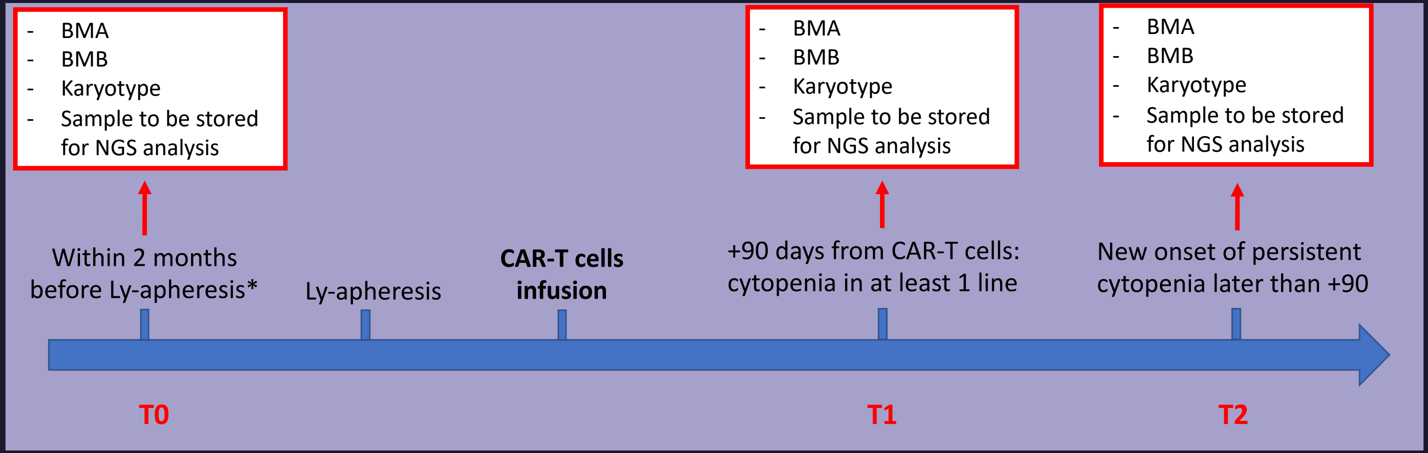
Eugenia Accorsi Buttini[1*], Mirko Farina[1], Luisa Lorenzi[2], Nicola Polverelli[1], Vera Radici[1], Enrico Morello[1], Federica Colnaghi[1], Camillo Almici[3], Emilio Ferrari[3], Andrea Bianchetti[3], Alessandro Leoni[1,4], Federica Re[1,4], Katia Bosio[1,4], Simona Bernardi[1,4], Michele Malagola[1], Alessandro Re[5] and Domenico Russo[1]

Study of clonal hematopoiesis on patients undergoing CAR-T cells therapy

(ClonHema-CAR-T Study)

PI: Prof. D. Russo

✓ **Approved by Ethical Committee of Brescia (*NP 5554*)**



- BMA
- BMB
- Karyotype
- Sample to be stored for NGS analysis

Within 2 months before Ly-apheresis*

Ly-apheresis

CAR-T cells infusion

- BMA
- BMB
- Karyotype
- Sample to be stored for NGS analysis

+90 days from CAR-T cells: cytopenia in at least 1 line

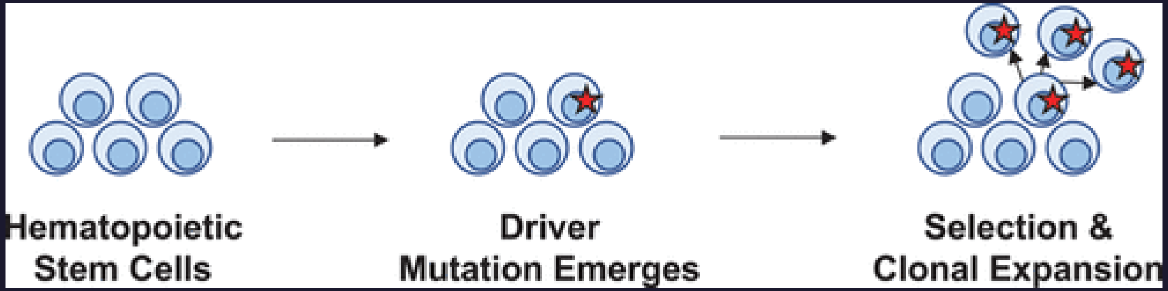
- BMA
- BMB
- Karyotype
- Sample to be stored for NGS analysis

New onset of persistent cytopenia later than +90

T0

T1

T2



GITMO

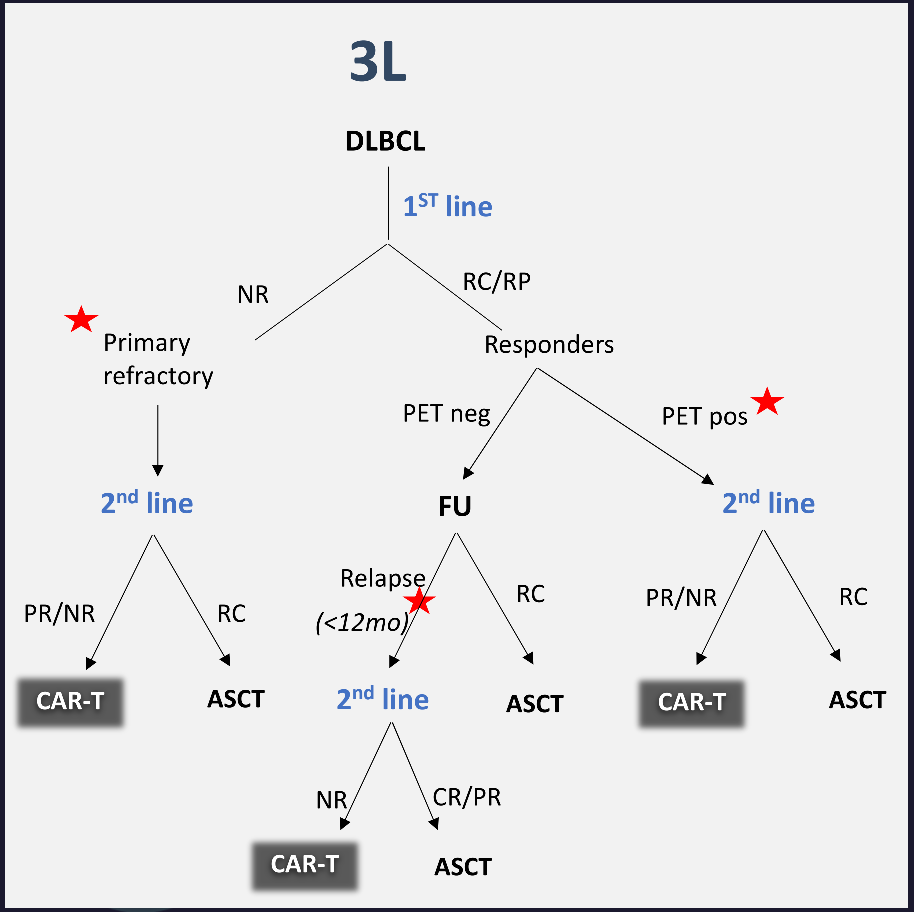
GRUPPO ITALIANO PER IL TRAPIANTO DI MIDOLLO OSSEO, CELLULE STAMINALI EMOPOIETICHE E TERAPIA CELLULARE

- *Bergamo – A. Rambaldi*
- *Brescia – D. Russo*
- *Firenze - R. Saccardi*
- *Milano (Humanitas Cancer Center) – S. Bramanti*
- *Milano (San Raffaele) – F. Ciceri*
- *Milano (Niguarda) – G. Grillo*
- *Napoli – F. Pane*
- *Padova – A. Biffi*
- *Palermo – M. Musso*
- *Pescara – M. Di Ianni*
- *Reggio Calabria – M. Martino*
- *Roma (Gemelli) – S. Sica*
- *Roma (Umberto I) – A.P. Iori*
- *Vicenza – C. Borghero*

……

Therapeutic algorithms evolution

PAST
FUTURE



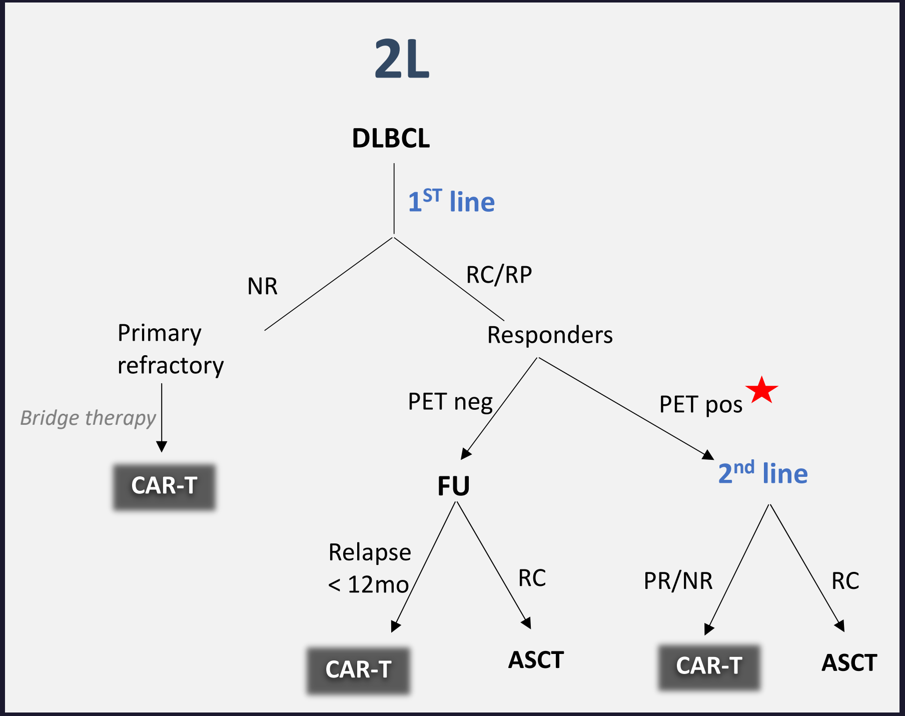
3L

DLBCL

1^{ST} line

NR ★ Primary refractory → 2^{nd} line
- PR/NR → **CAR-T**
- RC → **ASCT**

RC/RP Responders
- PET neg → **FU**
  - Relapse ★ *(<12mo)* → 2^{nd} line
    - NR → **CAR-T**
    - CR/PR → **ASCT**
  - RC → **ASCT**
- PET pos ★ → 2^{nd} line
  - PR/NR → **CAR-T**
  - RC → **ASCT**



2L

DLBCL

1^{ST} line

NR Primary refractory → *Bridge therapy* → **CAR-T**

RC/RP Responders
- PET neg → **FU**
  - Relapse < 12mo → **CAR-T**
  - RC → **ASCT**
- PET pos ★ → 2^{nd} line
  - PR/NR → **CAR-T**
  - RC → **ASCT**

★ Early Ly-apheresis

Suggestions for the future

Car-t allogenic

iPSC as alternative source of CAR-T

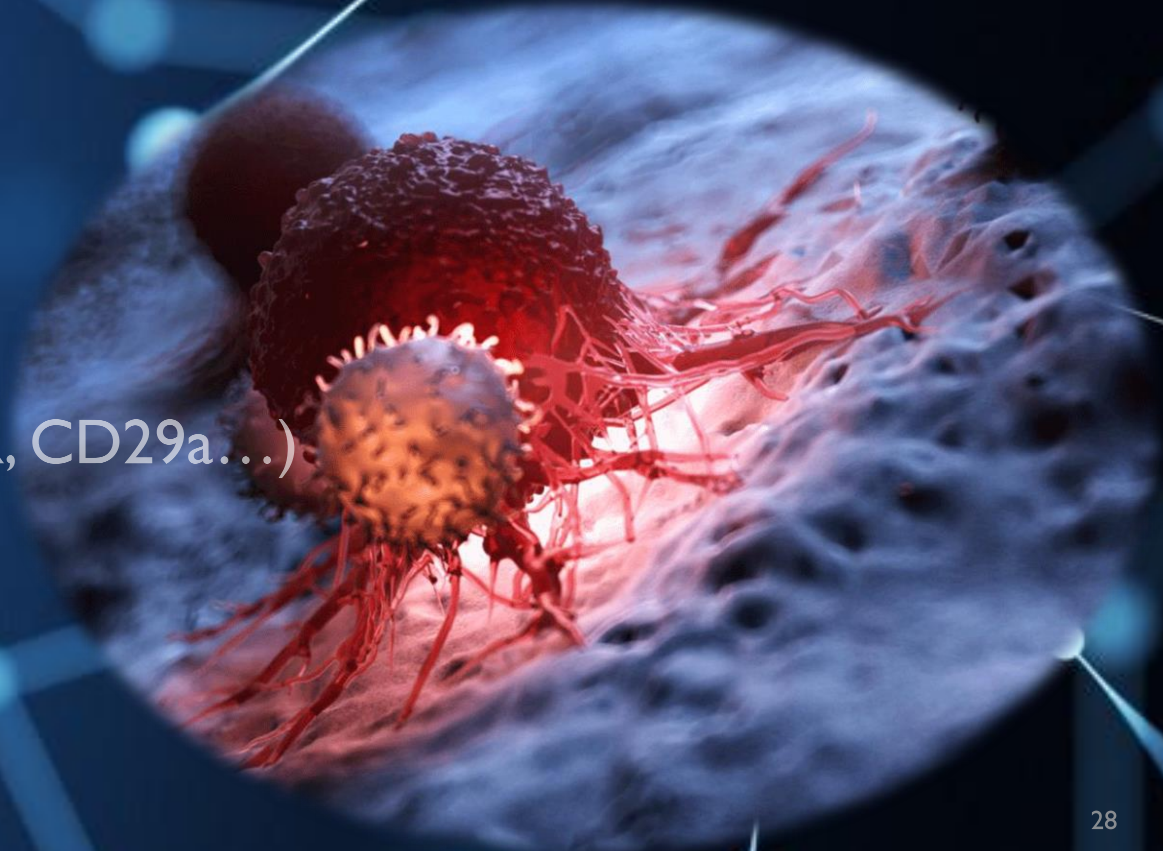
Nk CAR-T

New CAR-T constructs

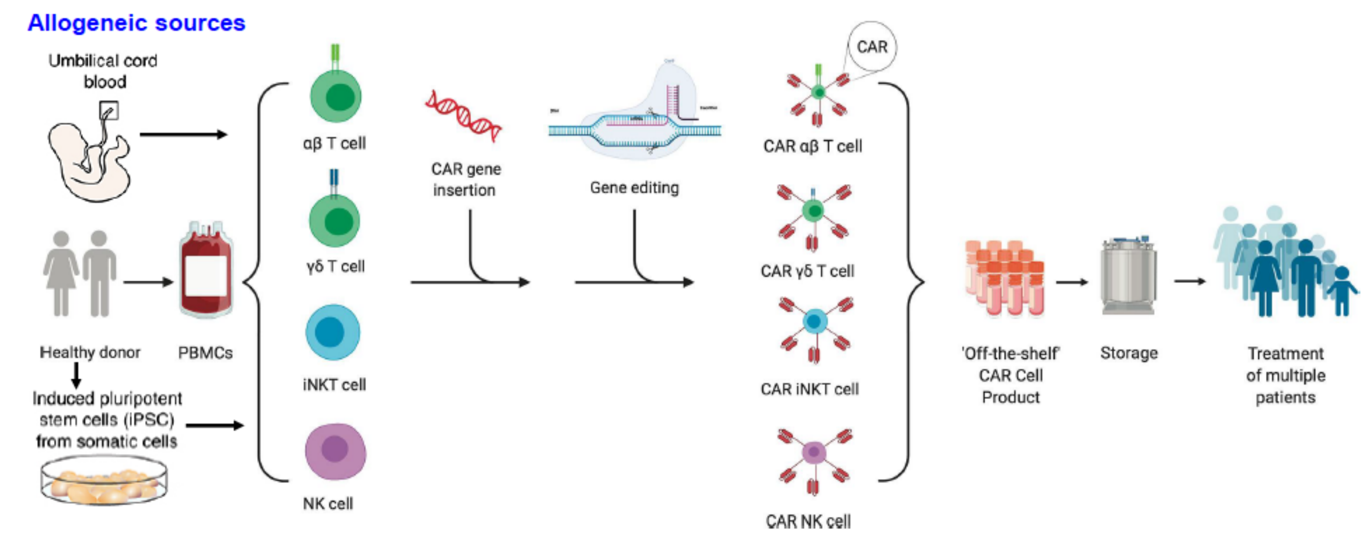
Targetting New Antigen (CD20, CD22, BAFF-R, CD29a…)

Outpatient

CAR-T earlier in treatment plan

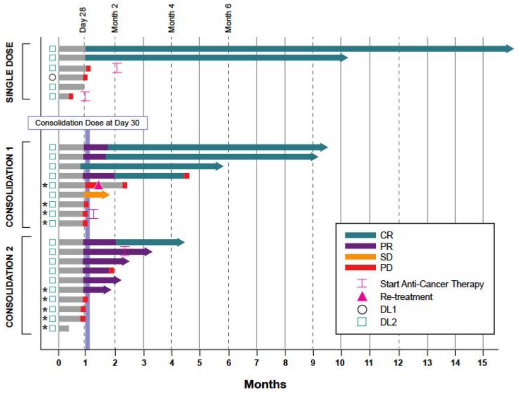


Efficacy in phase I allogeneic CAR-T trials in r/r DLBCL


Allogeneic sources
Umbilical cord blood
Healthy donor
PBMCs
Induced pluripotent stem cells (iPSC) from somatic cells
αβ T cell
γδ T cell
iNKT cell
NK cell
CAR gene insertion
Gene editing
CAR
CAR αβ T cell
CAR γδ T cell
CAR iNKT cell
CAR NK cell
'Off-the-shelf' CAR Cell Product
Storage
Treatment of multiple patients

ALLO-501 (CD19 αβ CAR)

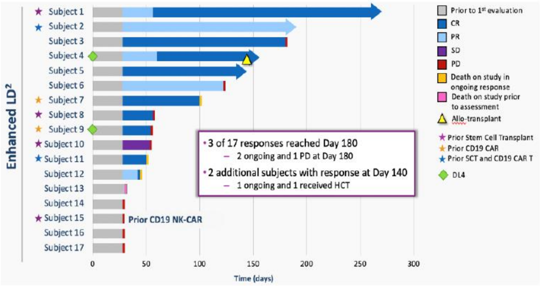
ORR/CR rate % = 48/28



Lekakis et al. *ASH* 2021, Abstract 649

PBCAR0191 (CD19 αβ CAR)

ORR/CR rate % = 69/56

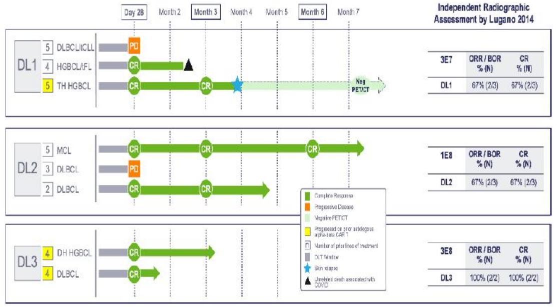


- 3 of 17 responses reached Day 180
  - 2 ongoing and 1 PD at Day 180
- 2 additional subjects with response at Day 140
  - 1 ongoing and 1 received HCT

Shah et al. *ASH* 2021, Abstract 302

ADI-001 (CD20 γδ CAR)

ORR/CR rate % = 75/75



Independent Radiographic Assessment by Lugano 2014

3E7	ORR / BOR % (N)	CR % (N)
DL1	67% (2/3)	67% (2/3)

1E8	ORR / BOR % (N)	CR % (N)
DL2	67% (2/3)	67% (2/3)

3E8	ORR / BOR % (N)	CR % (N)
DL3	100% (2/2)	100% (2/2)

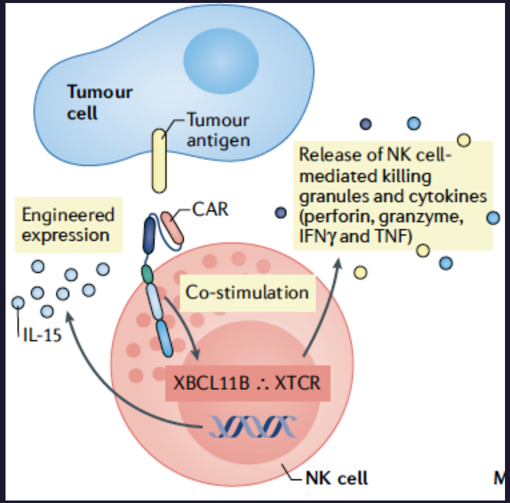
Neelapu et al. *ASCO* 2022, Abstract 7509

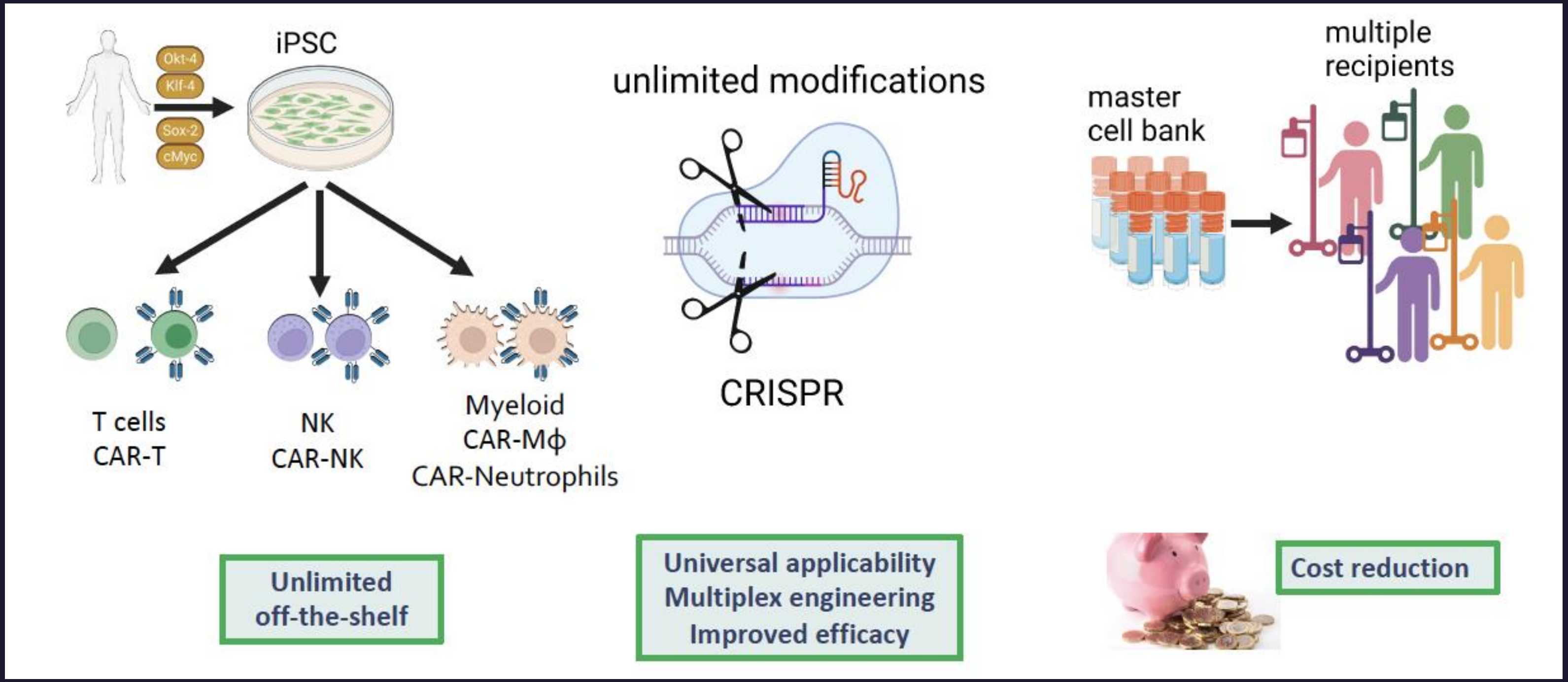
- No GvHD, Grade ≥3 NE or CRS in any of the trials
- Higher rate of grade ≥3 infections with enhanced LD

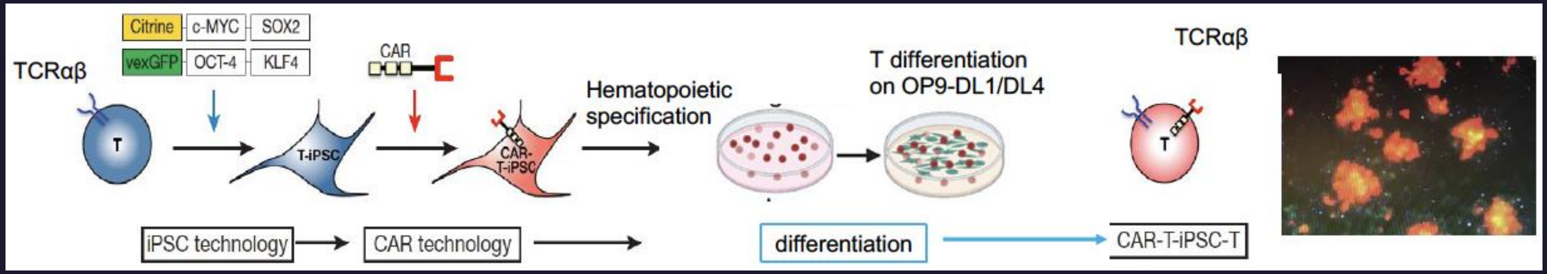
Alternative sources of CAR-T

iPSC

NK-CAR-T


Tumour cell
Tumour antigen
CAR
Engineered expression
Release of NK cell-mediated killing granules and cytokines (perforin, granzyme, IFNγ and TNF)
Co-stimulation
IL-15
XBCL11B ∴ XTCR
NK cell

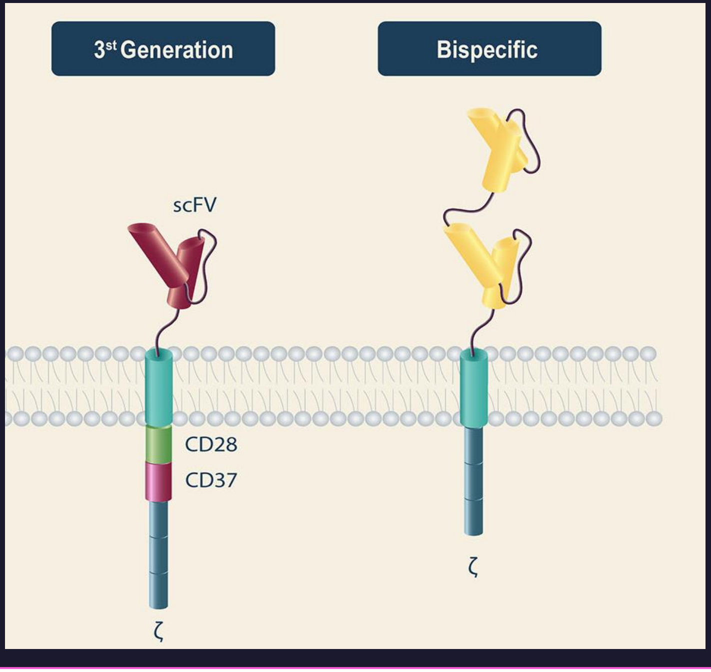

iPSC
Okt-4
Klf-4
Sox-2
cMyc
T cells
CAR-T
NK
CAR-NK
Myeloid
CAR-Mϕ
CAR-Neutrophils
unlimited modifications
CRISPR
master cell bank
multiple recipients
Unlimited off-the-shelf
Universal applicability
Multiplex engineering
Improved efficacy
Cost reduction

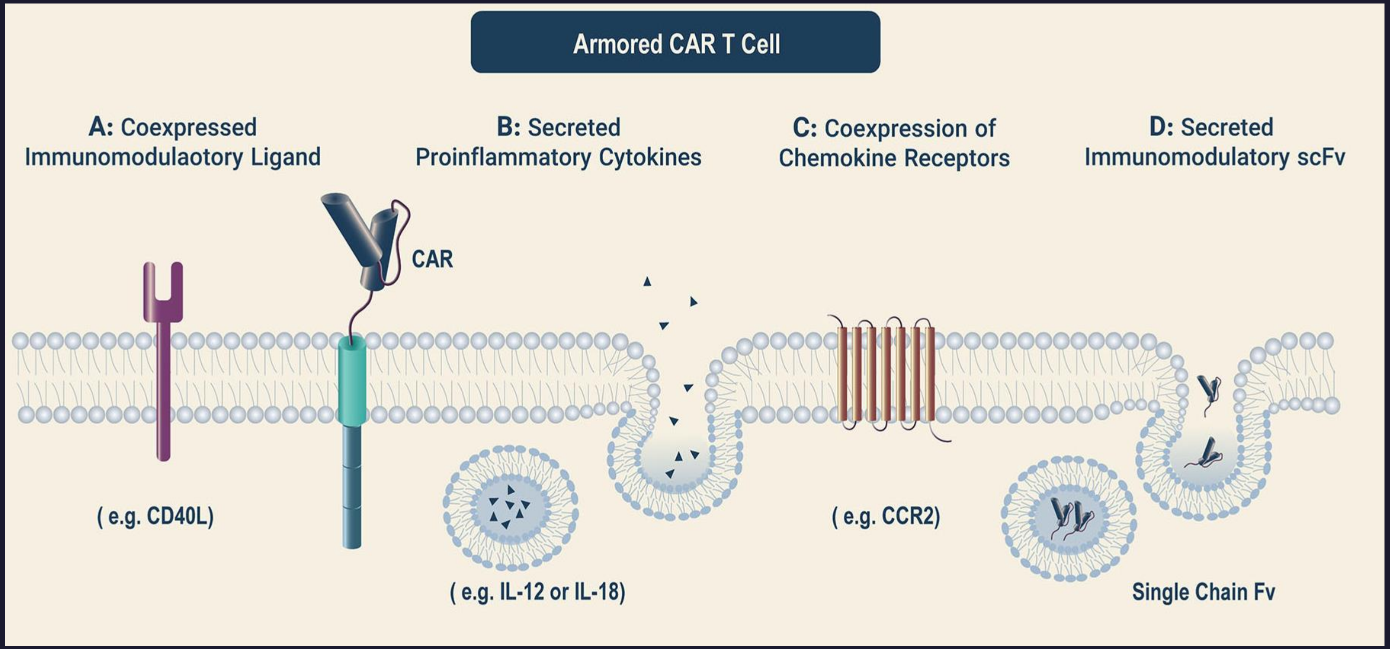

TCRαβ
Citrine
c-MYC
SOX2
vexGFP
OCT-4
KLF4
T
T-iPSC
CAR
CAR-T-iPSC
Hematopoietic specification
T differentiation on OP9-DL1/DL4
TCRαβ
T
iPSC technology
CAR technology
differentiation
CAR-T-iPSC-T

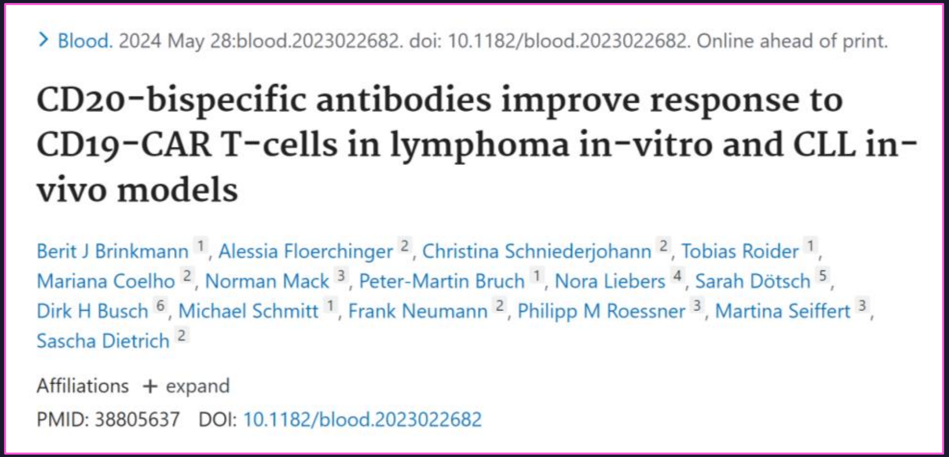
Larson et al., Nature Review 2021

CAR-T construct

Marofi et al., Frontiers in Imm. 2021


3rd Generation
Bispecific
scFV
CD28
CD37
ζ
ζ


Armored CAR T Cell
A: Coexpressed Immunomodulaotory Ligand
B: Secreted Proinflammatory Cytokines
C: Coexpression of Chemokine Receptors
D: Secreted Immunomodulatory scFv
CAR
(e.g. CD40L)
(e.g. IL-12 or IL-18)
(e.g. CCR2)
Single Chain Fv



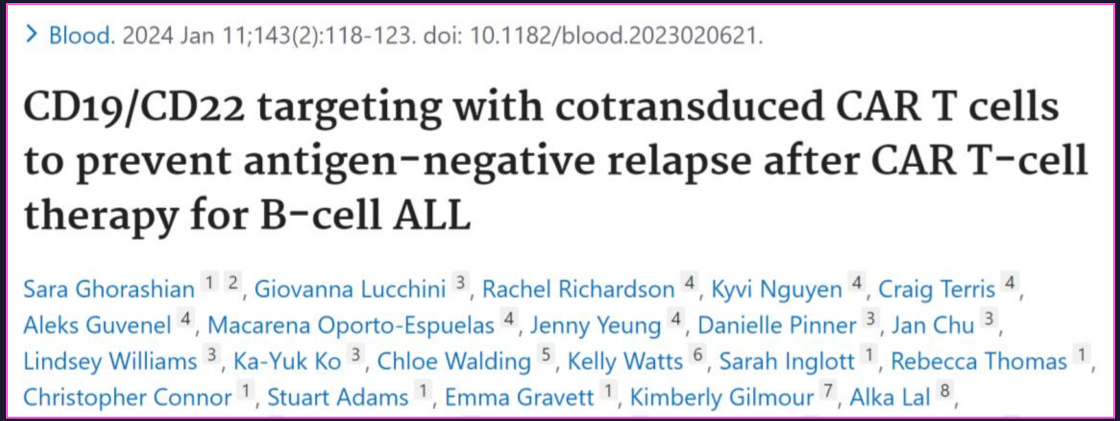
› Blood. 2024 May 28:blood.2023022682. doi: 10.1182/blood.2023022682. Online ahead of print.

CD20-bispecific antibodies improve response to CD19-CAR T-cells in lymphoma in-vitro and CLL in-vivo models

Berit J Brinkmann [1], Alessia Floerchinger [2], Christina Schniederjohann [2], Tobias Roider [1], Mariana Coelho [2], Norman Mack [3], Peter-Martin Bruch [1], Nora Liebers [4], Sarah Dötsch [5], Dirk H Busch [6], Michael Schmitt [1], Frank Neumann [2], Philipp M Roessner [3], Martina Seiffert [3], Sascha Dietrich [2]

Affiliations + expand

PMID: 38805637 DOI: 10.1182/blood.2023022682

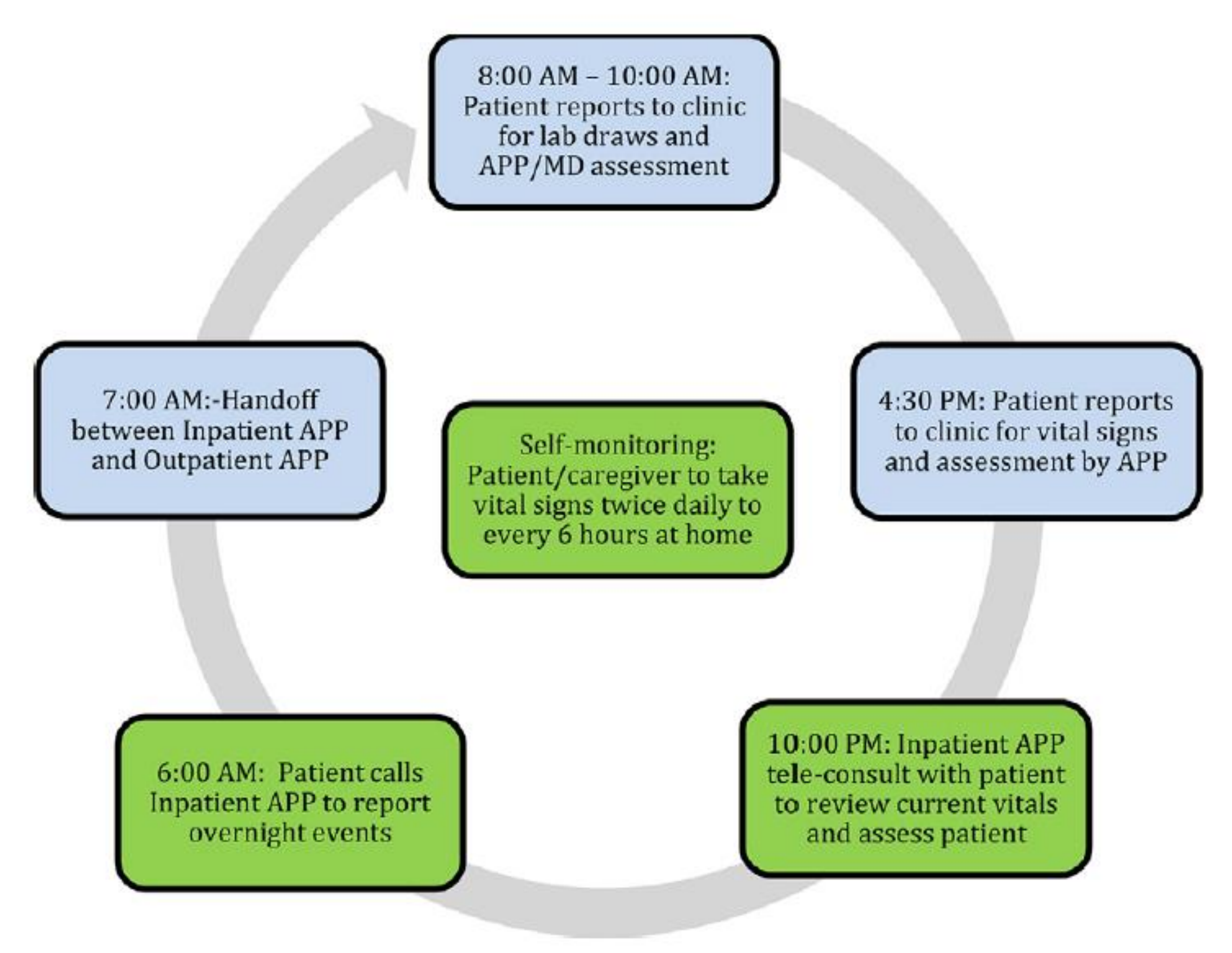


› Blood. 2024 Jan 11;143(2):118-123. doi: 10.1182/blood.2023020621.

CD19/CD22 targeting with cotransduced CAR T cells to prevent antigen-negative relapse after CAR T-cell therapy for B-cell ALL

Sara Ghorashian [1] [2], Giovanna Lucchini [3], Rachel Richardson [4], Kyvi Nguyen [4], Craig Terris [4], Aleks Guvenel [4], Macarena Oporto-Espuelas [4], Jenny Yeung [4], Danielle Pinner [3], Jan Chu [3], Lindsey Williams [3], Ka-Yuk Ko [3], Chloe Walding [5], Kelly Watts [6], Sarah Inglott [1], Rebecca Thomas [1], Christopher Connor [1], Stuart Adams [1], Emma Gravett [1], Kimberly Gilmour [7], Alka Lal [8],

Outpatient CAR-T therapy



8:00 AM – 10:00 AM: Patient reports to clinic for lab draws and APP/MD assessment

4:30 PM: Patient reports to clinic for vital signs and assessment by APP

10:00 PM: Inpatient APP tele-consult with patient to review current vitals and assess patient

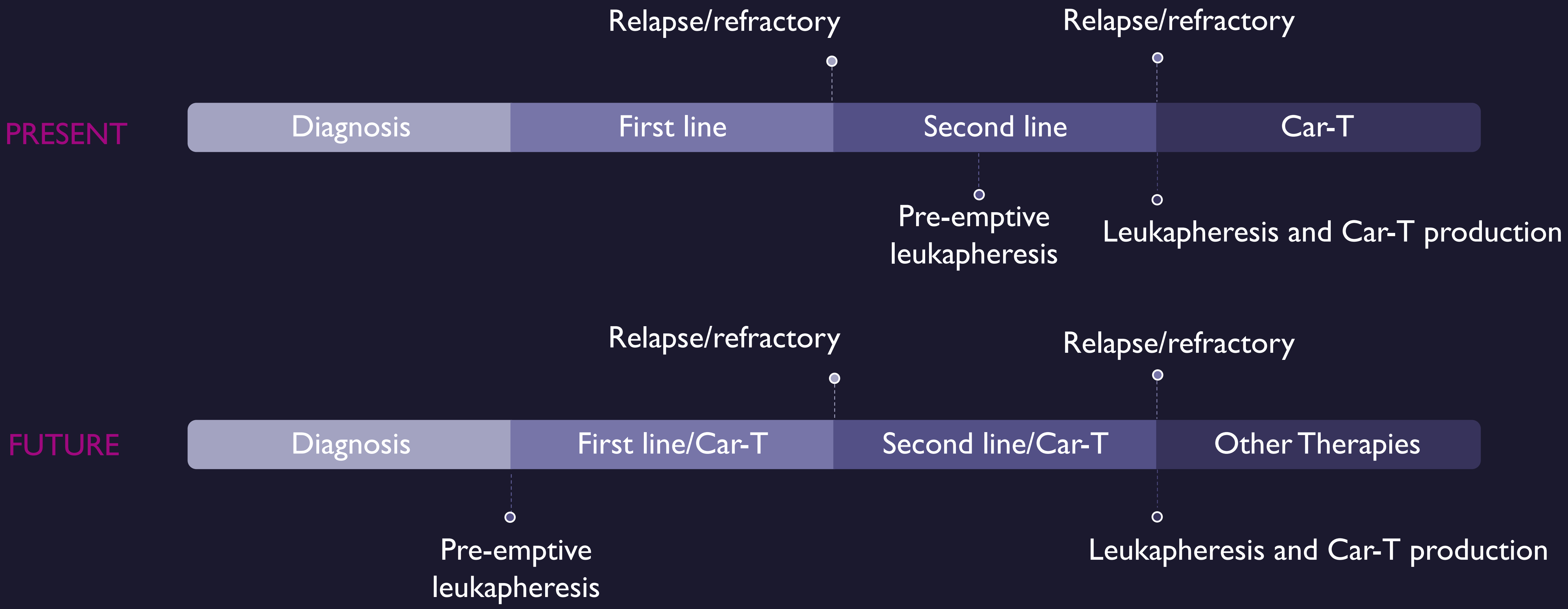
6:00 AM: Patient calls Inpatient APP to report overnight events

7:00 AM:-Handoff between Inpatient APP and Outpatient APP

Self-monitoring: Patient/caregiver to take vital signs twice daily to every 6 hours at home

McGann et al, Transp Cell Ther. 2022;
Alexander et al., Transplant Cell Ther. 2021

CAR-T earlier in the treatment plan



PRESENT

Diagnosis | First line | Second line | Car-T

Relapse/refractory

Relapse/refractory

Pre-emptive leukapheresis

Leukapheresis and Car-T production

FUTURE

Diagnosis | First line/Car-T | Second line/Car-T | Other Therapies

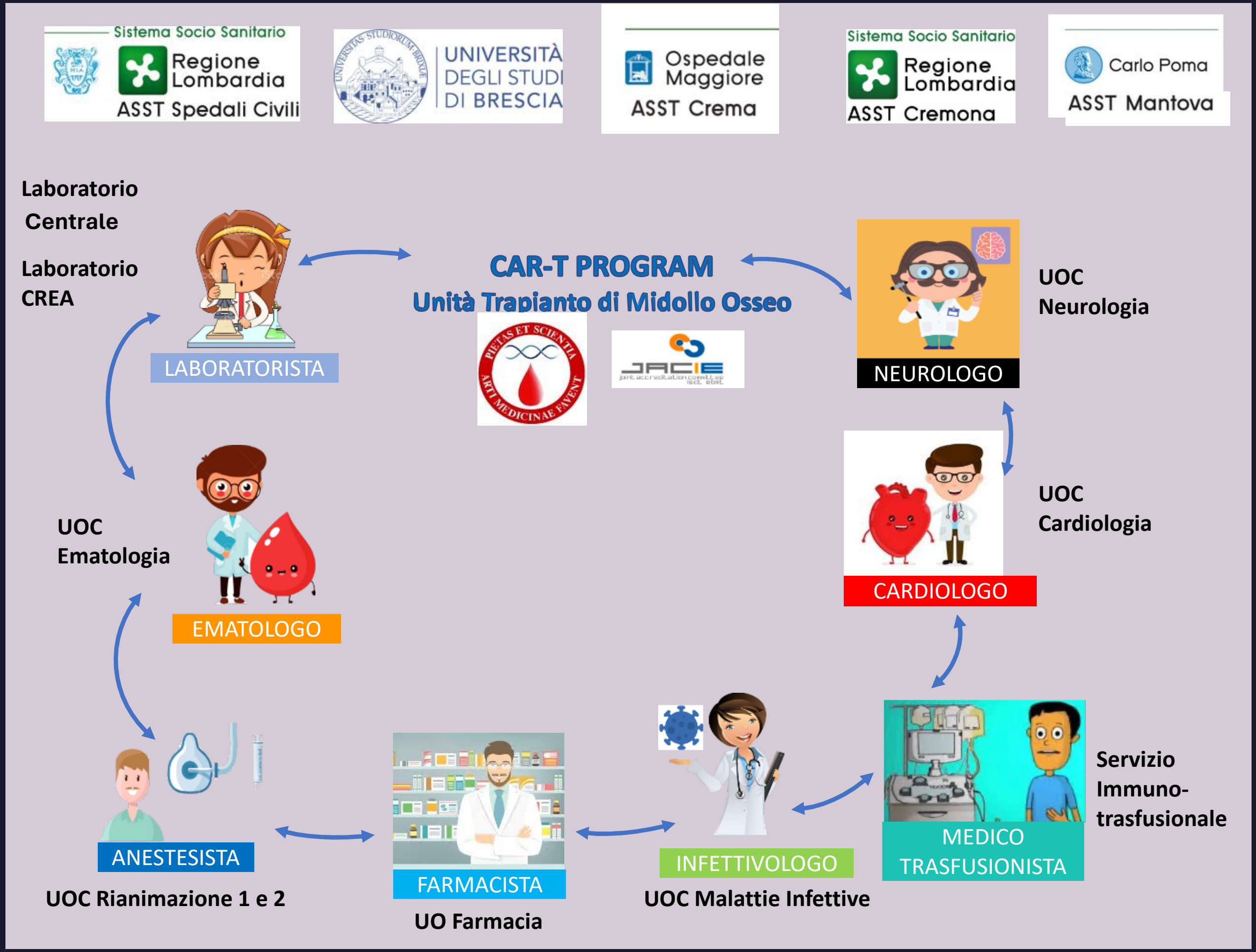
Relapse/refractory

Relapse/refractory

Pre-emptive leukapheresis

Leukapheresis and Car-T production

Car-T administration at ASST Spedali Civili


Sistema Socio Sanitario
Regione Lombardia
ASST Spedali Civili
UNIVERSITÀ DEGLI STUDI DI BRESCIA
Ospedale Maggiore
ASST Crema
Sistema Socio Sanitario
Regione Lombardia
ASST Cremona
Carlo Poma
ASST Mantova
PIETAS ET SCIENTIA
ARTI MEDICINAE FAVENT
JACIE

CAR-T PROGRAM

Unità Trapianto di Midollo Osseo

Laboratorio Centrale

Laboratorio CREA

LABORATORISTA

UOC Neurologia

NEUROLOGO

UOC Cardiologia

CARDIOLOGO

UOC Ematologia

EMATOLOGO

Servizio Immuno-trasfusionale

MEDICO TRASFUSIONISTA

INFETTIVOLOGO

UOC Malattie Infettive

FARMACISTA

UO Farmacia

ANESTESISTA

UOC Rianimazione 1 e 2

Summary

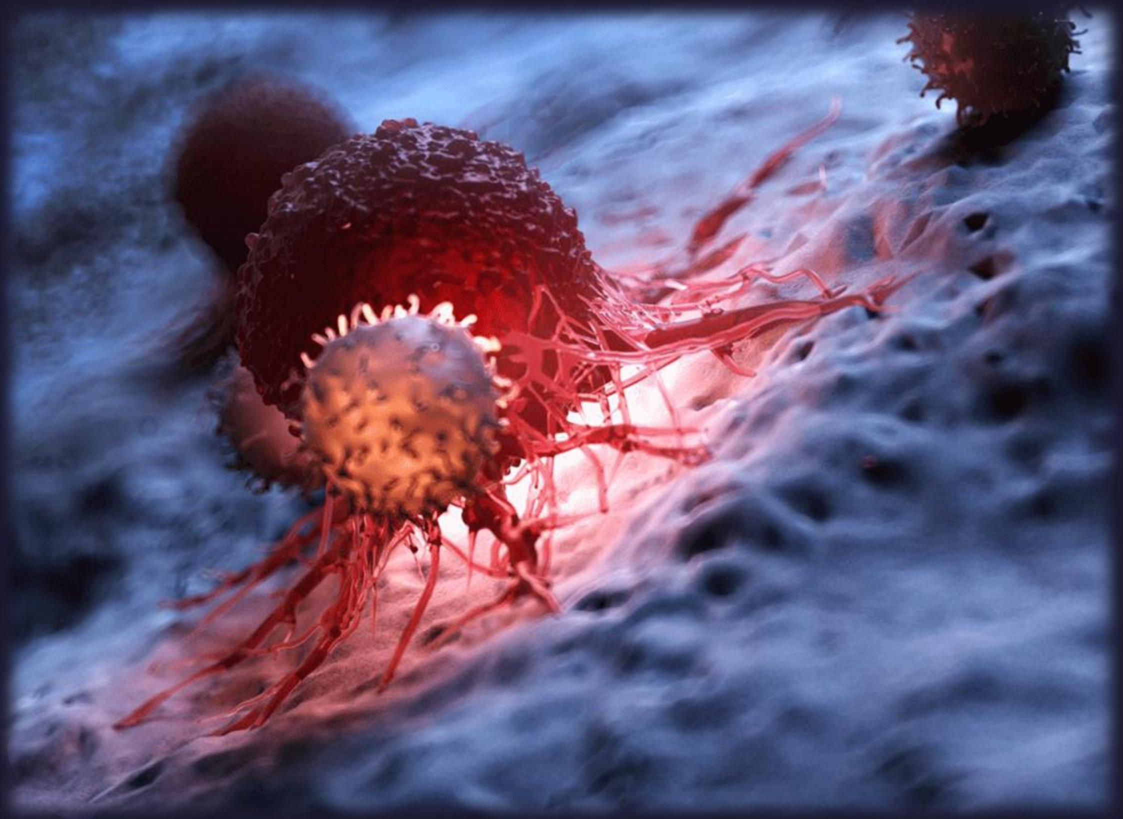
- Anti CD19 CAR-T cells are **highly effective therapy in DLBCL patients with ≥ 3 line of treatment** (FDA; EMA; AIFA approval) both in pivotal trials and real life experience. Axi-cel and Liso-cel have higher efficacy compared with SOC (HD chemo+ASCT) in primary refractory DLBCL patients or in patients who relapse within 12 months of 1st line of treatment. CAR-T cells are the only available therapy for some lymphomas.

- **Patients, disease and CAR-T cells characteristics may influence CAR-T cell efficacy.**

- **The toxicity** of Car-T is an important aspect that must be considered and discussed in a multidisciplinary team.

- **The future** of CAR-T therapy (… and lymphomas patients) is **bright**: new CAR-T constructs, CAR-T from alternative source, outpatient administration, use of CAR-T earlier in treatment plan.

Thank You for your attention!



simona.bernardi@unibs.it