STUDI BIOLOGICI ED EVIDENZE SPERIMENTALI SULLE CAR-T

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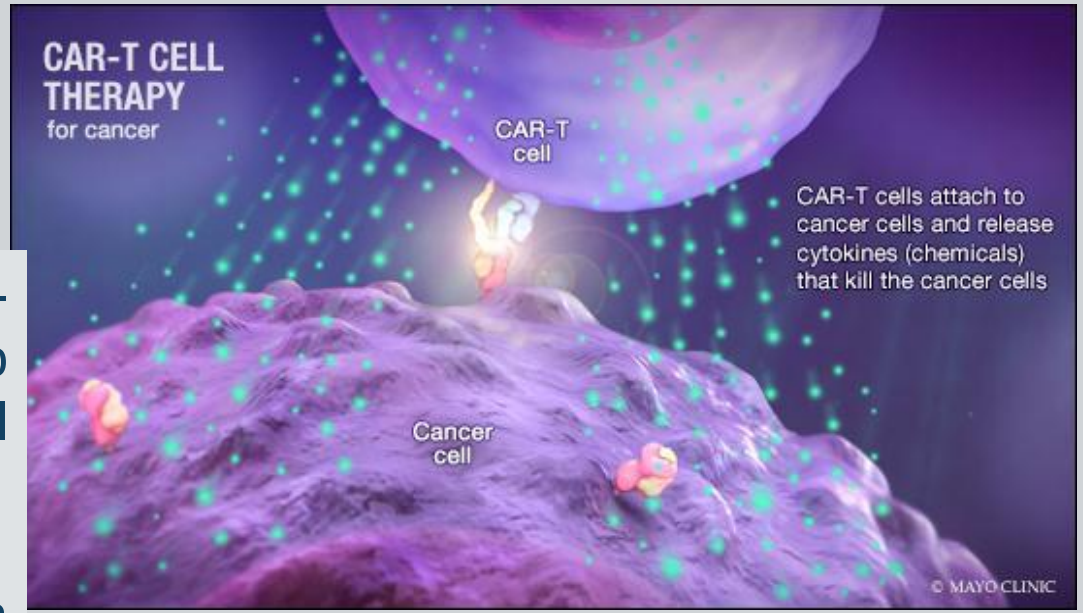
AGENDA

1. Cosa sono le CAR-T e come vengono prodotte
2. Efficacia e tossicità delle CAR-T
3. Gli studi biologici relativi
4. Le prospettive future

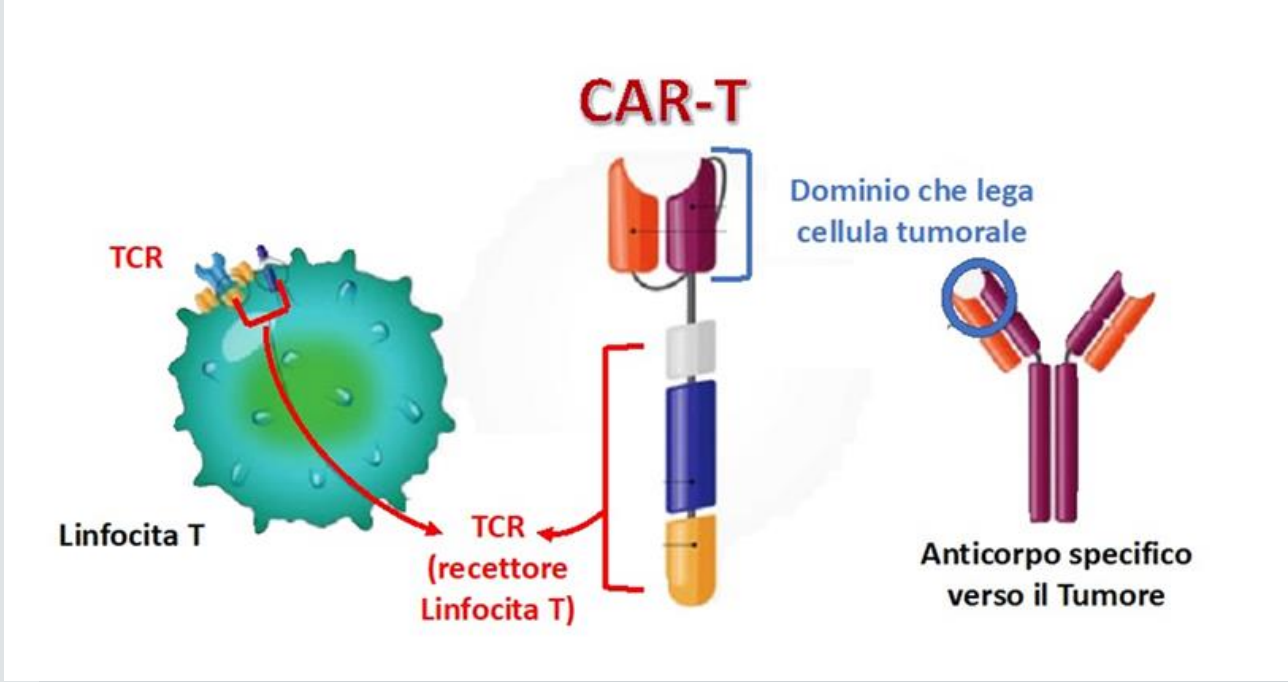
INTRODUZIONE

- L'eterogeneità clonale e sub-clonale intra-tumorale e il microambiente immunosoppressivo contribuiscono alle difficoltà di trattamento del tumore.
- L'immuno-oncologia è una strategia basata sull'utilizzo di cellule per trattare le metastasi e «rompere» la tolleranza immunologica verso il tumore.
- L'ingegneria basata su cellule T e l'immunità «sintetica» vengono applicate per ottenere remissioni durevoli nei pz con tumori refrattari al trattamento.



CAR-T: cosa sono?

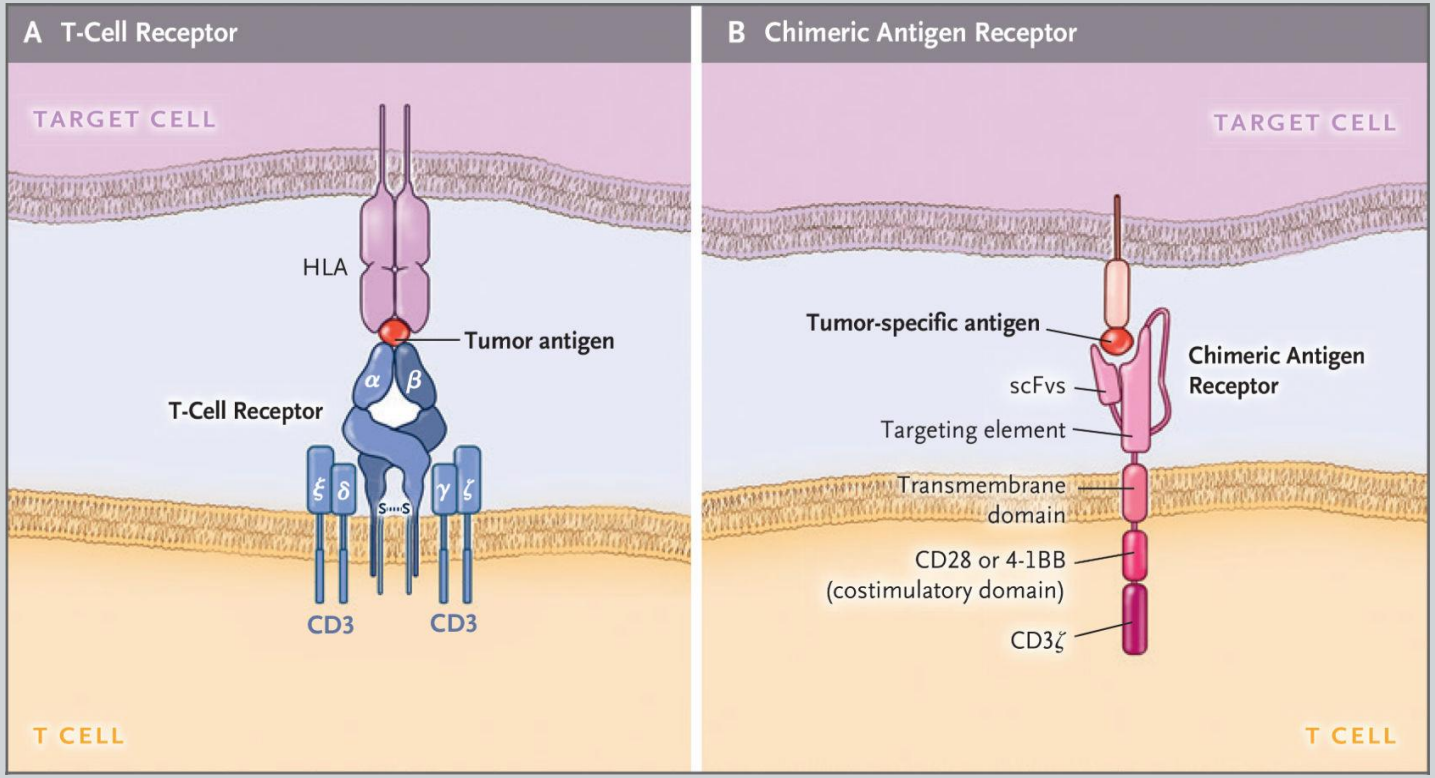
- LE CELLULE CAR-T SONO UNA TERAPIA INNOVATIVA PER IL TRATTAMENTO DELLE PATOLOGIE EMATOLOGICHE.
- LA TERAPIA SI BASA SULL'UTILIZZO DI LINFOCITI CHE VENGONO ISOLATI, INGEGNERIZZATI AL FINE DI ESPRIMERE IL CAR, E REINFUSI NEL PAZIENTE.
- LO SCOPO FINALE E' QUELLO DI RENDERE LE CELLULE CAR-T COMPETENTI CONTRO LA POPOLAZIONE CELLULARE MALIGNA



CAR-T: struttura

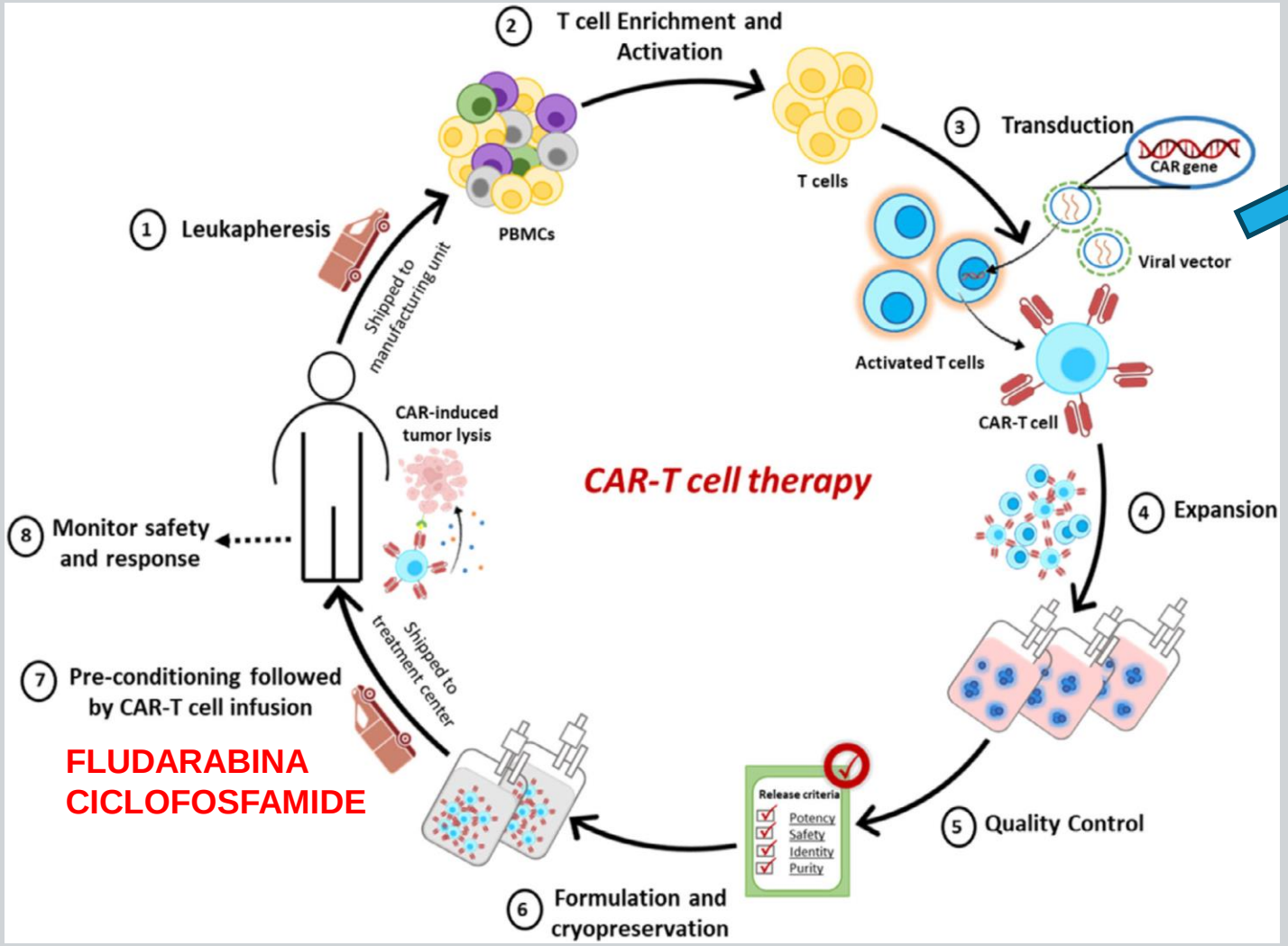
I CARs sono costituiti da:

- Catene variabili leggere e pesanti, derivate dalle Immunoglobuline, dirette contro gli antigeni tumorali.
- Un dominio transmembrana.
- Molecole segnale intracellulare che comprendono la catena zeta del complesso CD3 del TCR.
- Molecole co-stimolatorie, come CD28 o 4-1BB, nel dominio intracellulare che aumentano l'espansione e la potenza delle cellule T ingegnerizzate.



June CH, Sadelain M. N Engl J Med 2018;379:64-73

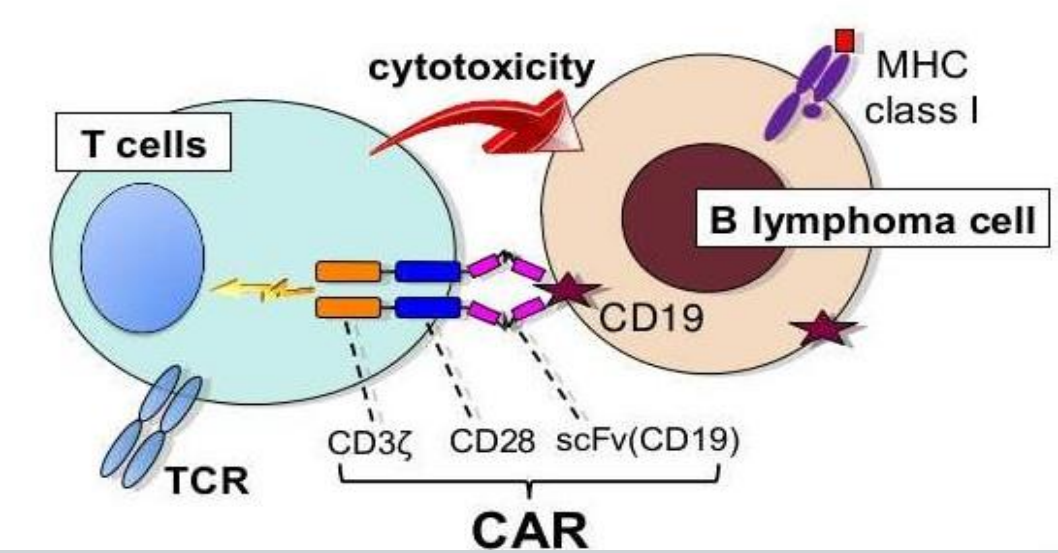
IL PROCESSO DI PRODUZIONE DI CAR-T

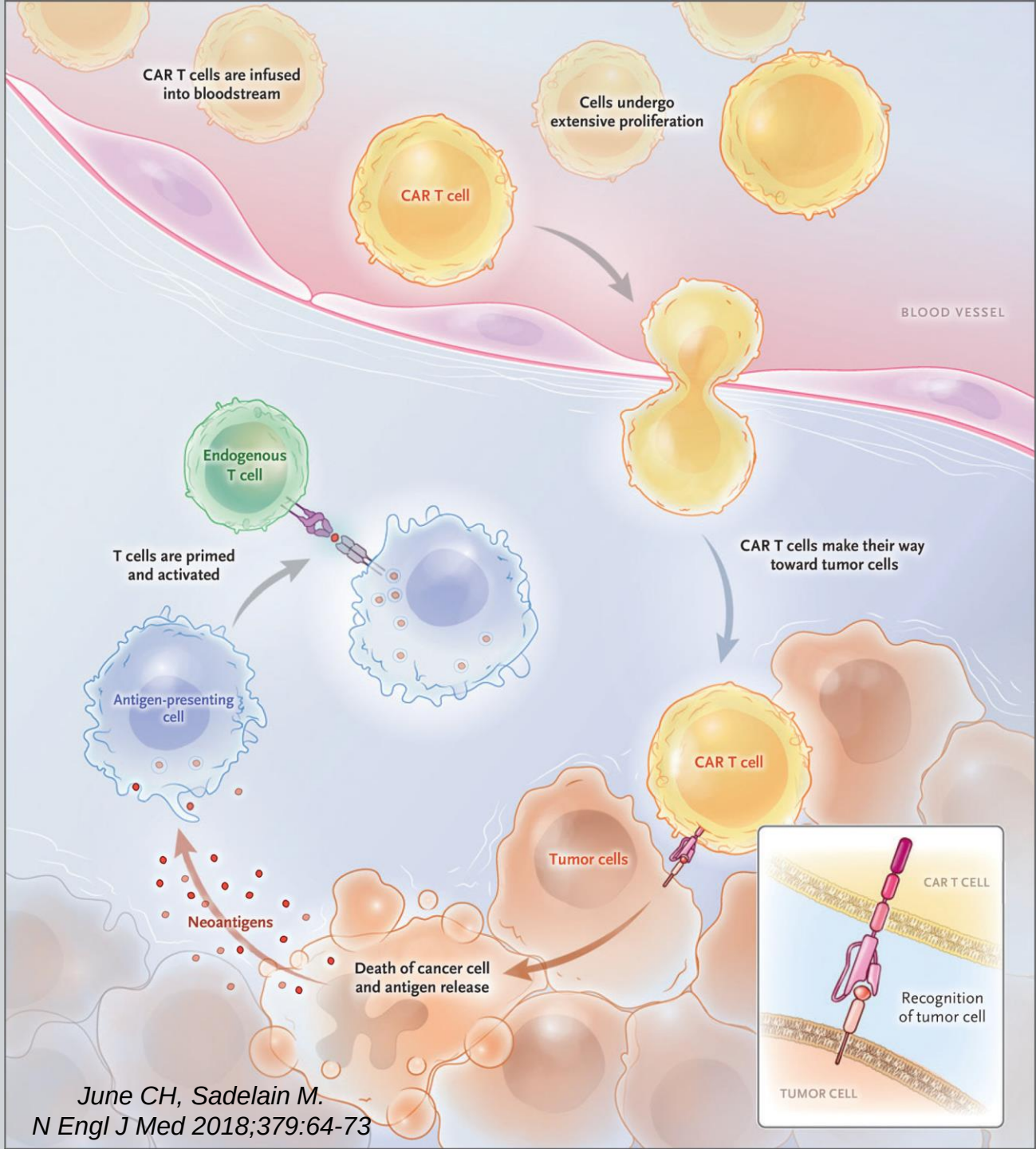


1) **VETTORI RETROVIRALI**: CELLULE ATTIVAMENTE IN DIVISIONE PER L'INTEGRAZIONE
2) **VETTORI LENTIVIRALI**: CELLULE NON IN DIVISIONE O CHE PROLIFERANO LENTAMENTE PER L'INTEGRAZIONE
3) **TRASPOSONI**

CAR-T: come funzionano?

Attecchimento, capacità di targettare il tumore, proliferazione estensiva dopo infusione.





June CH, Sadelain M.
N Engl J Med 2018;379:64-73

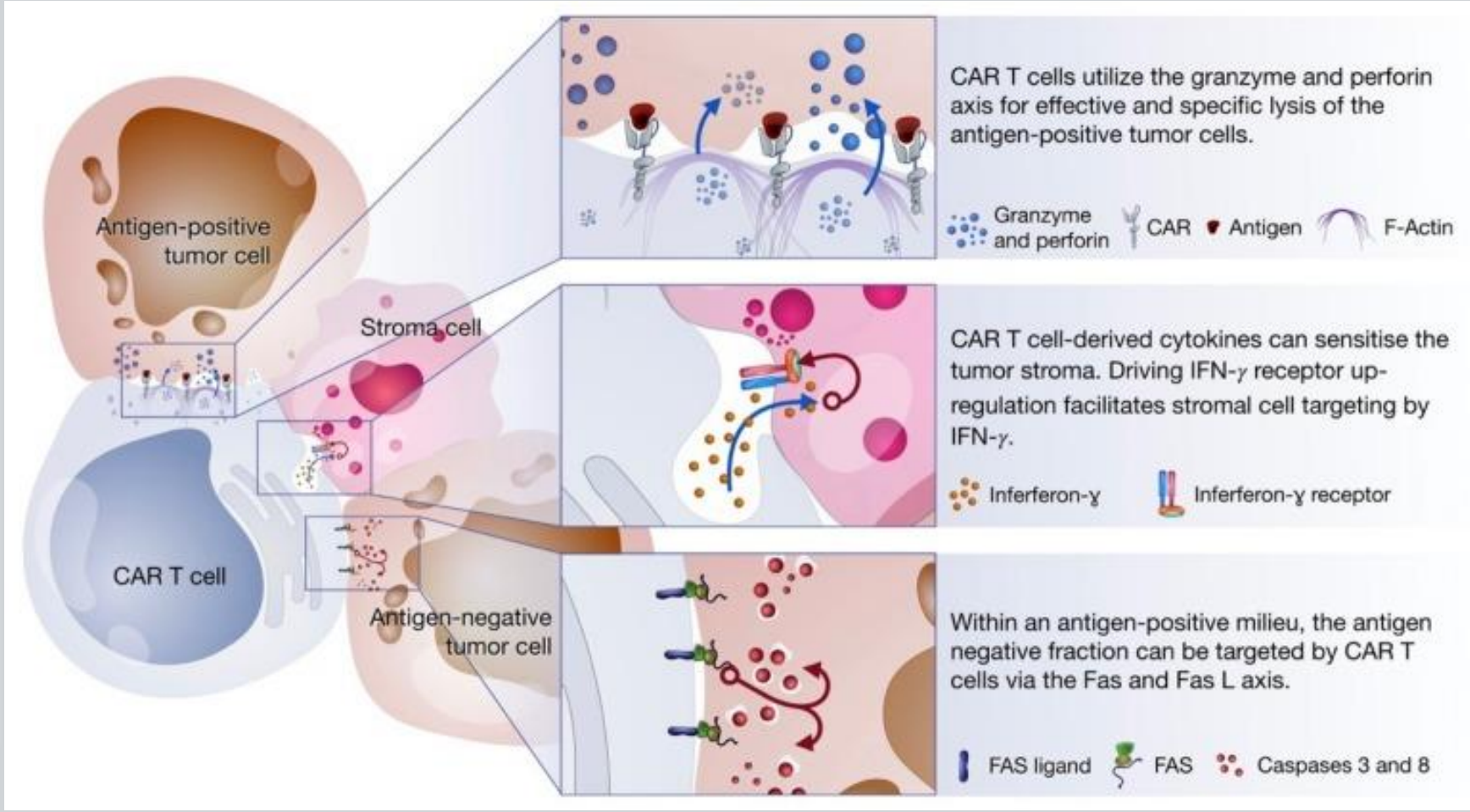
CAR-T: come funzionano?

Le CAR-T mediano la morte tumorale mediante tre meccanismi:

(1) perforina e granzimi: targetta la frazione antigene-positiva del tumore

(2) secrezione citochine: sensibilizzazione dello stroma tumorale

(3) Fas-FasL: targetta la frazione antigene-negativa del tumore



CAR-T: come funzionano?

- Riconoscono gli antigeni tumorali in maniera HLA- indipendente, non limitata alla presentazione dell'antigene;

- sono insensibili ai meccanismi di escape tumorale dovuti alla perdita dell'MHC;

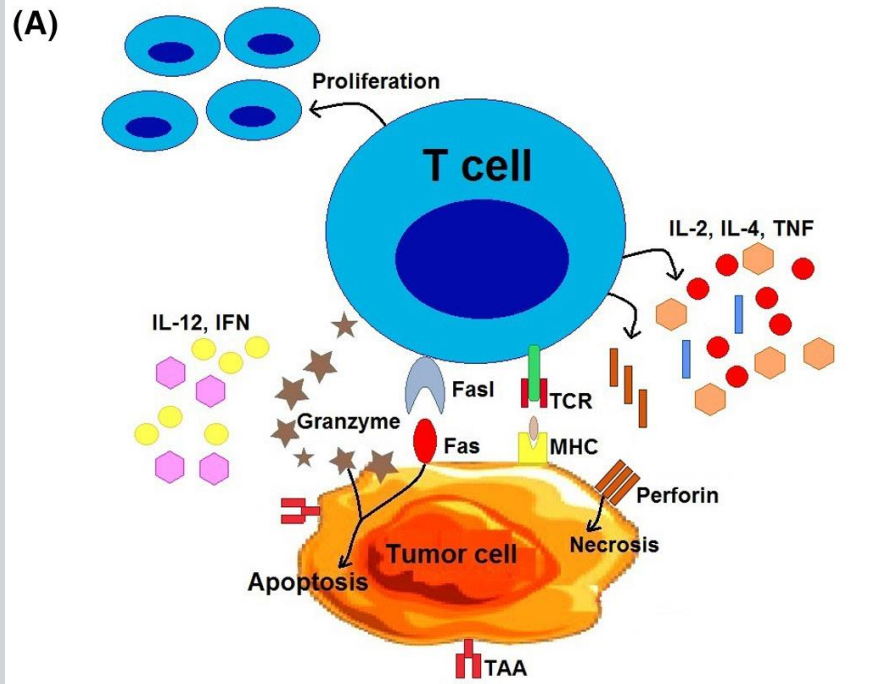
- lavorano in sinergia con la risposta immunitaria endogena;

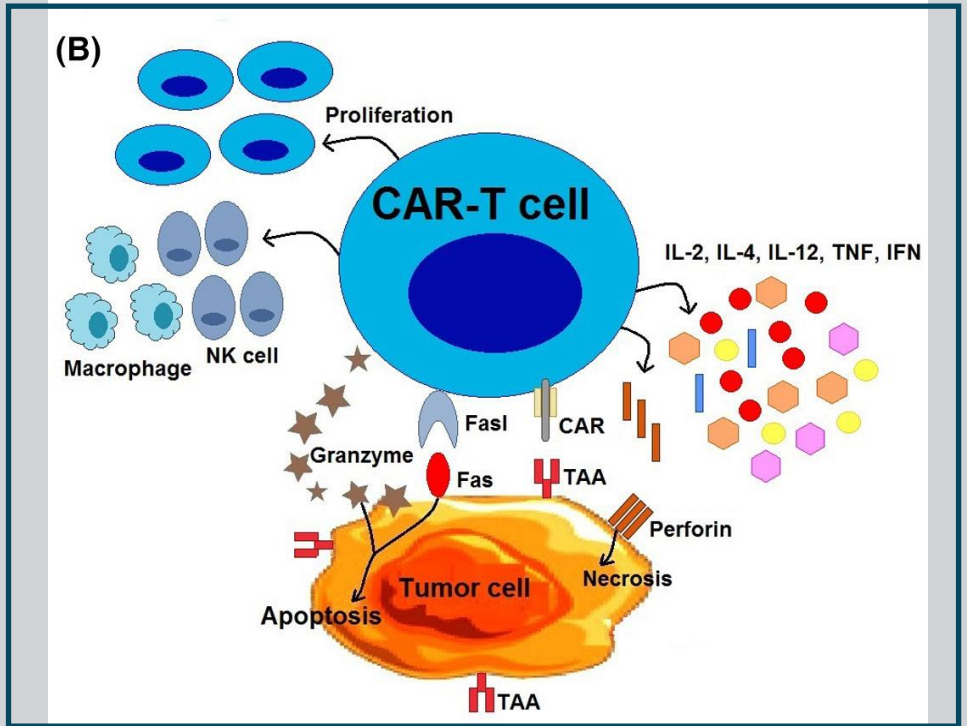
- targettano l'ecosistema tumorale:

1) **Interazione con le cellule della nicchia**: regolano le interazioni tra i cloni tumorali eterogenei e le cellule immunitarie;
2) **Microambiente tumorale immunosoppressivo**: rimodellano il microambiente attraverso il rilascio di fattori solubili che regolano la funzione delle cellule stromali e immunitarie;

- persistono a lungo termine in vivo: effetti antitumorali permanenti.

Abbasi S, et al. Cancer Med. 2023.

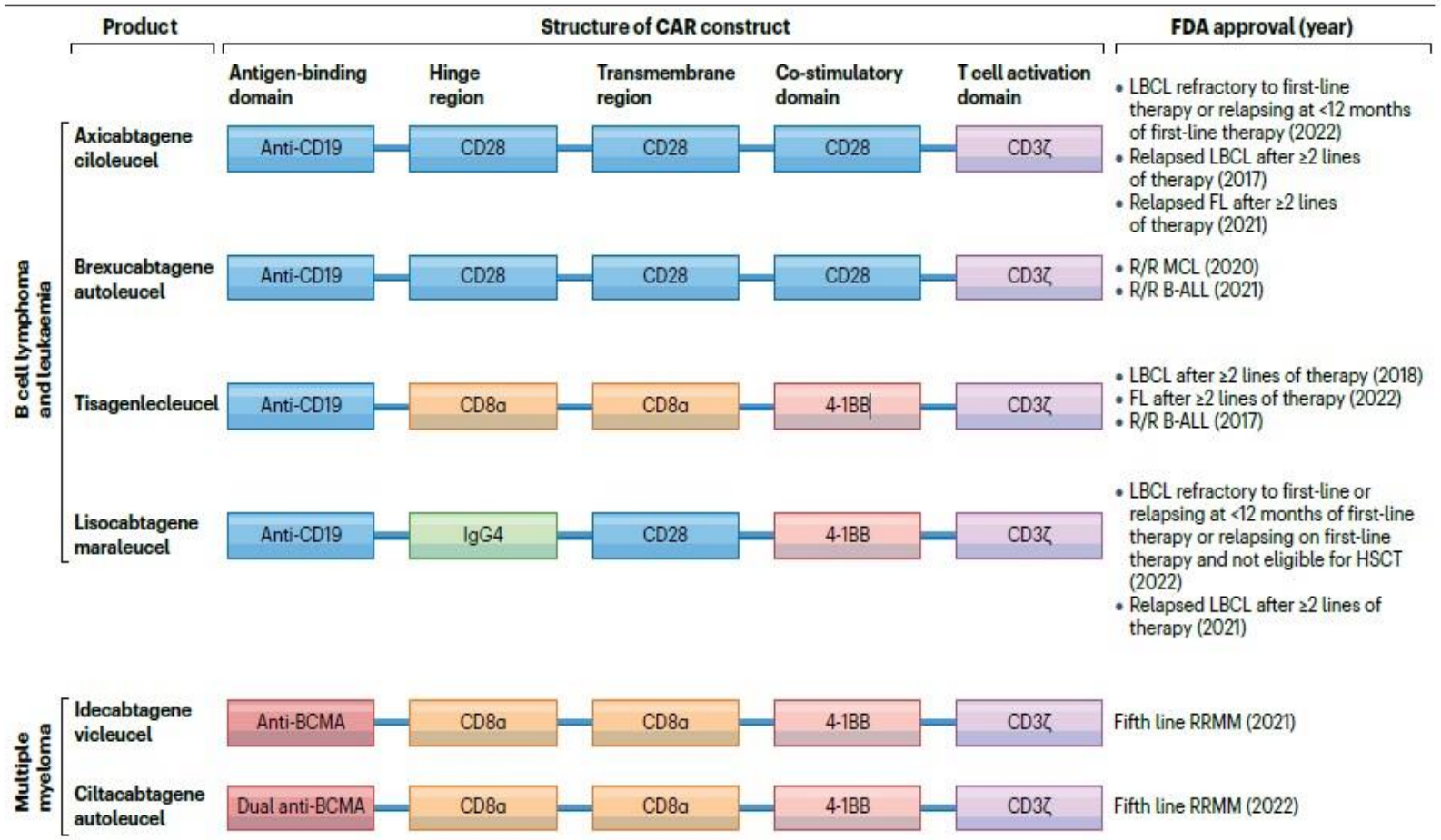




CAR-T: INDICAZIONI TERAPEUTICHE IN EMATOLOGIA

- LEUCEMIA LINFOBLASTICA ACUTA (LLA)
- LEUCEMIA LINFATICA CRONICA
- LEUCEMIA ACUTA MIELODIE (LAM) – non ancora approvato
- LINFOMA DIFFUSO A GRANDI CELLULE B
- LINFOMA MEDIASTINICO PRIMITIVO
- LINFOMA MANTELLARE
- MIELOMA MULTIPLO

CAR-T approvati



4 for for patients with B cell lymphomas

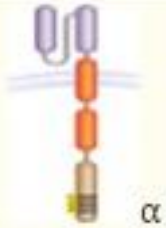
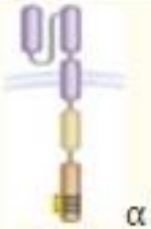
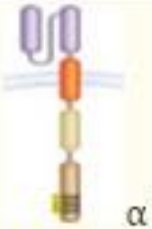
2 for patients with B cell acute lymphoblastic leukaemia (B-ALL)

2 for those with multiple myeloma (MM).

TOTAL: 6 CAR-T APPROVED second-generation CAR construct (anti-CD19, anti-BCMA)

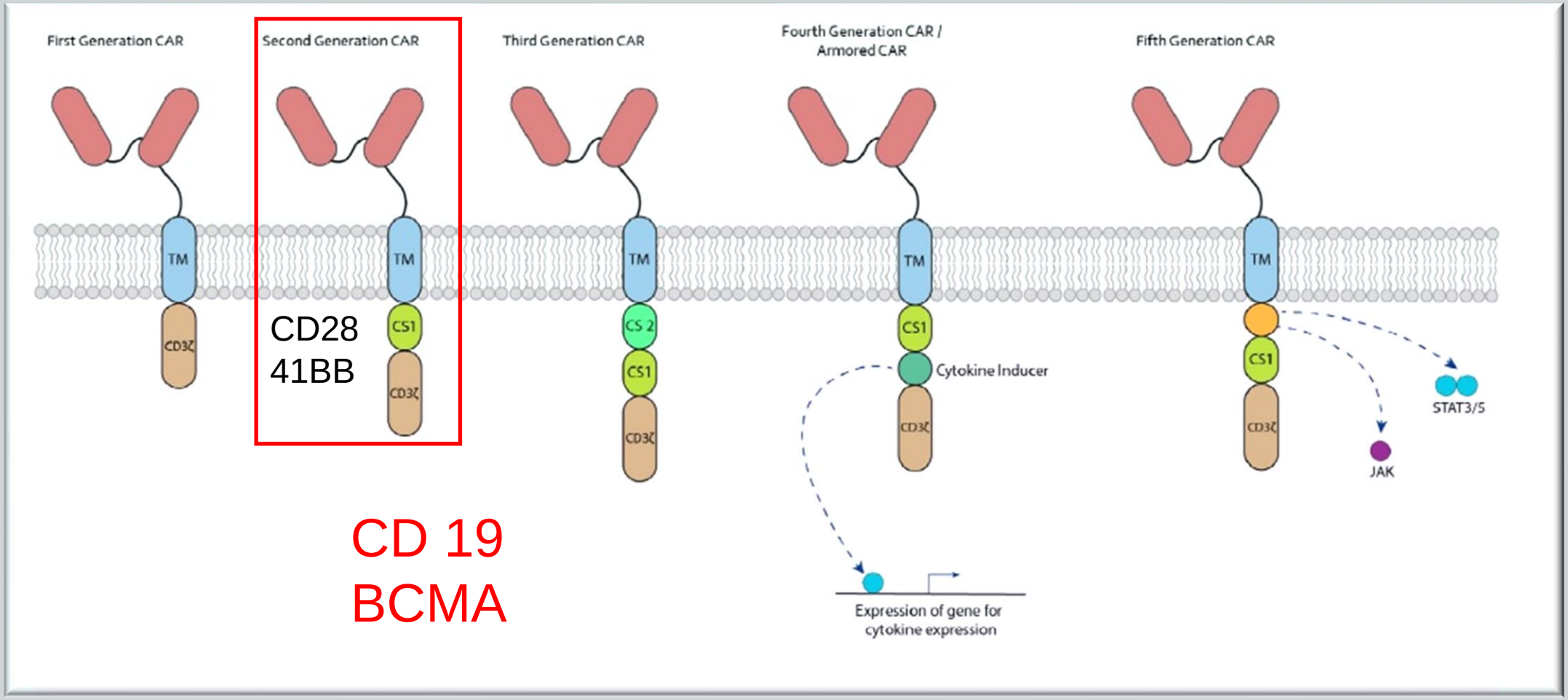
CAR-T approvati in Italia

AXI-CEL TISA-CEL LISO-CEL

	Axicabtagene ciloleucel ZUMA-1	Tisagenlecleucel JULIET	Lisocabtagene maraleucel TRANSCEND
CAR	 α CD19	 α CD19	 α CD19
Transmembrane domain	CD28	CD8	CD28
Co-stimulatory domain	CD28	4-1BB	4-1BB
T-cell activation domain	CD3ζ	CD3ζ	CD3ζ

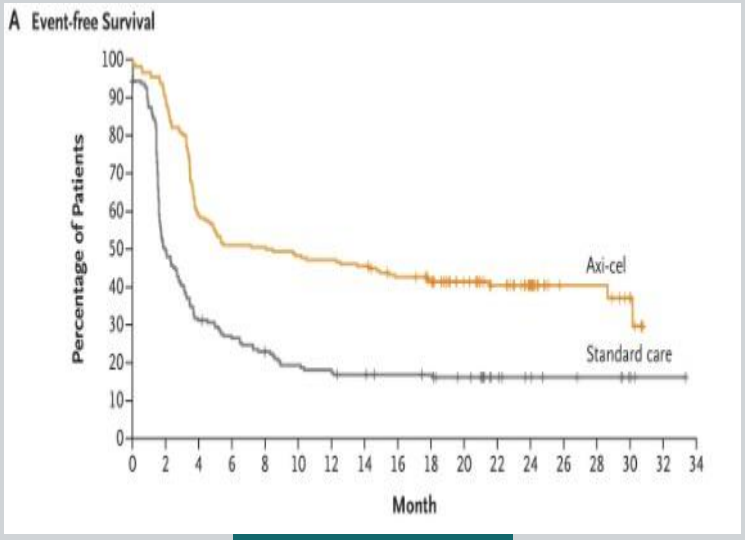
Westin JR et al. Am J Hematol. 2021

DIVERSE GENERAZIONI DI CAR-T



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

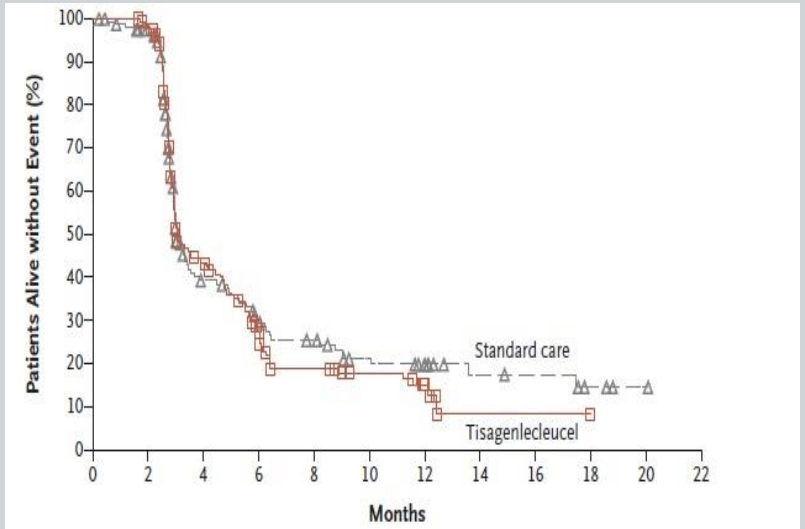
Large B Cell Lymphoma



AXI-CEL

EFS: 41% vs 16%
CR: 65% VS 32%
OS: 61% VS 52%

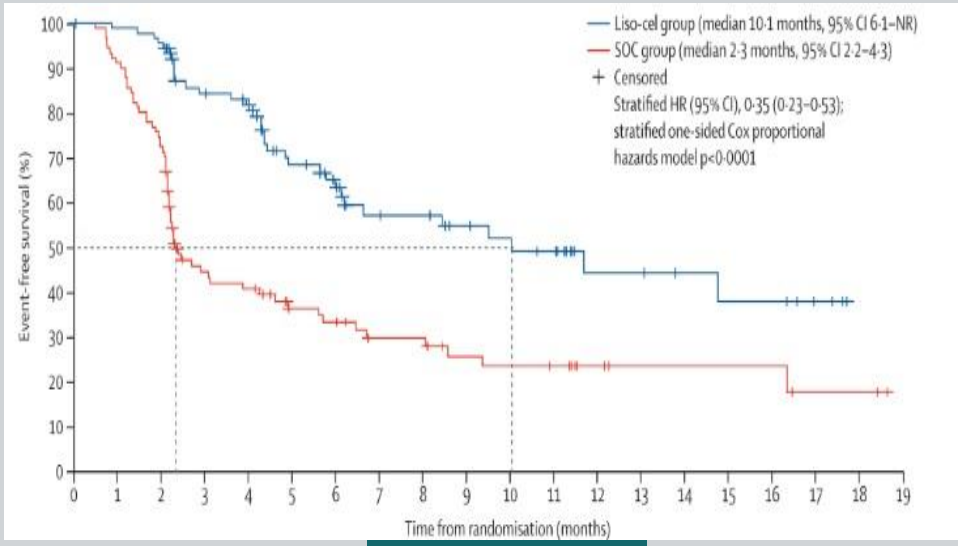
Locke et al., NEJM 2022



TISA-CEL

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

Bishop et al., NEJM 2022



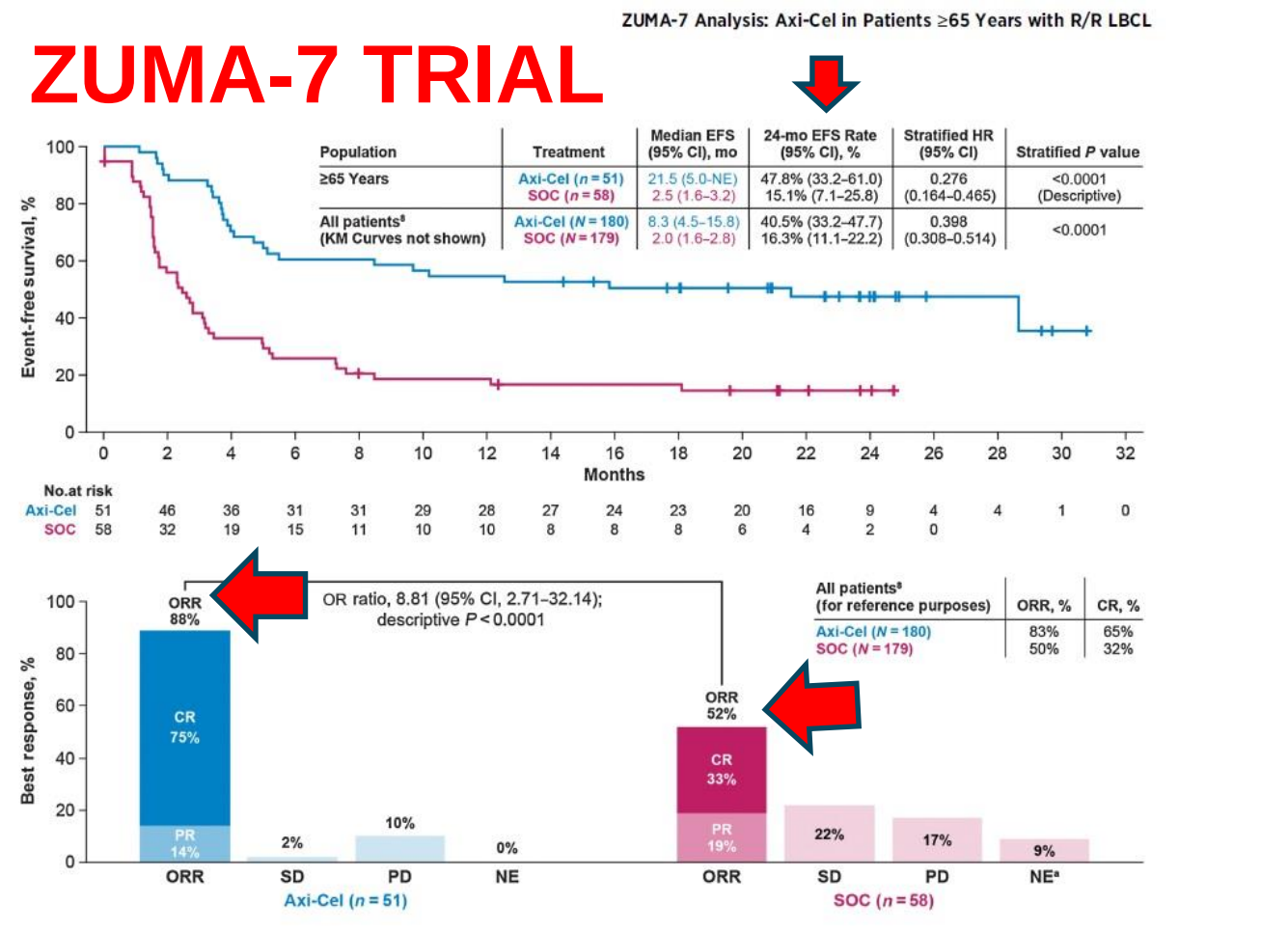
LISO-CEL

EFS: 63% vs 33%
CR: 66% vs 39%
OS: 86% vs 48%

Kamdar et al., Lancet 2022

AXI-CEL vs SOC in Relapsed/Refractory Large B Cell Lymphoma

ZUMA-7 TRIAL

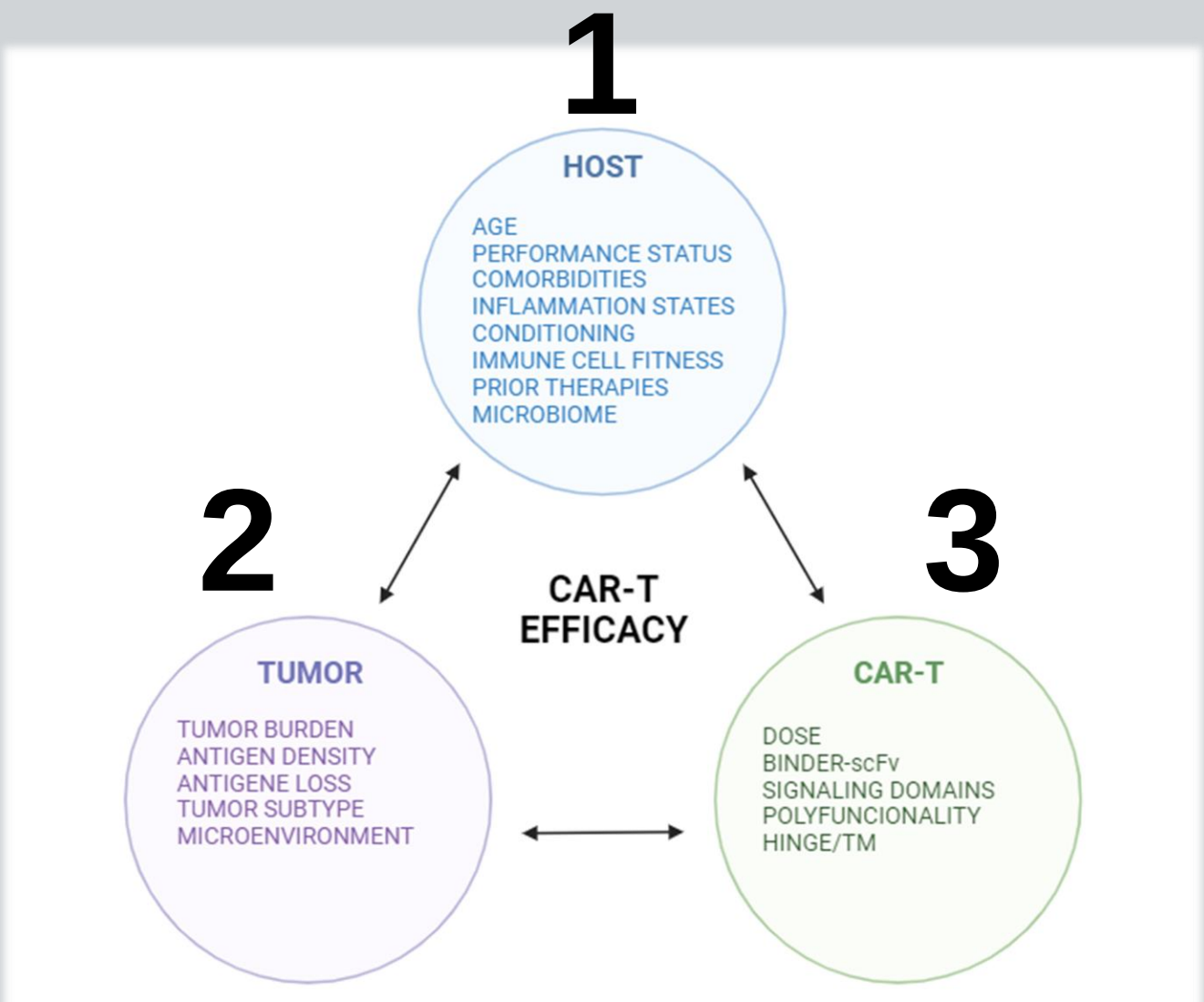


Median FU: 24 months

EFS: 47.8% VS 15.1%(Axi-Cel/SOC)
ORR: 88% VS 52%
OS: 64% VS 51%
PFS: 50% VS 30%

Westin JR et al. Clin Cancer Res. 2023.

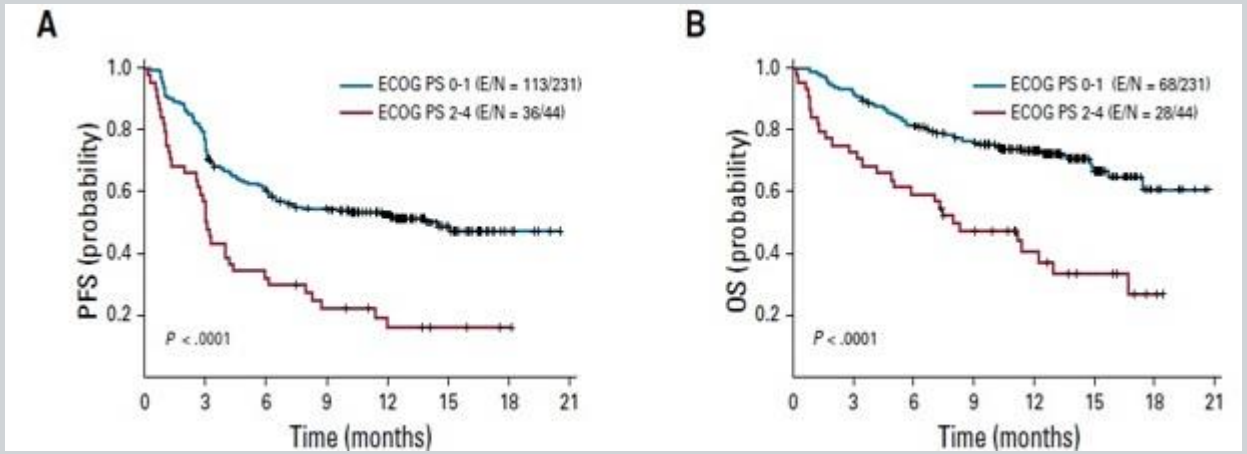
Fattori che influenzano la qualità delle CAR-T



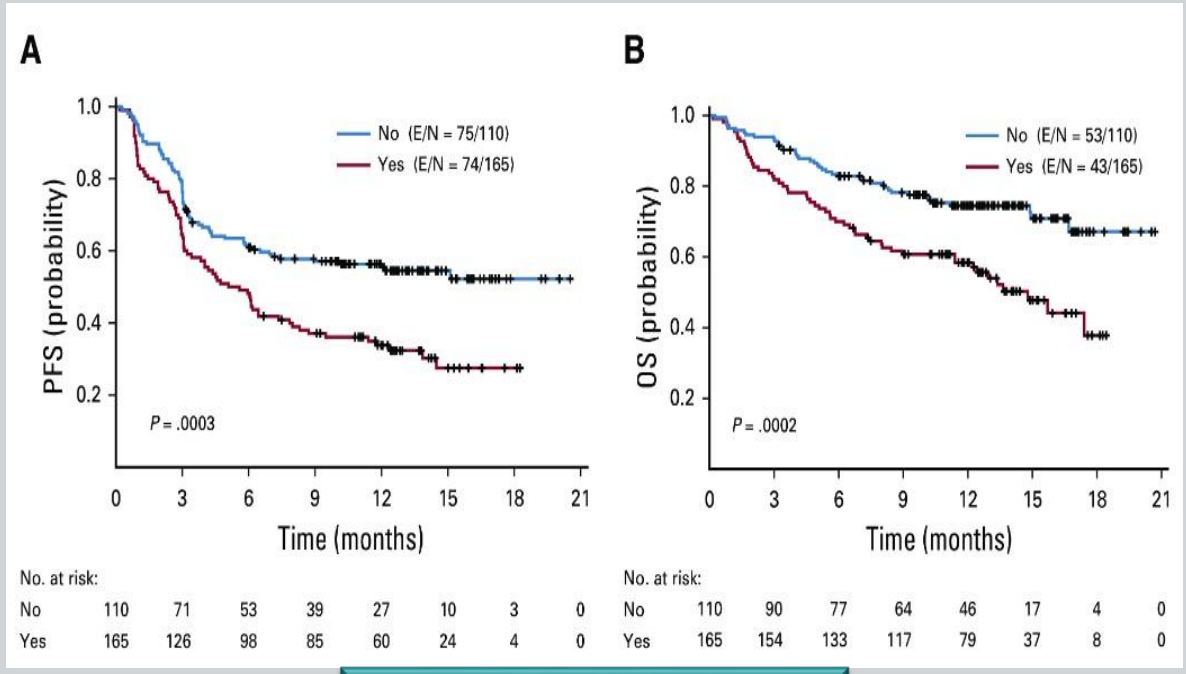
Efficacia delle CAR-T paziente-dipendente

R/R LBCL

AXI-CEL



ECOG PS



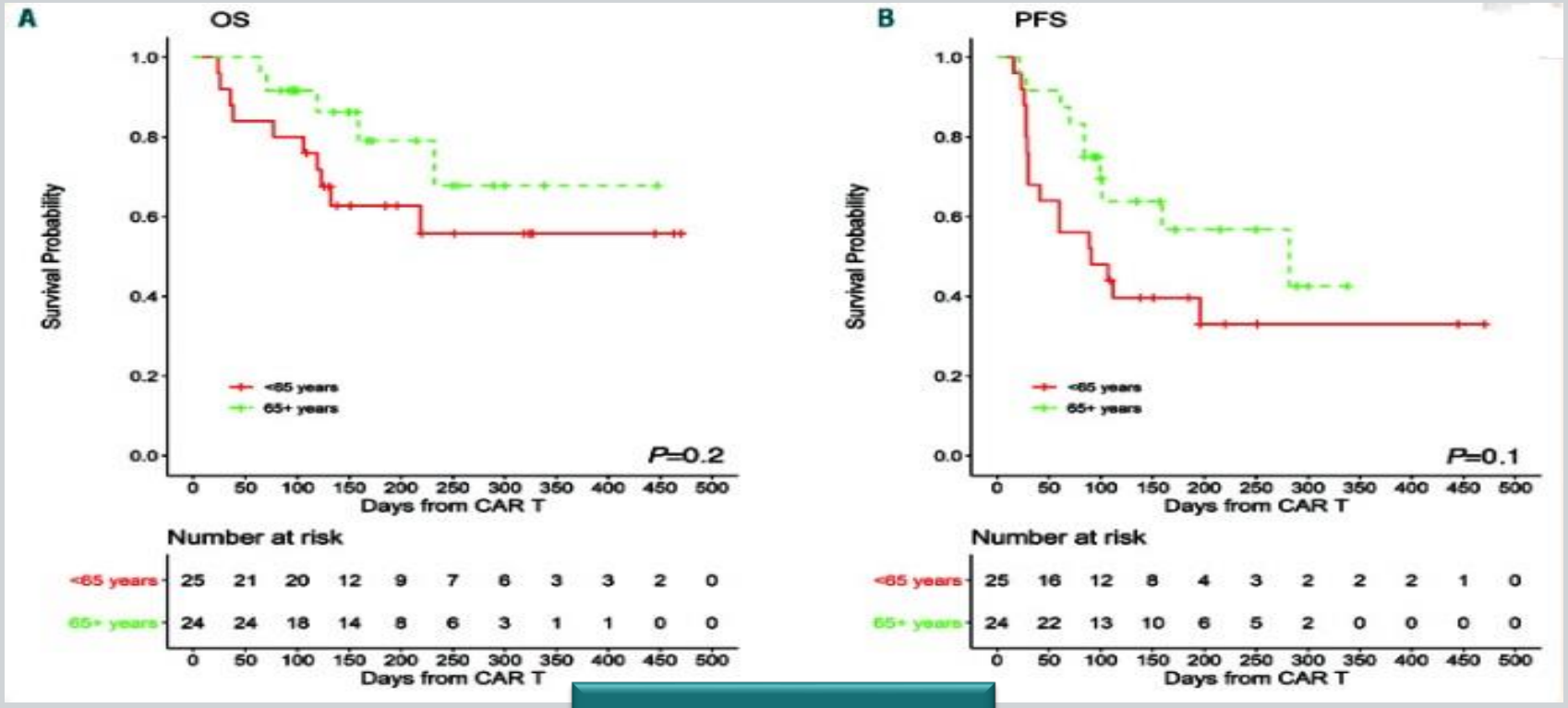
Comorbidities

Nastoupil et al., JCO 2020

Efficacia delle CAR-T paziente-dipendente

R/R LBCL

AXI-CEL
TISA-CEL



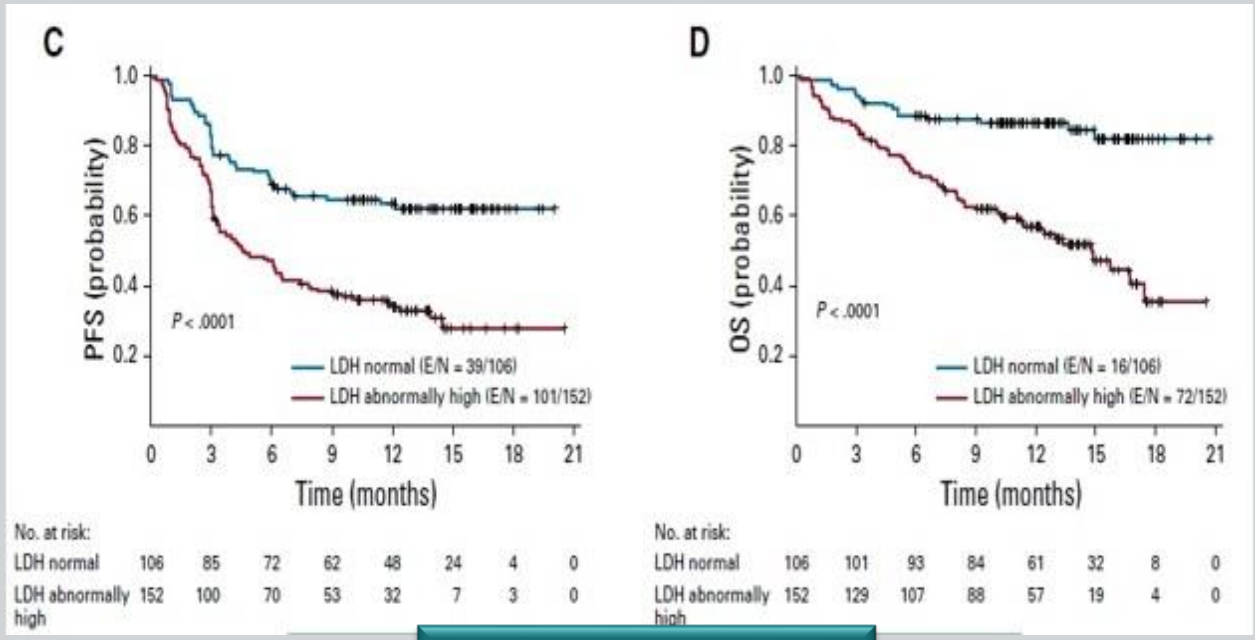
Age

Lin et al., Haematologica 2021

Efficacia delle CAR-T tumore-dipendente

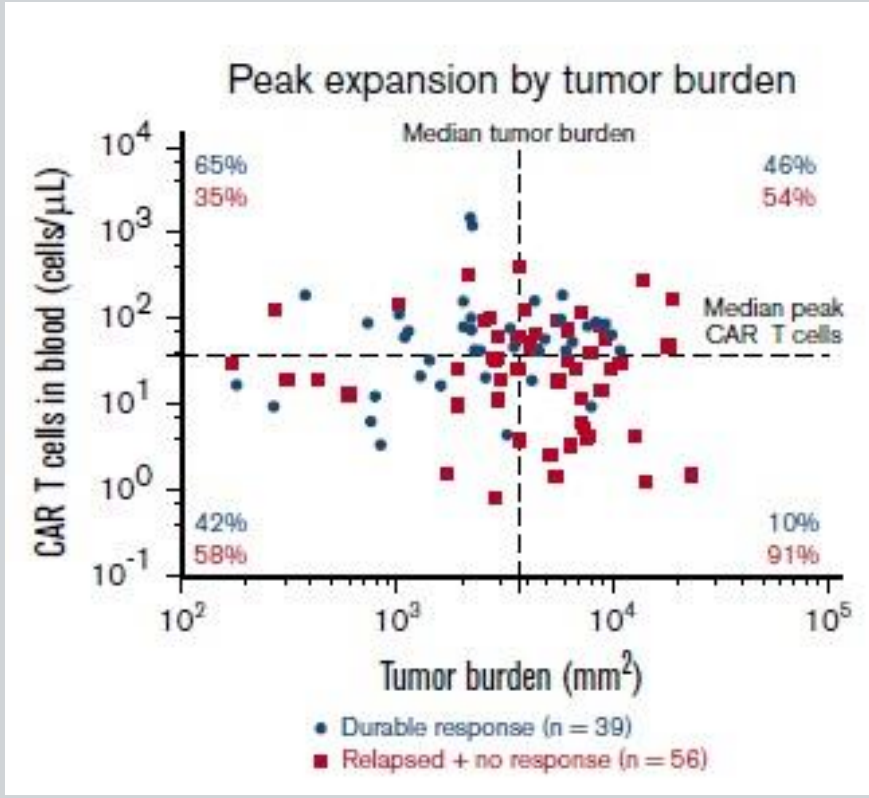
R/R LBCL

AXI-CEL



LDH
ZUMA-1 trial

Nastoupil et al., JCO 2020

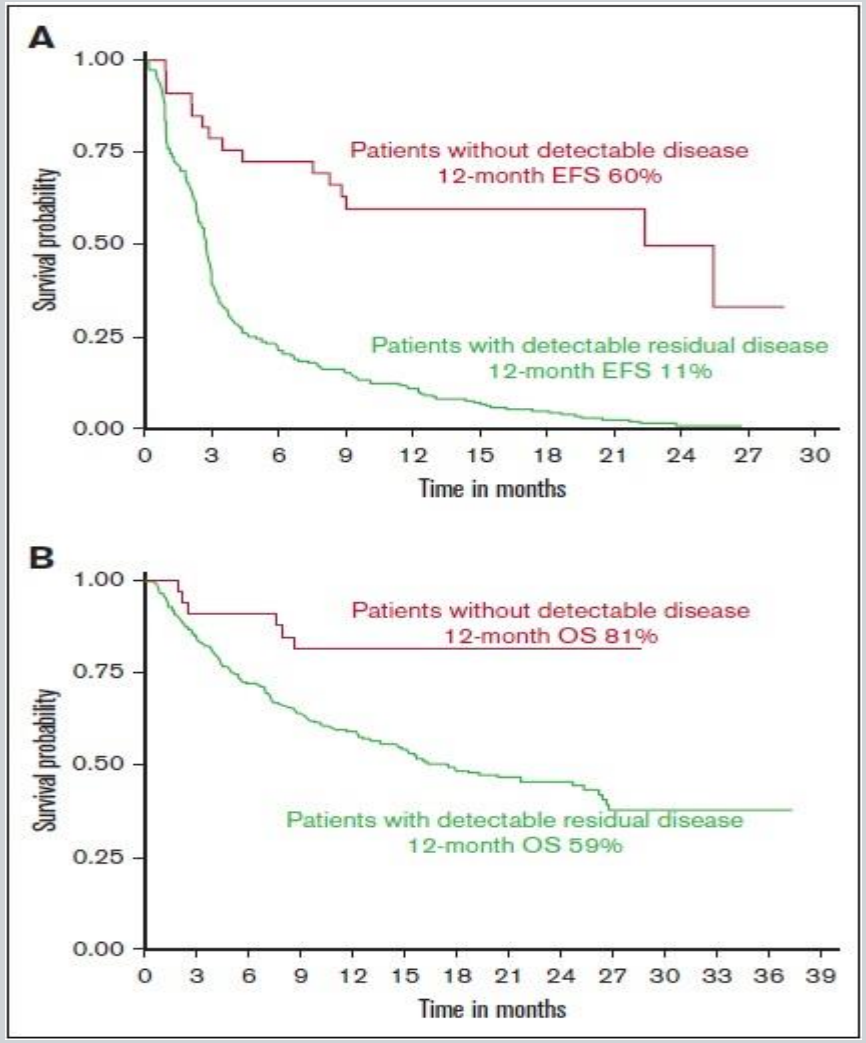


Tumor burden
ZUMA-1 trial

Locke FL et al. Blood Adv. 2020

Efficacia delle CAR-T tumore-dipendente

R/R LBCL



PET neg

FU 24 months
AXI-CEL
LISA-CEL

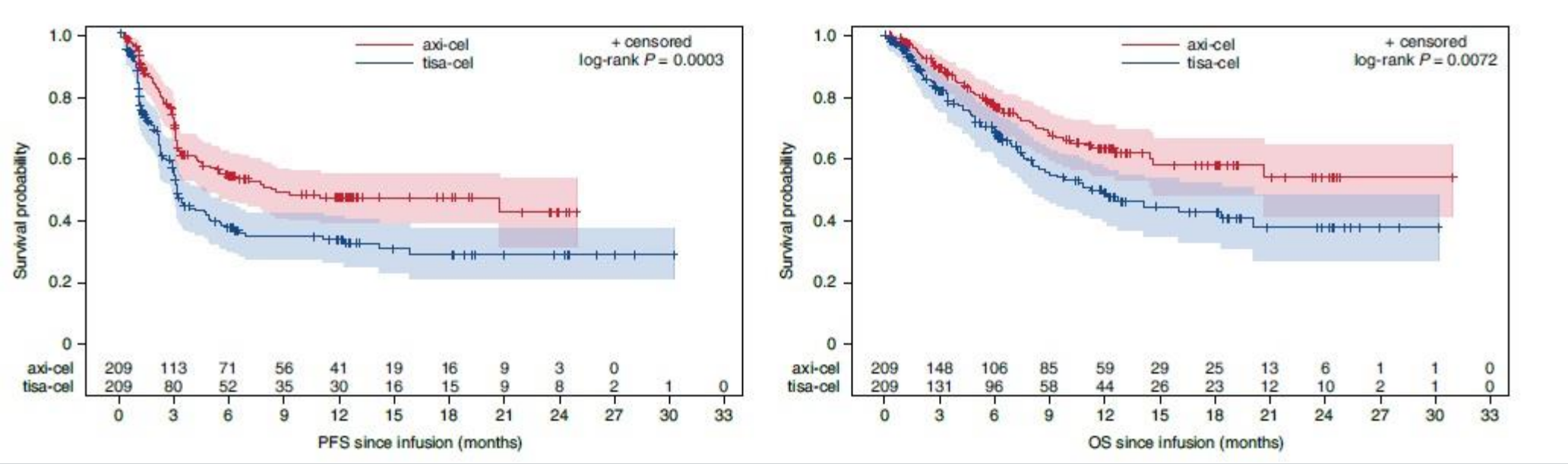
Wudhikarn et al., Blood Adv. 2022

Efficacia delle CAR-T tumore-dipendente

R/R LBCL

Real World data
AXI-CEL
TISA-CEL
DLBCL

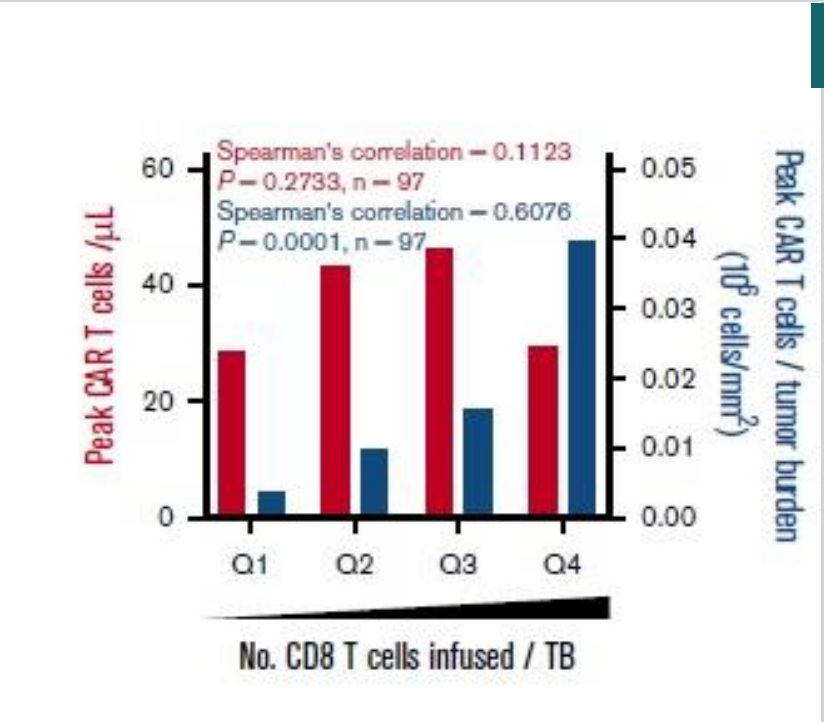
FU 24 months
PFS: 46.6% (Axi-cel), 33,2% (Tisa-Cel)
OS: 63.5% (Axi-cel), 48.8% (Tisa-Cel)

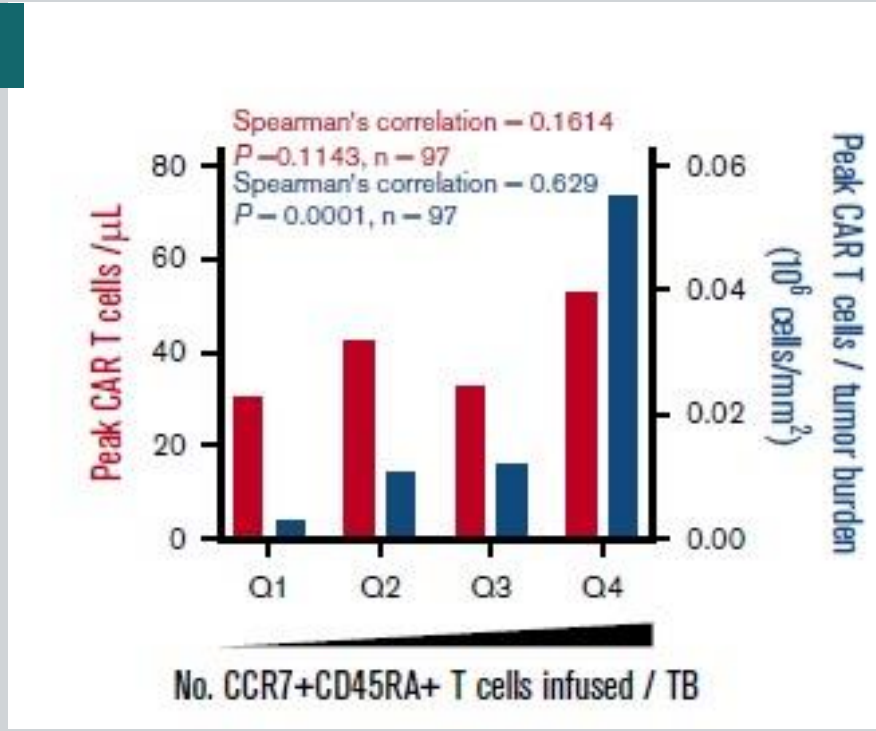


Bachy et al. Nature Medicine 2022

Efficacia CAR-T-dipendente

AXI-CEL





The number of CD8 and CCR7 CD45RA T cells commensurate with TB is critical to achieving durable response after axi-cel

Locke FL et al. Blood Adv. 2020

Efficacia CAR-T-dipendente

- Contaminating red blood cells, monocytes, and neutrophils in the starting material may **adversely affect T** cell expansion in culture as well as final CAR-T cell product characteristics *[Elavia N et al. Transfusion 2019].*
- Increased quantities of monocytes have been shown to be associated with **reduced T cell expansion**, while excess neutrophils may be associated with **reduced transduction efficiency** *[Stroncek DF et al. Cytotherapy 2016].*
- CD4+ cells were shown to **support** development of **CD8+** memory functions *[Sommermeyer, D. et al. Leukemia 2016].*
- CD4+ Treg cells **suppress** the efficacy of **CAR-T** cell therapy *[Golubovskaya V et al. Cancer 2016].*
- The persistence of CAR-T therapy was shown to be dependent on the number of CD4+ *[Sommermeyer, D. et al. Leukemia 2016].*

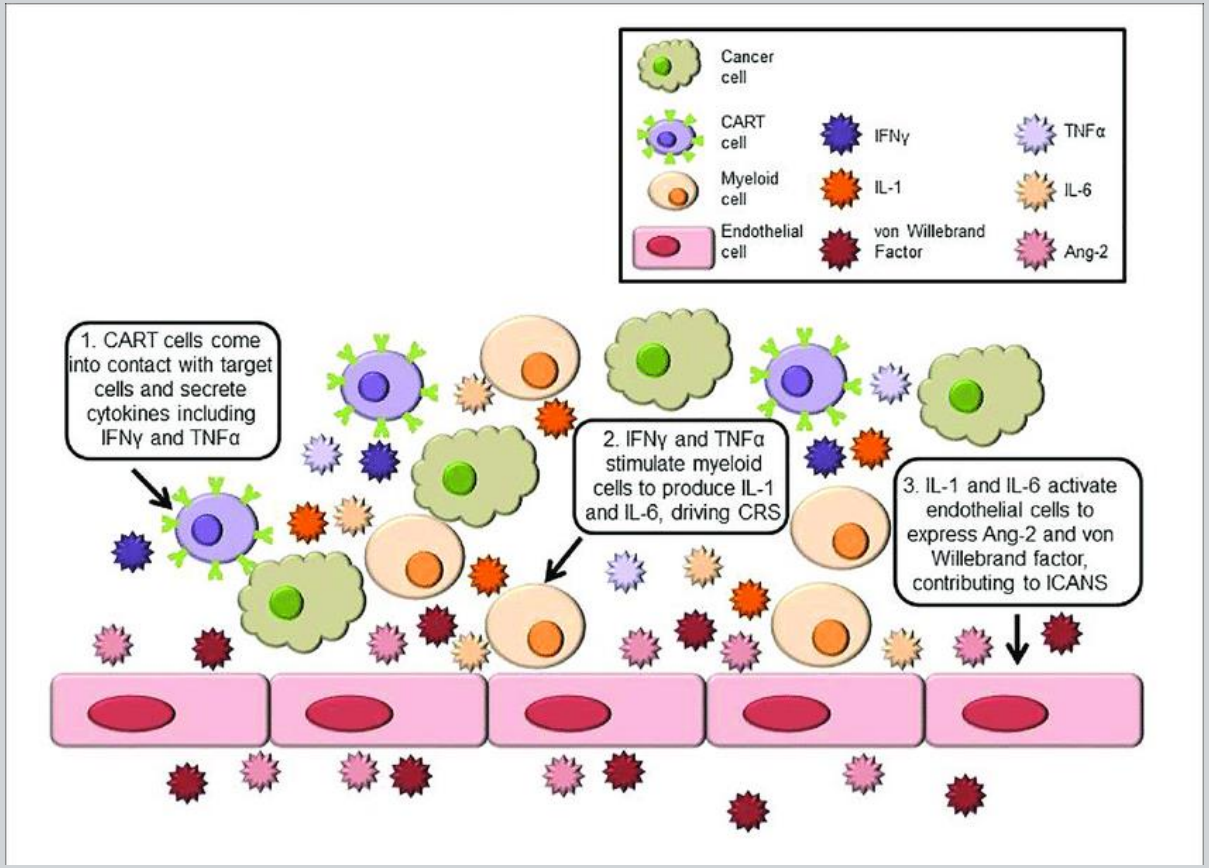
Employing a cell selection or enrichment step is a critical part of the manufacturing process.

Manufacturing Phase	Variable
Collection	Patient characteristics (autologous products)
	Peripheral blood cell composition
	Apheresis procedure and collection efficiency
Cell enrichment	Method of cell enrichment
	Quantity of contaminating cells
	Lymphocyte phenotypes
Cell stimulation	Method of cell stimulation
	Duration of cell stimulation
	Presence of insoluble reagents
Gene transfer	CAR construct design
	Method of gene delivery
	Timing of transduction
	Use of transduction enhancers
	Vector envelope (viral vectors)
Cell expansion	Cytokine selection
	Culture systems
	Duration of expansion
Cryopreservation	Cryoprotectant type and concentration
	Method and rate of freezing

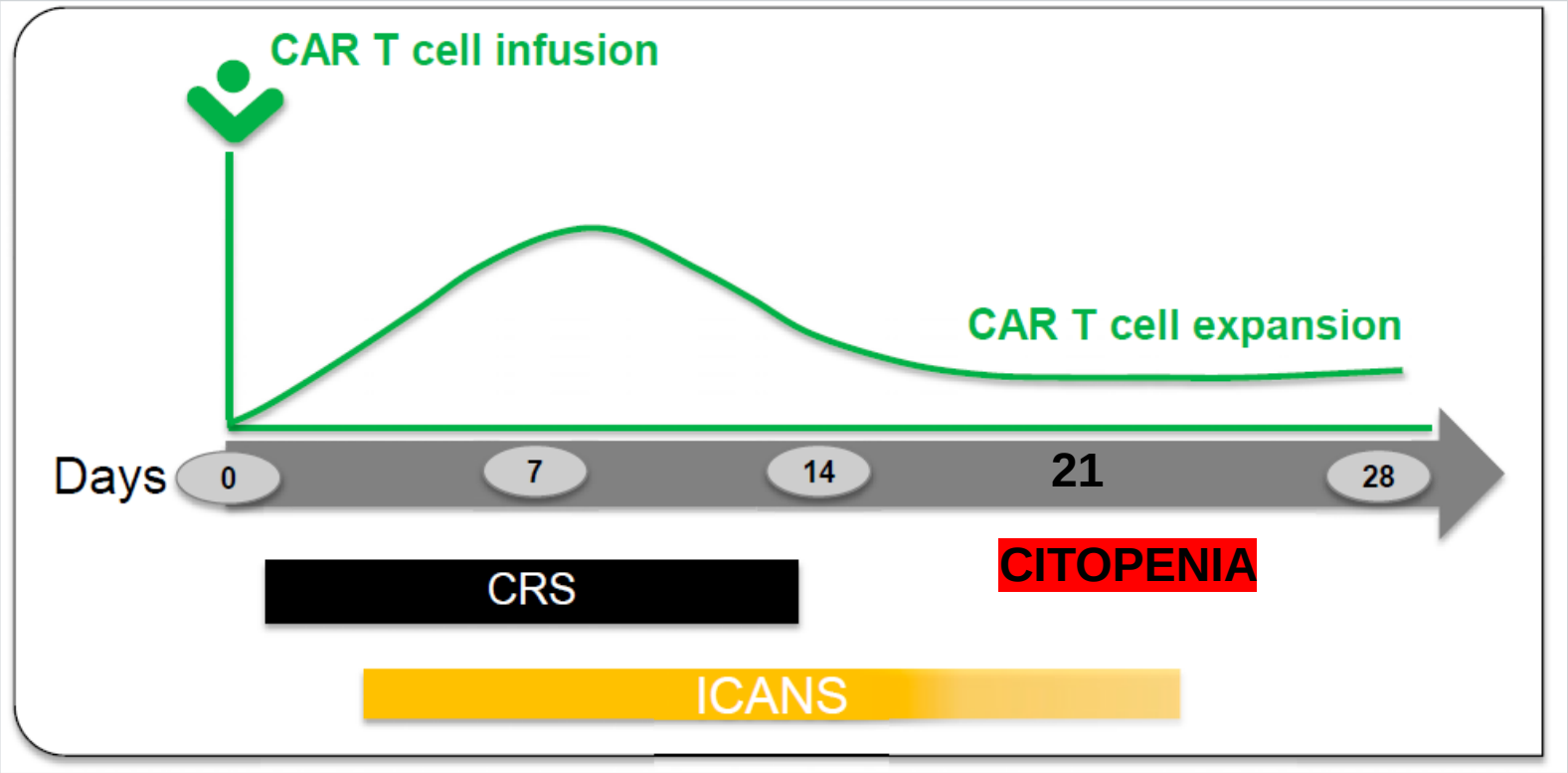
Reddy OL et al. Semin Hematol. 2020

Tossicità a breve termine post CD19 CAR-T

1. **CRS** (Cytokine Release Syndrome)
2. **ICANS** (Immune effector Cell-associated Neurotoxicity Syndrome)
3. **Cytopenia**



Tossicità a breve termine post CD19 CAR-T



Manifestazioni cliniche

CRS

- Il più frequente
- Iper-attivazione linfociti T endogeni o infusi
- Febbre, astenia, ipotensione, ipossia
- Insufficienza multiorgano
- Sindrome da attivazione macrofagica (MAS)

ICANS

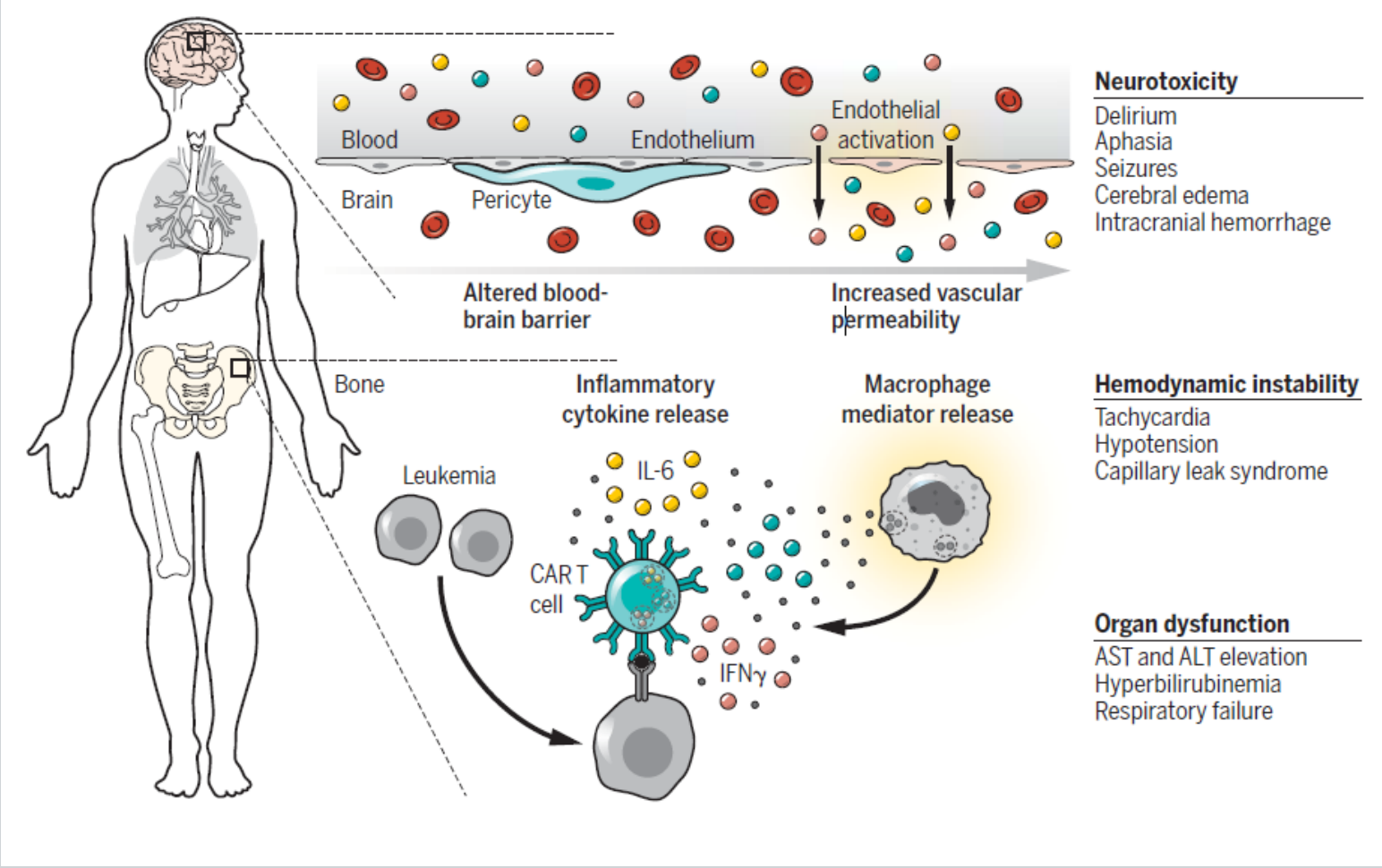
- Il secondo più frequente
- Coinvolge il SNC
- Sintomi neurologici, tra cui encefalopatia
- Attivazione linfociti T endogeni o infusi o altre cellule immunitarie

Cytopenia

“on-target, off-tumor” effect o tossicità auto-immune

- Aplasia delle cellule B, che risulta in citopenia e ipogammaglobulinemia.
- Generalmente nei primi 30 giorni dall’infusione, ma può richiedere mesi per risolversi

Meccanismi patogenetici alla base della CRS e dell'ICANS



Laboratorio

CRS

- Transaminasi
- Coaugulopatie
- Aumento di Ferritina e Proteina C reattiva
- Aumento livelli di lattati nel sangue

ICANS

Valutazione microbiologica e della coagulazione per diagnosi differenziale di infezioni e infarto

Cytopenia

Esame emocromocitometrico per monitoraggio cellulare SP

Sievers et al., Front Onc 2020

Tossicità delle CAR-T a lungo termine

frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 20 January 2023
DOI 10.3389/fonc.2023.1036455

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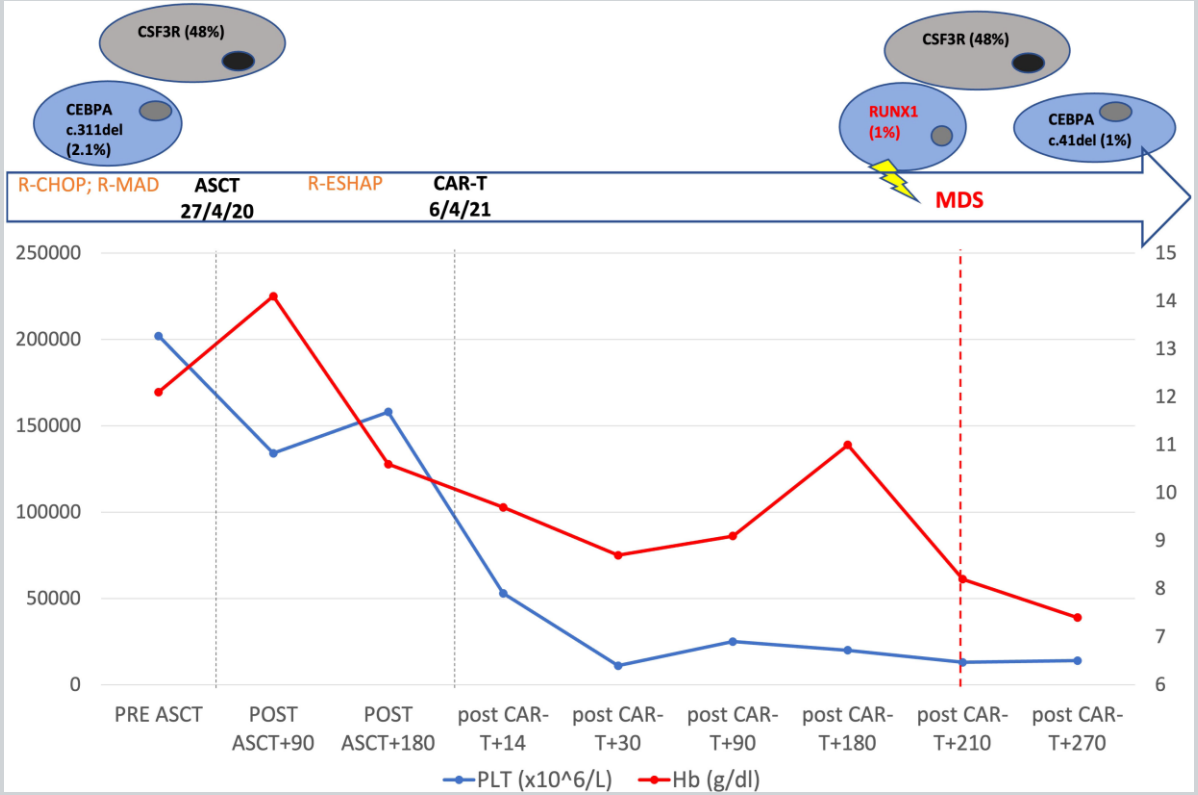
SPECIALTY SECTION
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

RECEIVED 04 September 2022
ACCEPTED 02 January 2023

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]



Studio dell'ematopoiesi clonale nei pazienti in trattamento con terapia CAR-T

ClonHema-CAR-T Study

PI: Prof. D. Russo

Approved by Ethical Committee of Brescia (NP 5554)

Study Duration:

Accrual: 3 years. Minimum follow up: 1 year

- Observational multicenter prospective and retrospective study
- Patients with relapsed/refractory DLBCL, PMBCL, MCL or ALL or new AIFA registered disease undergoing CAR-T therapy
- ASST Spedali Civili di Brescia and GITMO Centers accredited for CAR T-cell therapy
- Axi-cel (Yescarta) or tisa-cel (Kymriah) or Brexucabtagene Autoleucel (Tecartus) therapy according to the Italian Medicines Agency (AIFA)

GITMO

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

ClonHema-CAR-T: il razionale

- The pathophysiology underlying prolonged cytopenia is still unclear: could be the development of MDS or AML?
- CAR-T cells therapy had previously underwent ASCT.
- **MDS or AML onset may be to**:

(1) previous treatments, as ASCT
(2) clonal hematopoiesis of indeterminate potential (CHIP)
(3) an impairment of immunosurveillance related either to the lymphoma or to B-cells aplasia induced by CAR-T cell treatment.

Secondary MDS or AML clearly related to CAR T-cells <u>are still not described</u>.

AIMS

- **Evaluation of clonal hematopoiesis (CH-CHIP) before and post CAR-T cells infusion**
- **Evaluation of myelodysplastic syndrome (MDS), Acute Myeloid Leukemia (AML) or other hematological neoplasia onset in patients who underwent CAR-T cells therapy.**

Studio ClonHema-CAR-T

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

Within 2 months before Ly-apheresis*

Ly-apheresis

CAR-T cells infusion

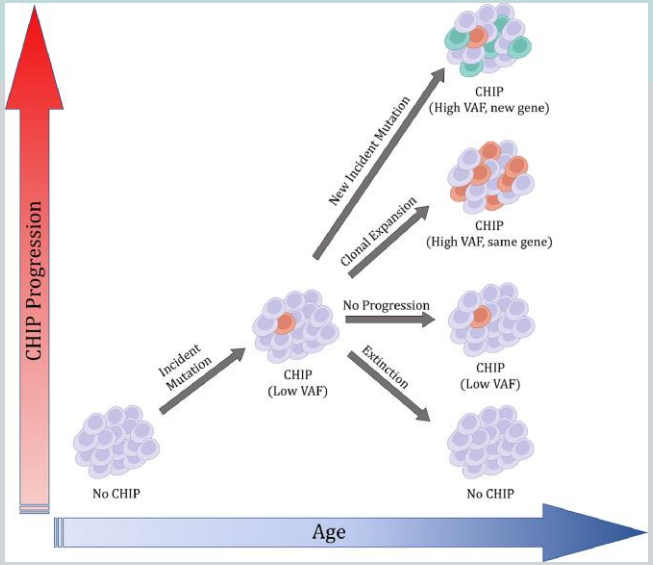
- BMA
- BMB
- Karyotype
- Sample to be stored for NGS analysis

+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**



Resistenza alla terapia CAR-T

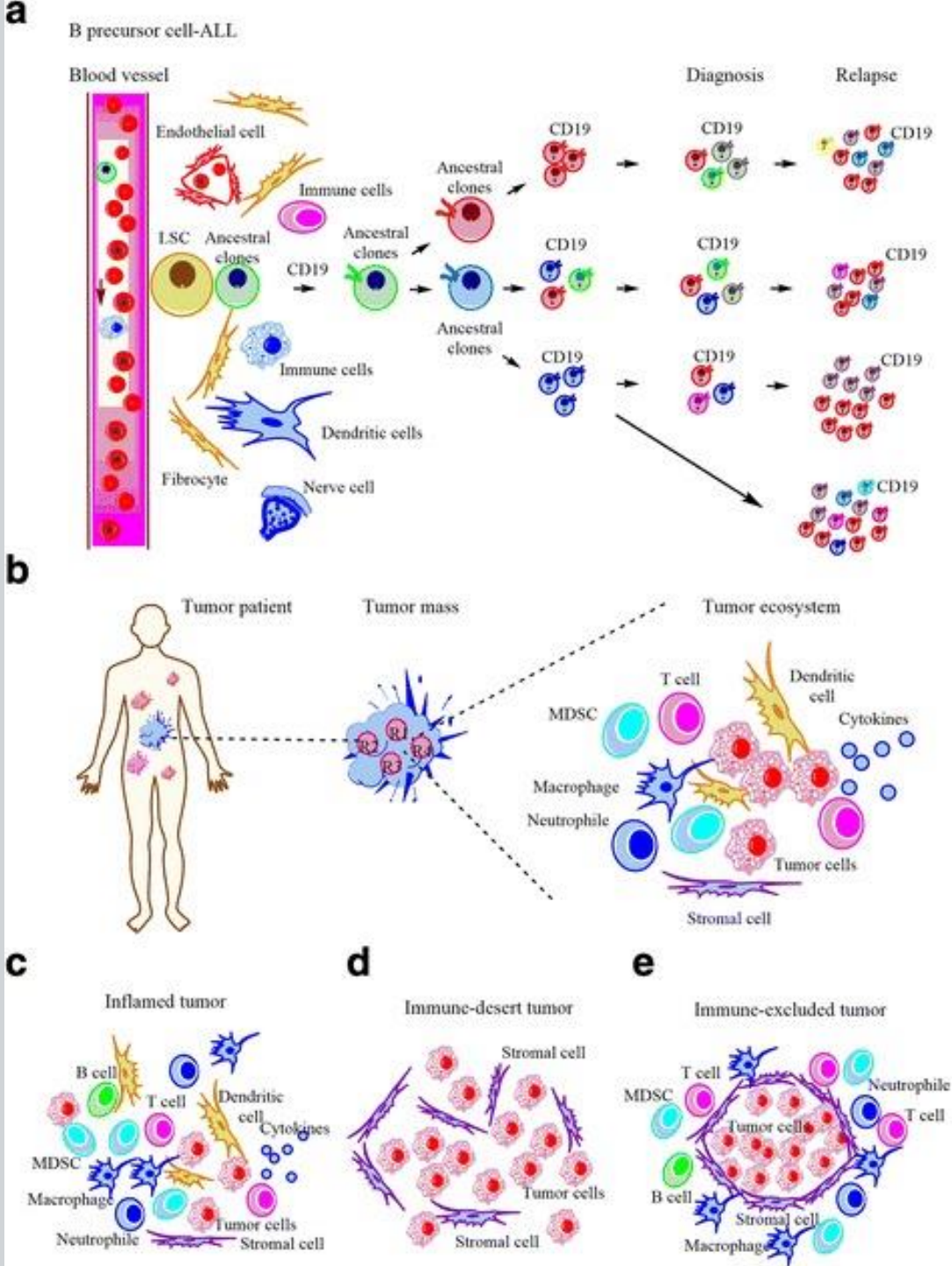
- **Espressione di una variante tumorale del CD19**:

- perdita dell'antigene o epitopo CD19
- splicing alternativo, mutazioni in omozigosi o frameshift bi-allelici del CD19 (varianti del CD19 che portano ad una mancato riconoscimento da parte del CAR);

- **Ecosistema tumorale**:

- interazione tra le cellule maligne, immunitarie, stromali, endoteliali e le citochine;

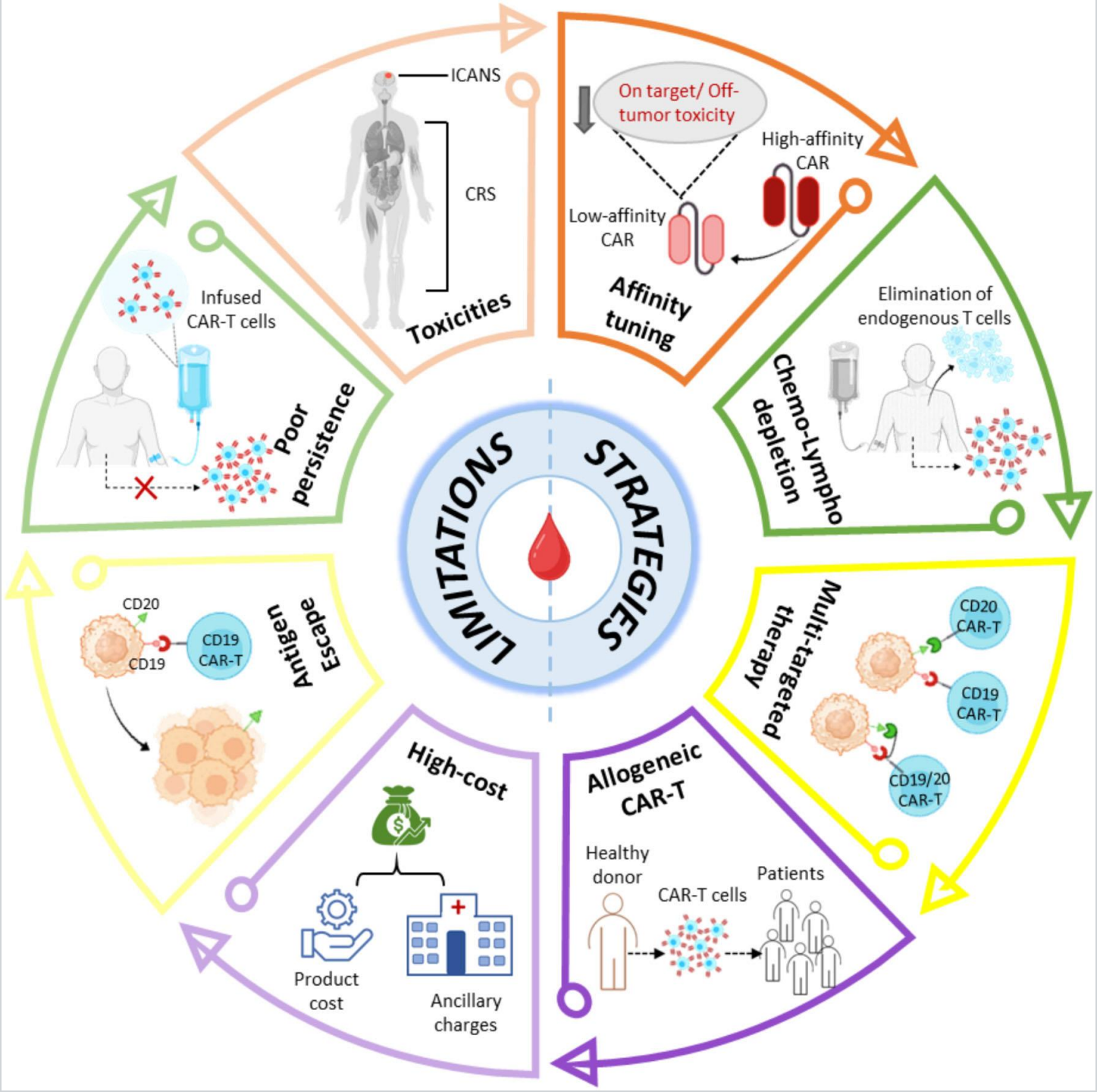
- **Fenotipi immunitari tumorali differenti**



Baixin Ye et al. Molecular Cancer (2018) 17:32

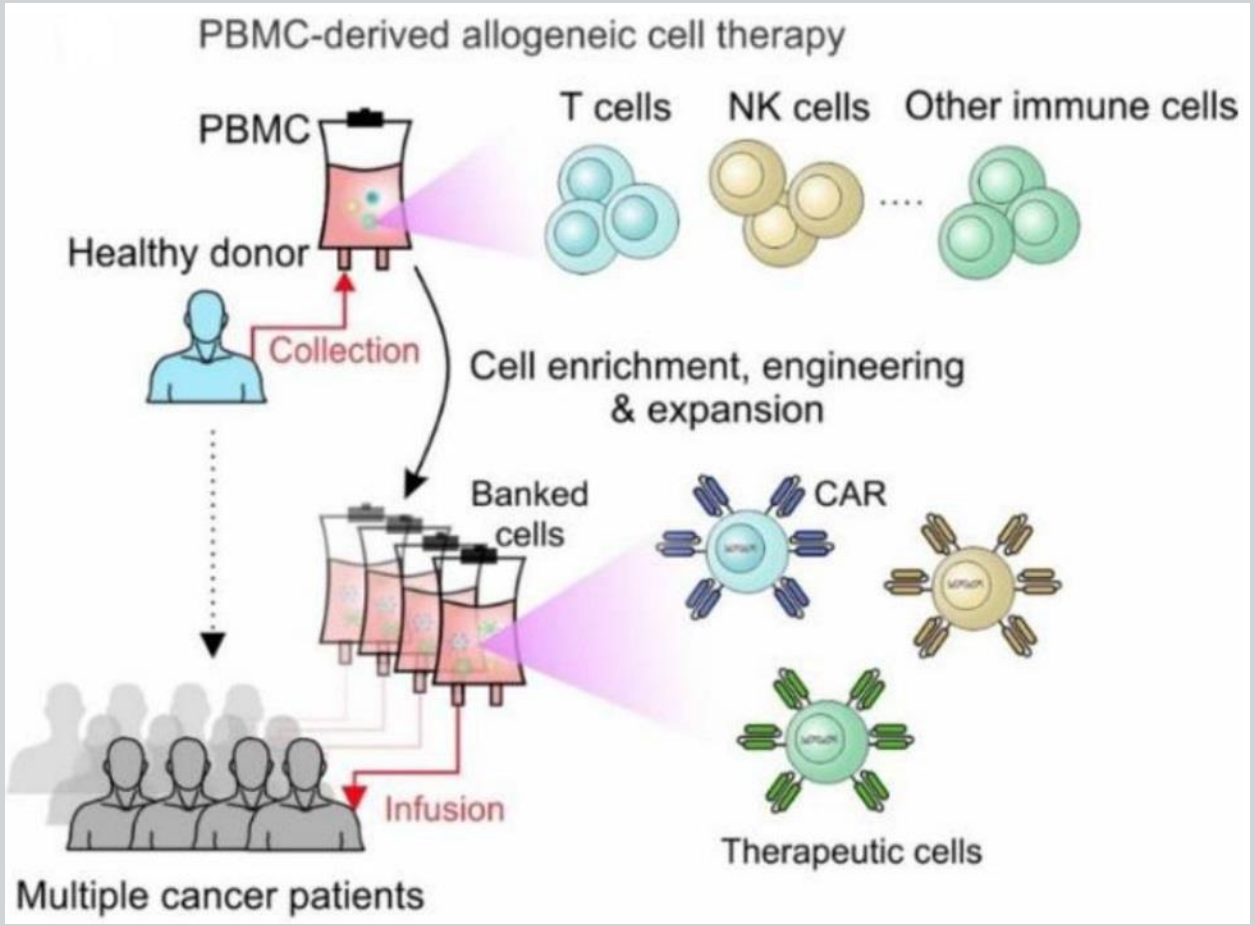
SUGGERIMENTI PER IL FUTURO

- Car-t allogenic
- iPSC as alternative source of CAR-T
- Nk CAR-T
- New CAR-T constructs
- Targeting New Antigen (CD20, CD22, BAFF-R, CD29a…)
- CAR-T earlier in treatment plan



Cellule CAR-T alternative

ALLOGENIC CAR-T

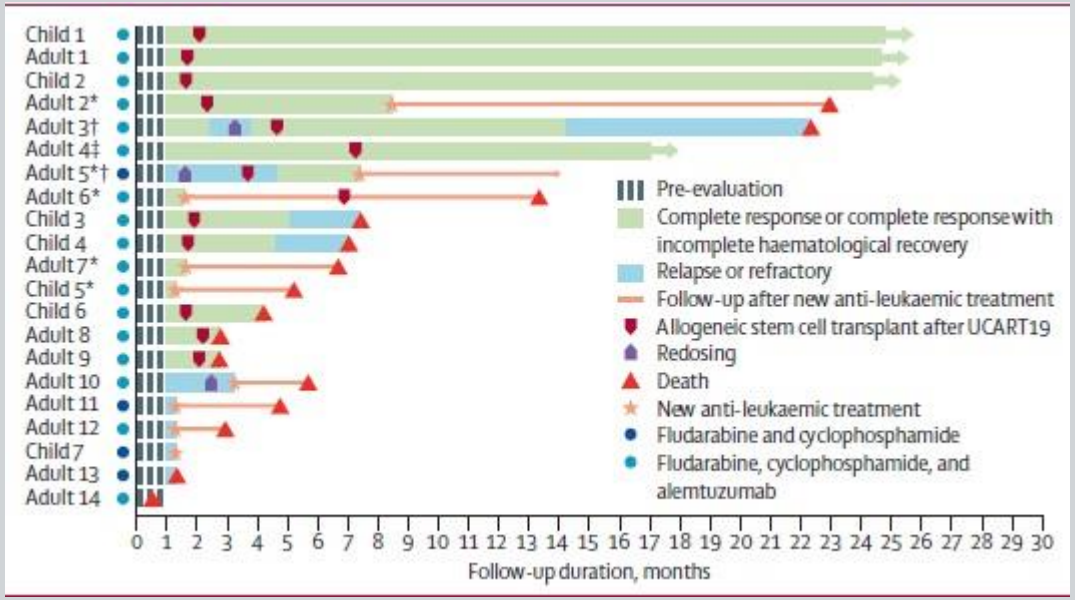


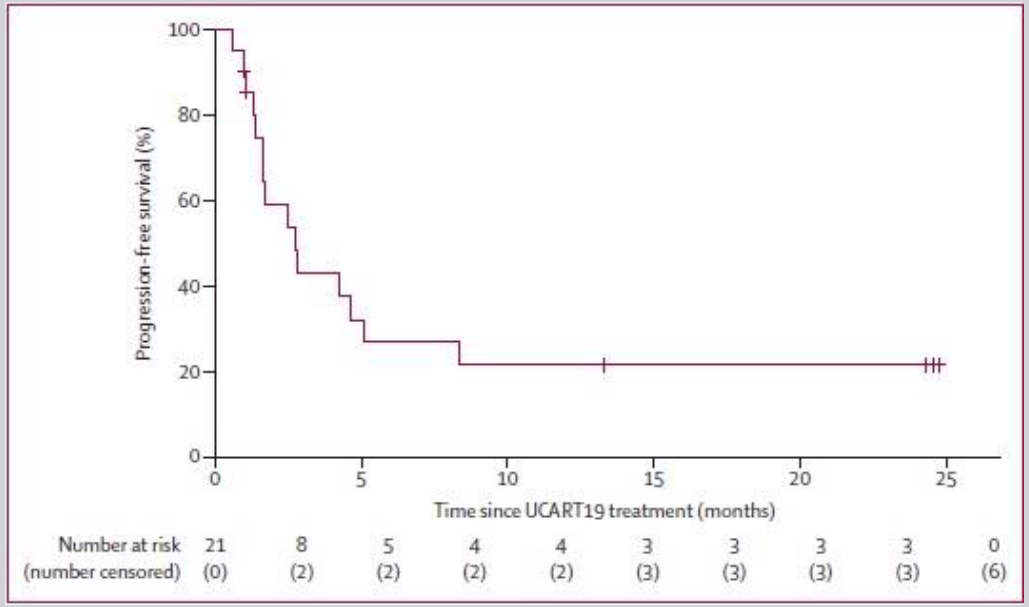
Zhou Y et al. Cancers 2022

CAR-T allogeniche

Study Ph1 of UCART19 in patients with R/R B-ALL

Allogeneic T cells expressing anti-CD19 CAR



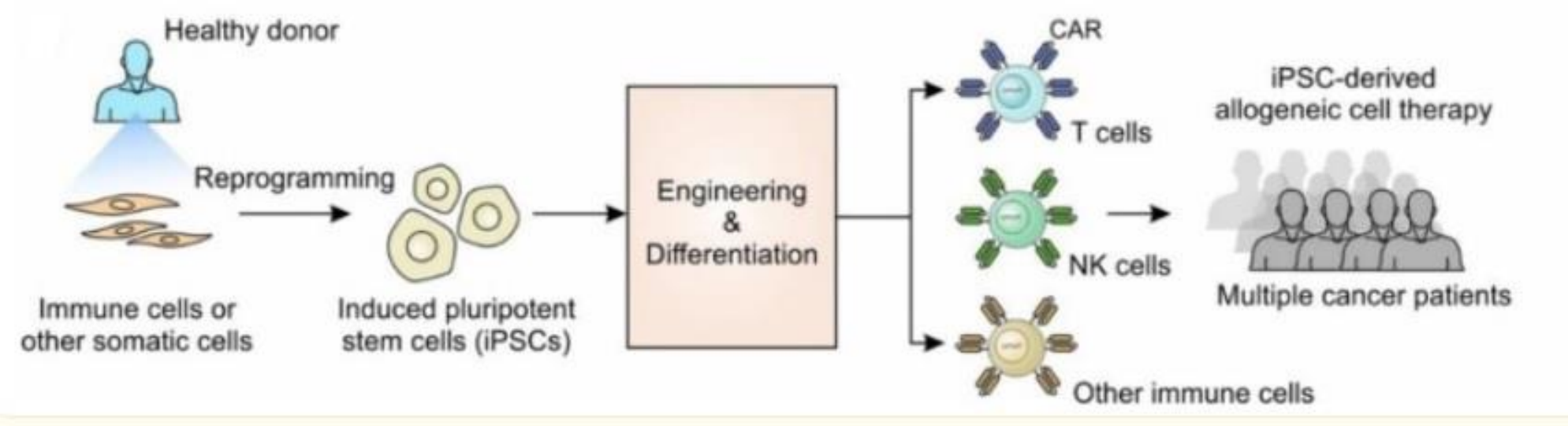


PFS @ 6 mo 27%
OS @ 6 mo 55%

Benjamin R et al. Lancet. 2020

Cellule CAR-T alternative

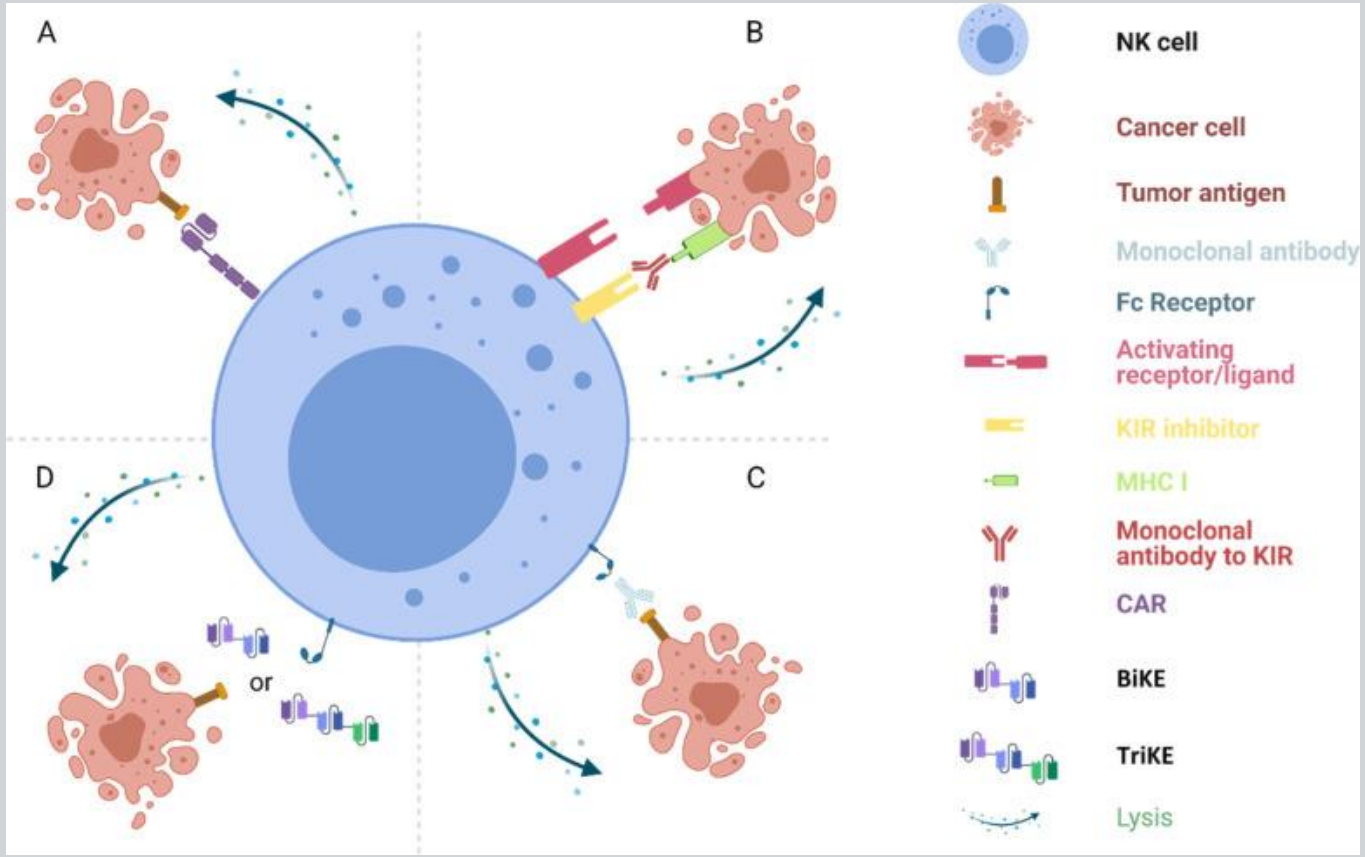
iPSC



Zhou Y et al. Cancers 2022

Cellule CAR-T alternative

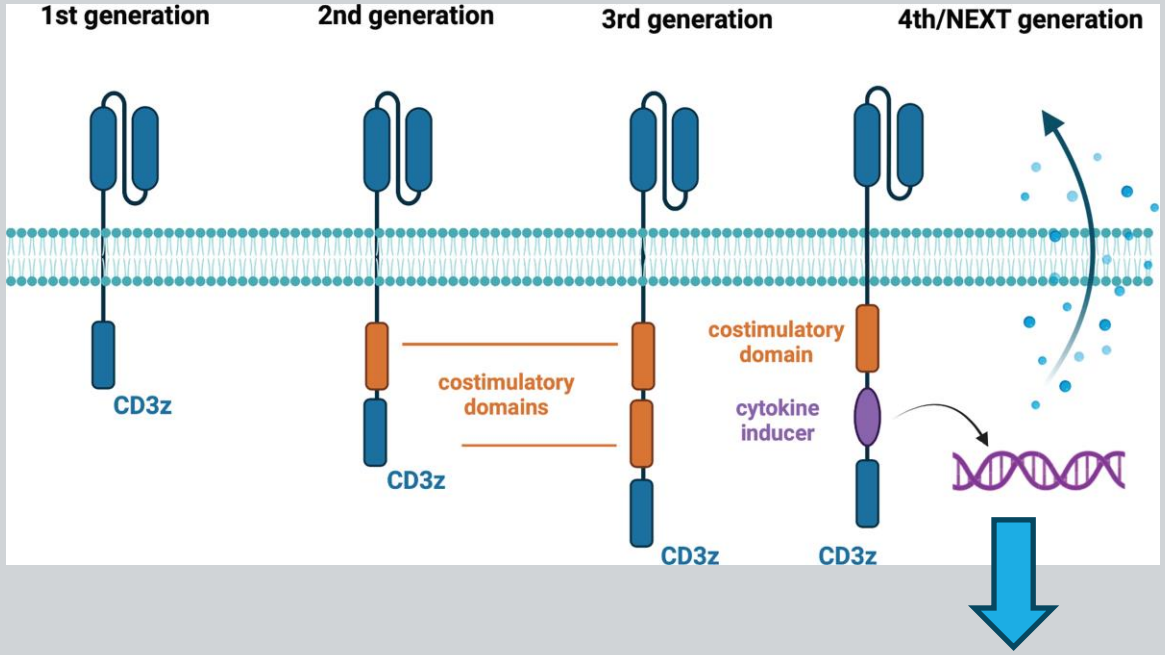
NK CAR-T

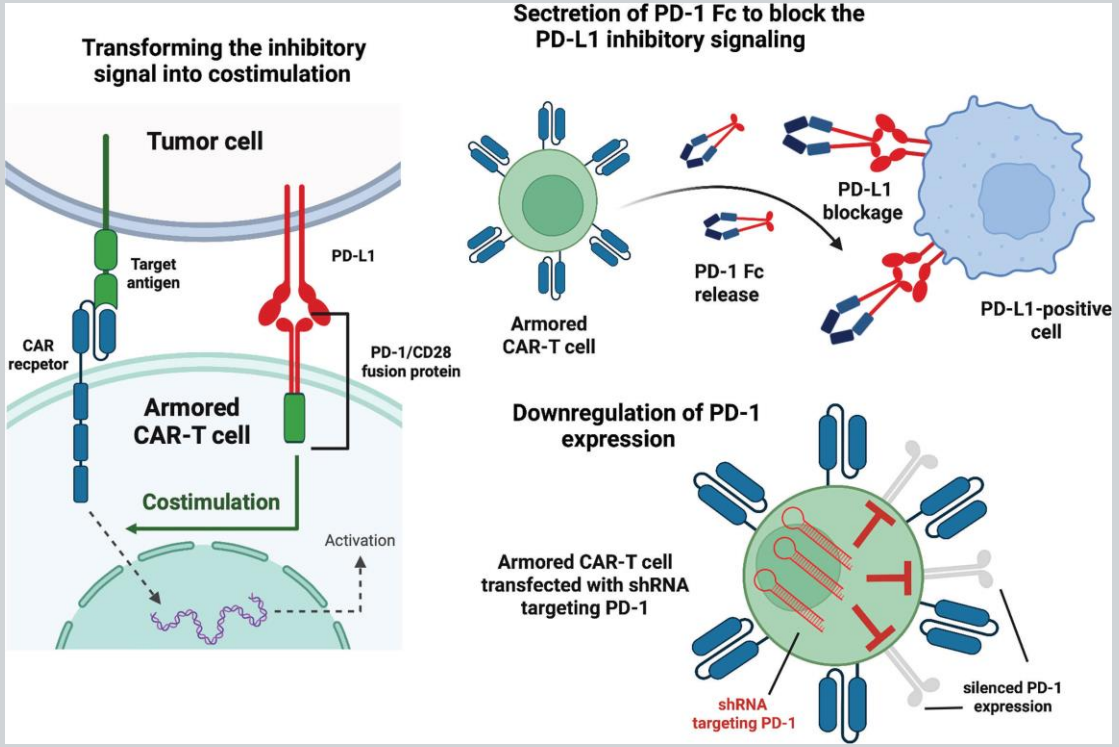


- NK cells can directly recognize target cells in the absence of MHC.
- Do not induce GVHD, CRS, ICANS.
- NK cells have multiple mechanisms to target and eliminate cancer cells in addition to the CAR pathway.

Pan K et al. J Exp Clin Cancer Res. 2022

CAR-T costrutti





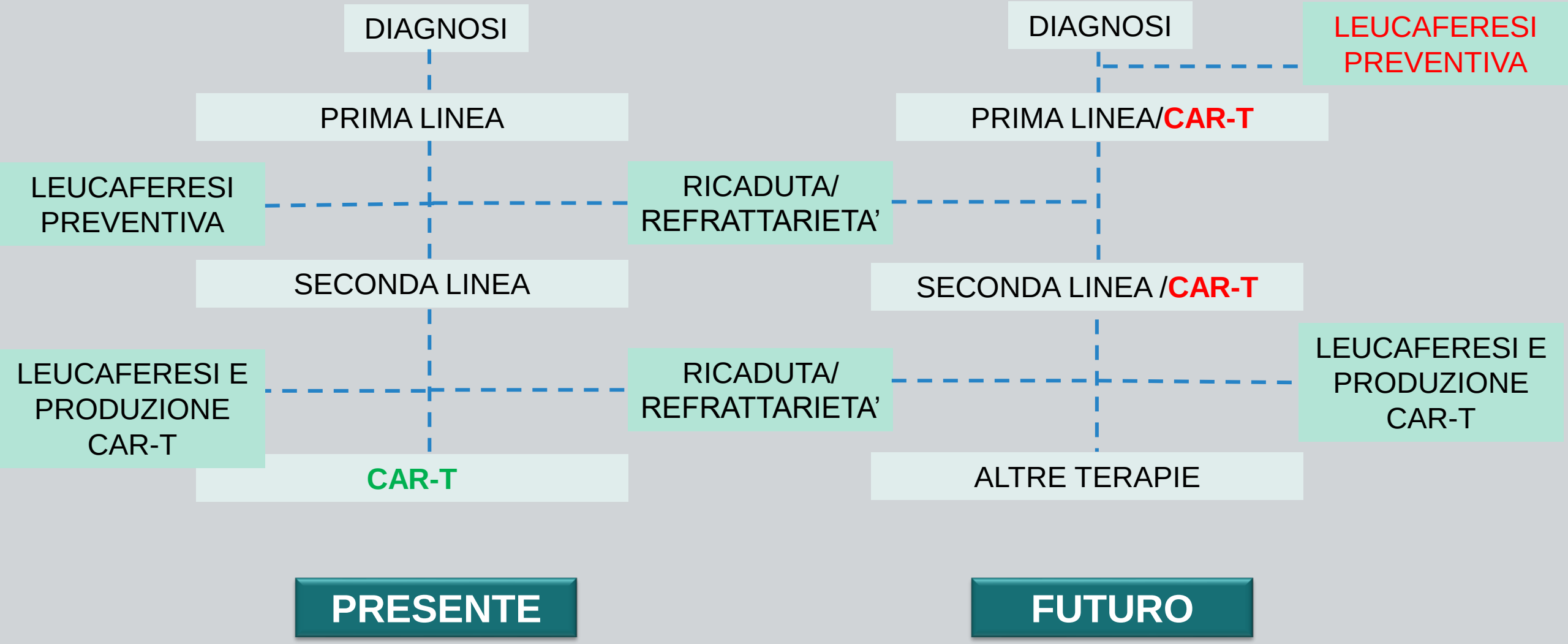
Immune checkpoint modulation

Armored CAR-T cells
Cytokine-expressing CAR-T cells
Switchable CAR-T cells
Universal CAR-T cells

NEX GENERATION OF CAR-T CELLS

Tomasik J et al. Front Immunol. 2022

Somministrazione precoce di cellule CAR-T



«Fitness» dei linfociti

cancers

MDPI

Article

Timely Leukapheresis May Interfere with the "Fitness" of Lymphocytes Collected for CAR-T Treatment in High Risk DLBCL Patients

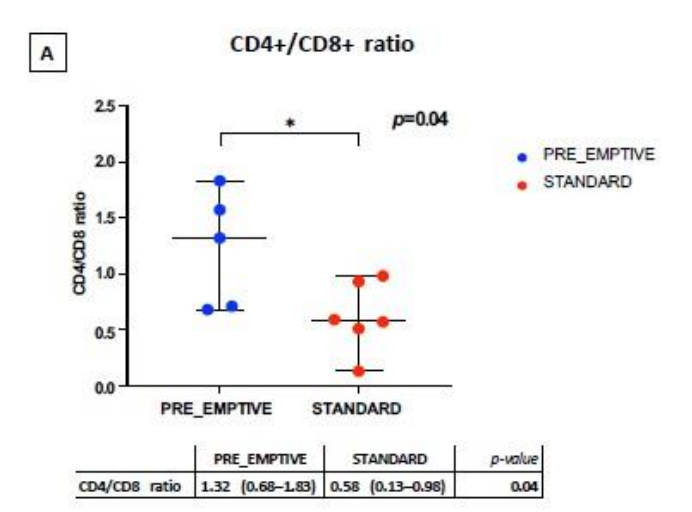
Mirko Farina [1,*,†], Marco Chiarini [2,†], Camillo Almici [3], Eugenia Accorsi Buttini [1], Francesco Zuccalà [4], Simone Piva [5], Irene Volonghi [6], Loris Poli [6], Simona Bernardi [1,7], Federica Colnaghi [1], Federica Re [1,7], Alessandro Leoni [1], Nicola Polverelli [1], Alessandro Turra [1], Enrico Morello [1], Anna Galvagni [2], Daniele Moratto [2], Duilio Brugnoni [2], Chiara Cattaneo [8], Emilio Ferrari [3], Andrea Bianchetti [3], Michele Malagola [1], Alessandro Re [8] and Domenico Russo [1]

- "***Pre-emptive***" lymphocyte apheresis (Ly-apheresis) may preserve the fitness of lymphocytes for CAR-T cell infusion:

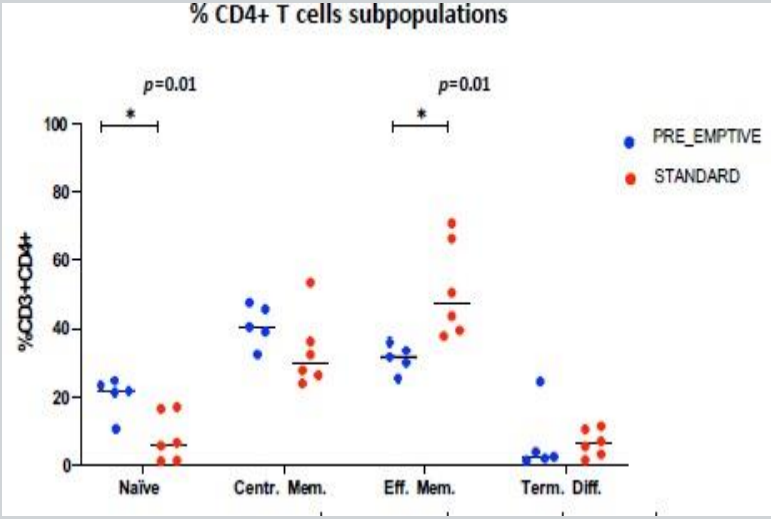
(1) higher CD4/CD8 ratio
(2) higher of CD4/CD8 naïve T cells
(3) lower CD8+ terminally differentiated T

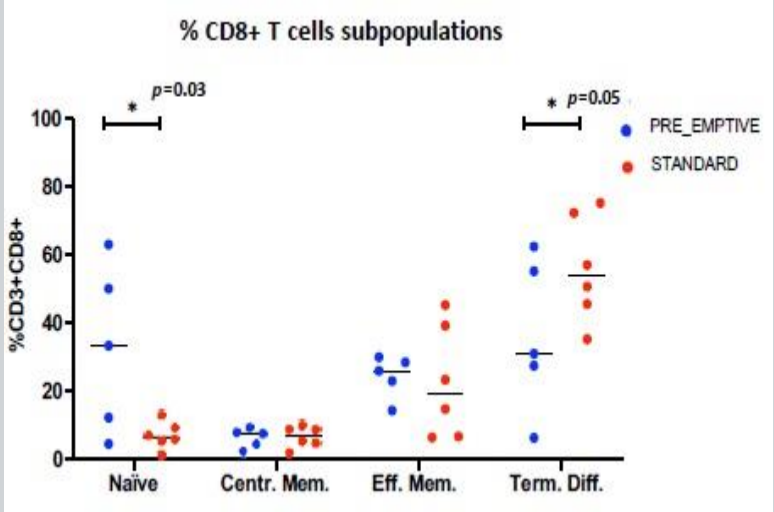
→ «fit» lymphocyte

Bio-CAR-T study on pre and post-infusion CAR-T cell Therapy (Bio-CAR-T BS Study)
PI: Prof. Domenico Russo



	PRE_EMPTIVE	STANDARD	p-value
CD4/CD8 ratio	1.32 (0.68–1.83)	0.58 (0.13–0.98)	0.04





TAKE HOME MESSAGES

- ✓ La terapia CAR-T CD19 è efficace nei pazienti con DLBCL con ≥ 3 line di trattamento (approvazioni FDA; EMA; AIFA).
- ✓ Axi-cel, Tiso-cell, Liso-cel hanno una maggiore efficacia rispetto alla SOC (alto dosaggio chemioterapia + ASCT) in pazienti con DLBCL primario refrattario o in pazienti che recidivano in 12 mesi dalla prima linea di trattamento.
- ✓ La terapia con CAR-T è l'unica per alcuni linfomi.
- ✓ Le caratteristiche dei pazienti, della malattia e delle cellule CAR-T possono influenzare l'efficacia stessa della terapia CAR-T.
- ✓ La tossicità delle CAR-T è un importante aspetto da considerare.
- ✓ Il futuro della terapia con CAR-T prevede nuovi costrutti di CAR-T, CAR-T da fonti alternative, utilizzo delle CAR-T precocemente nel piano di trattamento.