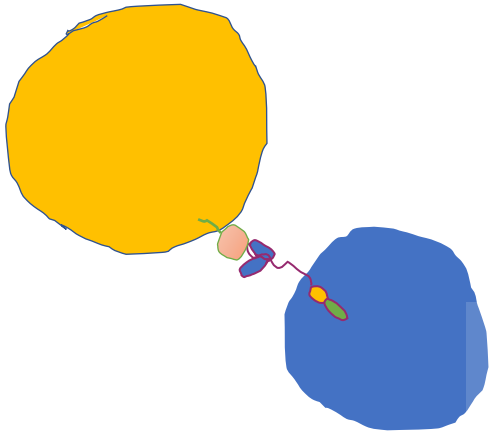

Transposon-based system for CAR T cell therapy

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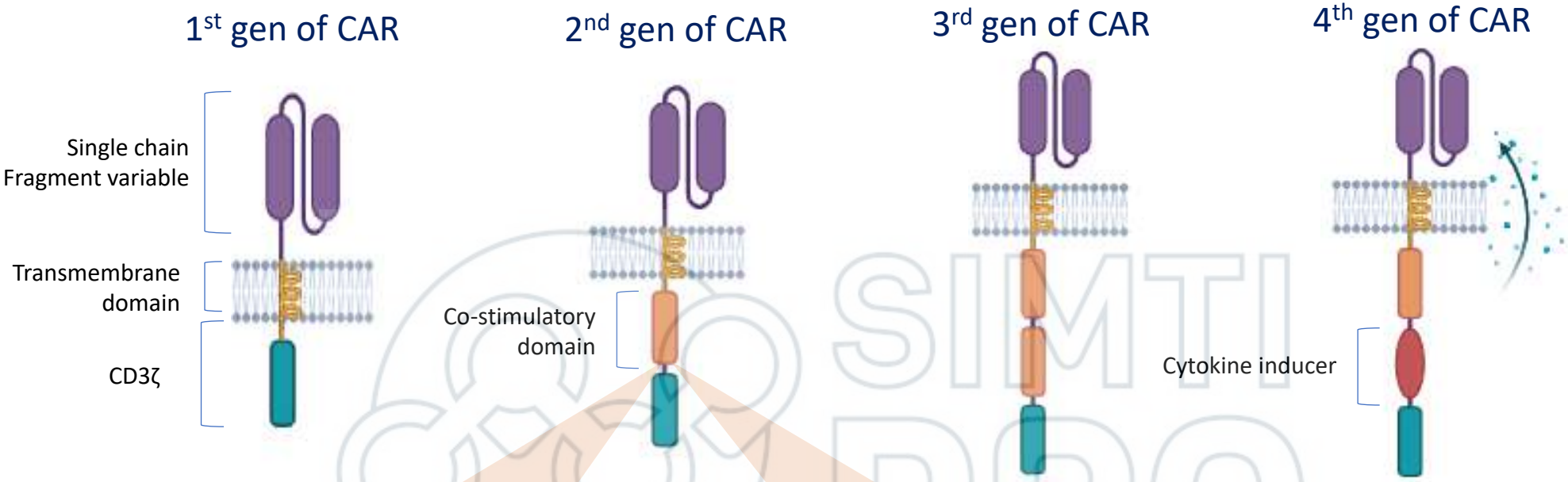
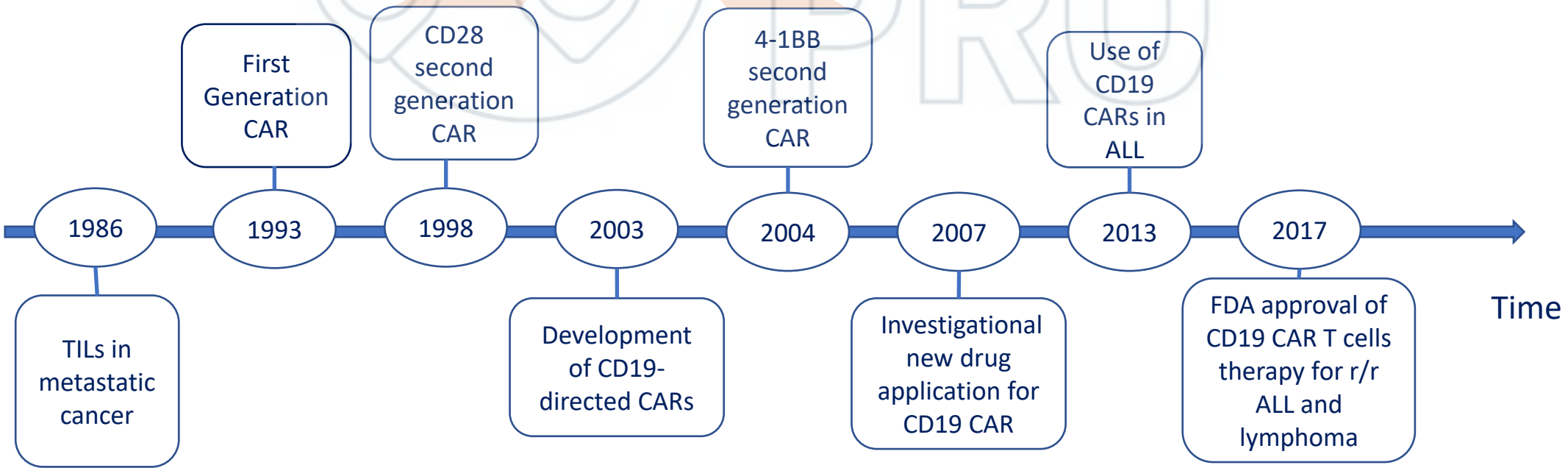
Fondazione IRCCS San Gerardo dei Tintori
Sistema Socio Sanitario Regione Lombardia
Università degli Studi di Milano Bicocca

Chimeric Antigen Receptor (CAR) T cell breakthrough





Clinical development of CAR T cells: translating innovative treatment concepts

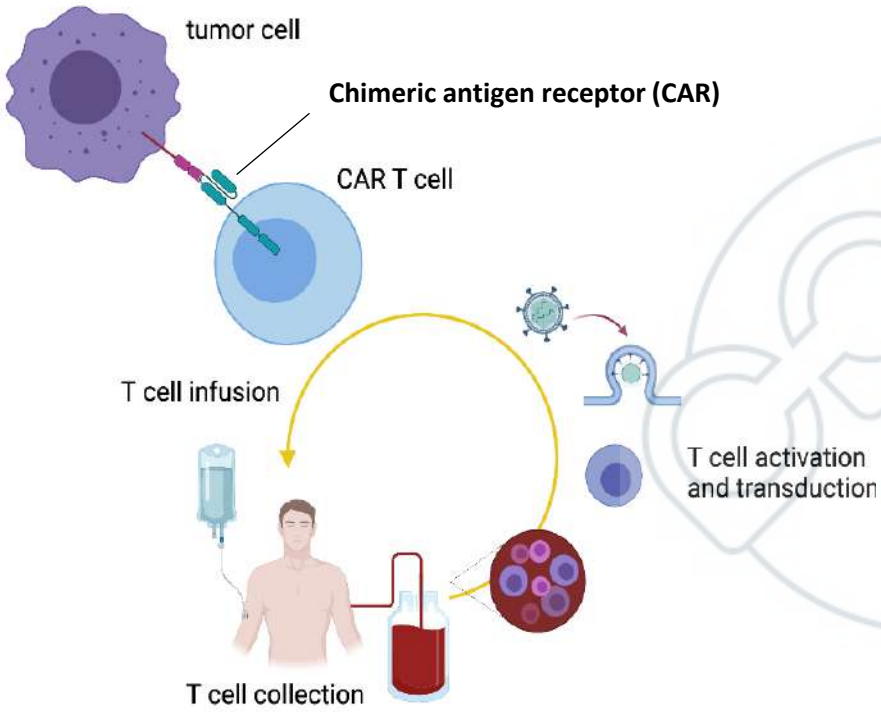


TABLE 1 | Commercial CAR T products and their indication and availability worldwide.

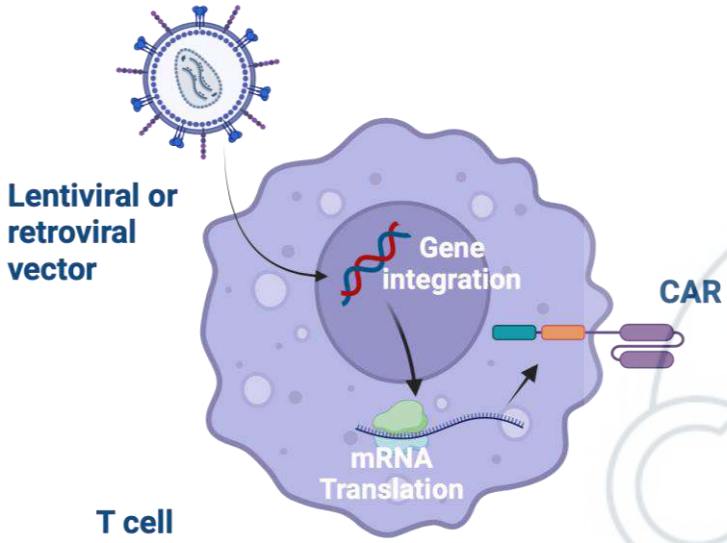
Active substance	Name	Indications	Manufacturer	Approvals	Target	Costimulatory domain
tisagenlecleucel	Kymriah	Pediatric and young adult R/R acute lymphoblastic leukemia; Adult R/R DLBCL	Novartis	FDA, EMA, Health Canada, Swissmedic, Japan's MHLW, Singapore's HSA, Australian TGA, UK's NICE	CD19	CD137
axicabtagene ciloleucel	Yescarta	R/R large B-cell lymphoma (DLBCL, PMBCL, high grade B-cell lymphoma, DLBCL arising from FL)	Kite Pharma	FDA, EMA, Health Canada, Swissmedic, Japan's MHLW, China's NMPA, Australian TGA, UK's NICE	CD19	CD28
brexucabtagene autoleucel	Tecartus	Mantle cell lymphoma; Adult lymphoblastic leukemia	Kite Pharma	FDA, EMA, Swissmedic, UK's NICE, Health Canada	CD19	CD28
lisocabtagene maraleucel	Breyanzi	R/R large B-cell lymphoma	BMS and Juno Therapeutics	FDA, Japan's MHLW, EMA	CD19	CD137
idecabtagene vicleucel	Abecma	Multiple myeloma	BMS and Bluebird Bio	FDA, EMA, Health Canada, Swissmedic, Japan	BCMA	CD137
ciltacabtagene autoleucel	CARVYKTI	Multiple myeloma	Janssen and Johnson & Johnson	FDA	BCMA	CD137
relmacabtagene autoleucel	Carteyva	R/R large B-cell lymphoma	JW Therapeutics	China's NMPA	CD19	CD137

MHLW, Ministry of Health, Labor and Welfare; HAS, Health Sciences Authority; TGA, Therapeutic Goods Administration; NMPA, National Medical Products Administration; NICE The National Institute for Health and Care Excellence.

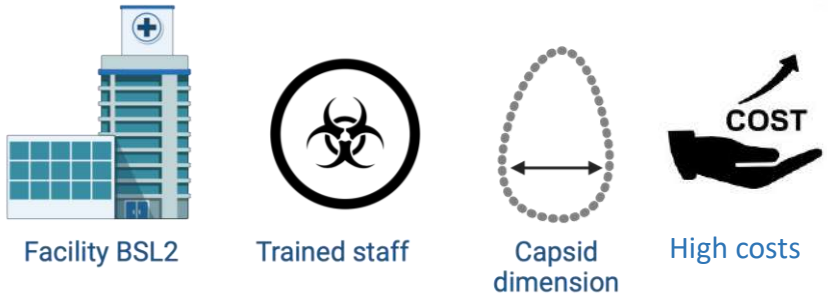
Moretti A et al., Front. Immunol. 9 June 2022

Issue and criticalities of CAR T cell products

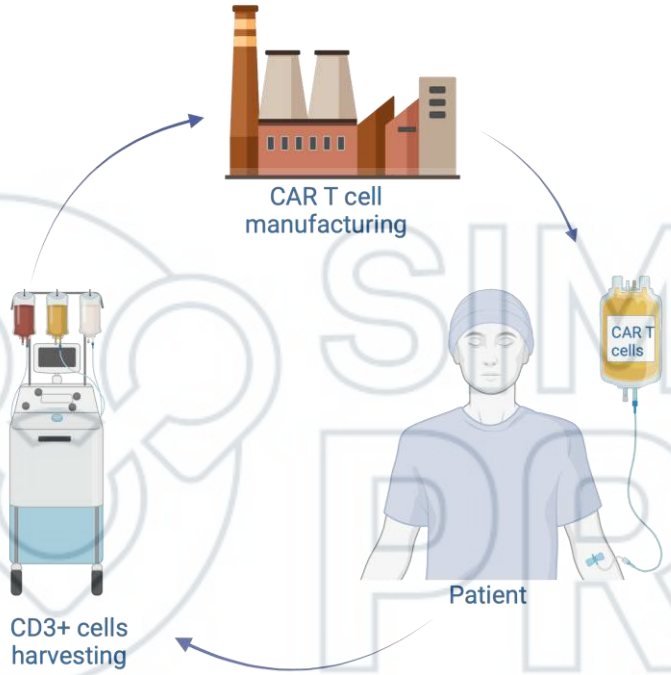
A) Viral vector



Limitations:



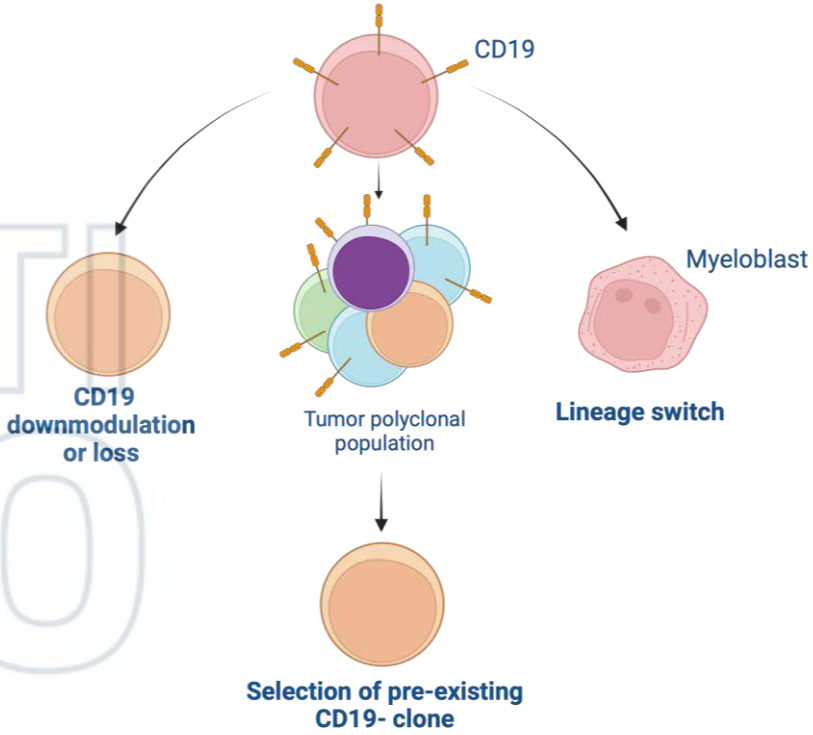
B) Autologous source

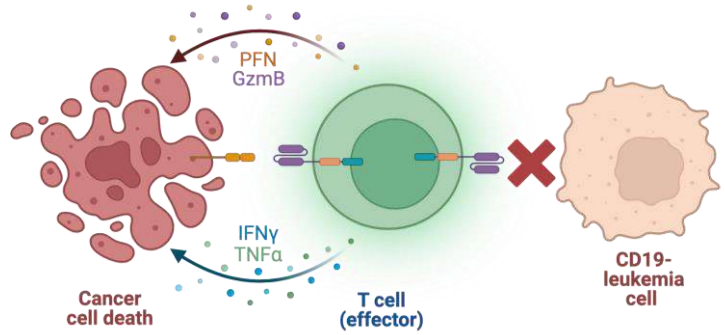


Issue:

- Previous therapy
- Lymphopenia
- Anergic immune system
- Failure of manufacturing
- Failure to expand

C) Emerging of CD19 negative relapse





Access problems for ALL in children: an EU view

- **Failures of drug development in childhood B-ALL**
  - Tecartus (Zuma4): ALL cohort closed
  - JCAR017: Trial closed
  - UCART 19 (Pfizer/Servier/Cellectis): product abandoned after adult and ped trial
- **New developments with available commercial drug refused by pharma**
  - Donor derived for pts in relapse post HSCT
  - First Relapse (despite being the main indication of HSCT in ALL)
- **No access to new platform of manufacturing with more persisting CART cells**
- **No widely available CAR for T-ALL even in clinical trial**
  - Current closure of Wugen trial in T-ALL (4 centers in EU)
  - Medisix trial still to be opened and oligocentric
  - Base edited CART only in UK
- **Countries underserved** (eastern EU, Turkey and more)

Baruchel A. , EHA Ped.Hem.Onc2024

blood®
Blood Spotlight

CAR T-cell therapy in multiple myeloma: mission accomplished?

Leo Rasche,[1,2] Michael Hudecek,[1,3] and Hermann Einsele[1]

…ide-cel is available and reimbursed in 5 countries (United States, France, Switzerland, Japan, and Germany) cilta-cel only in 2 countries (United States and Germany).

The advancement of scalable, virus-free, automated manufacturing will increase the number of available products.

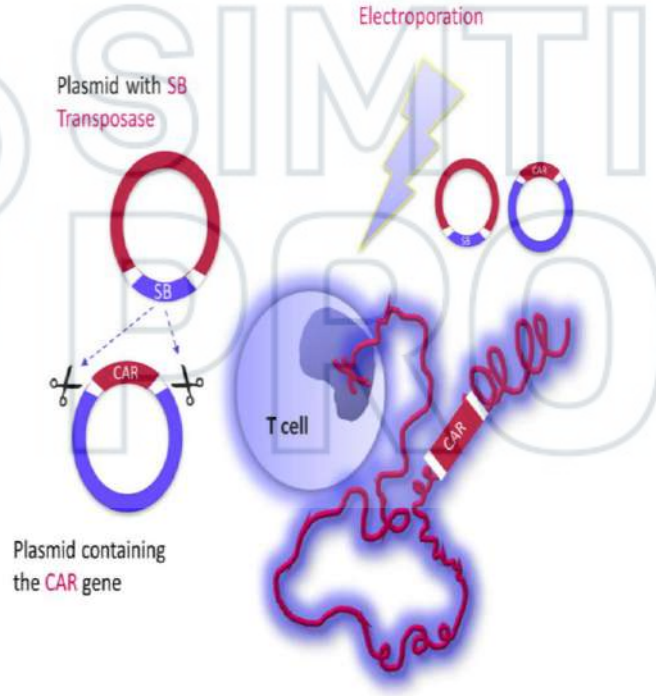
A non-viral Sleeping Beauty (SB) allogeneic platform: CARCIK CD19

Allo-T cells differentiated as Cytokine induced killer cells (CIK)

Cytotoxic T cells enriched in $CD3^{+}CD56^{+}$
Minimal GvHD after allogeneic CIK
Able to reach leukemia-infiltrated tissues
Rambaldi A (2015) Leukemia 29(1):1-10; Introna M (2017) Biol Blood Marrow Transplant. 23(12):2070-8

Sleeping Beauty (SB) transposon

a non-viral vector derived from the Tc1/mariner family of DNA transposon
validated in clinics for CAR T
Ivics Z (1997) Cell 91(4):501-10; Kebriaei P (2016) J Clin Invest. 126(9):3363-76



Biondi, Magnani (2017) J Autoimmun 85:141-152; Moretti A. et al Frontiers in Immunology 2022

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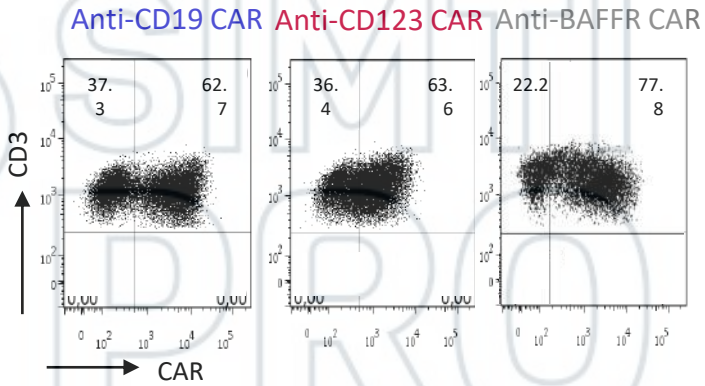


An improved SB transposon platform
Magnani CF (2016) Oncotarget 7(32): 51581-51597;
No. EP20140192371 "Improved method for the generation of genetically modified cells"; Turazzi (2018) Br J Haematol 182(6):939-943; Magnani CF (2018) Hum Gen Ther 29(5):602-613; Rotiroti MC et al., in press, Molecular Therapy 2020

Phase I/II trial with non-viral anti-CD19 CAR T cells in B-ALL post HSCT

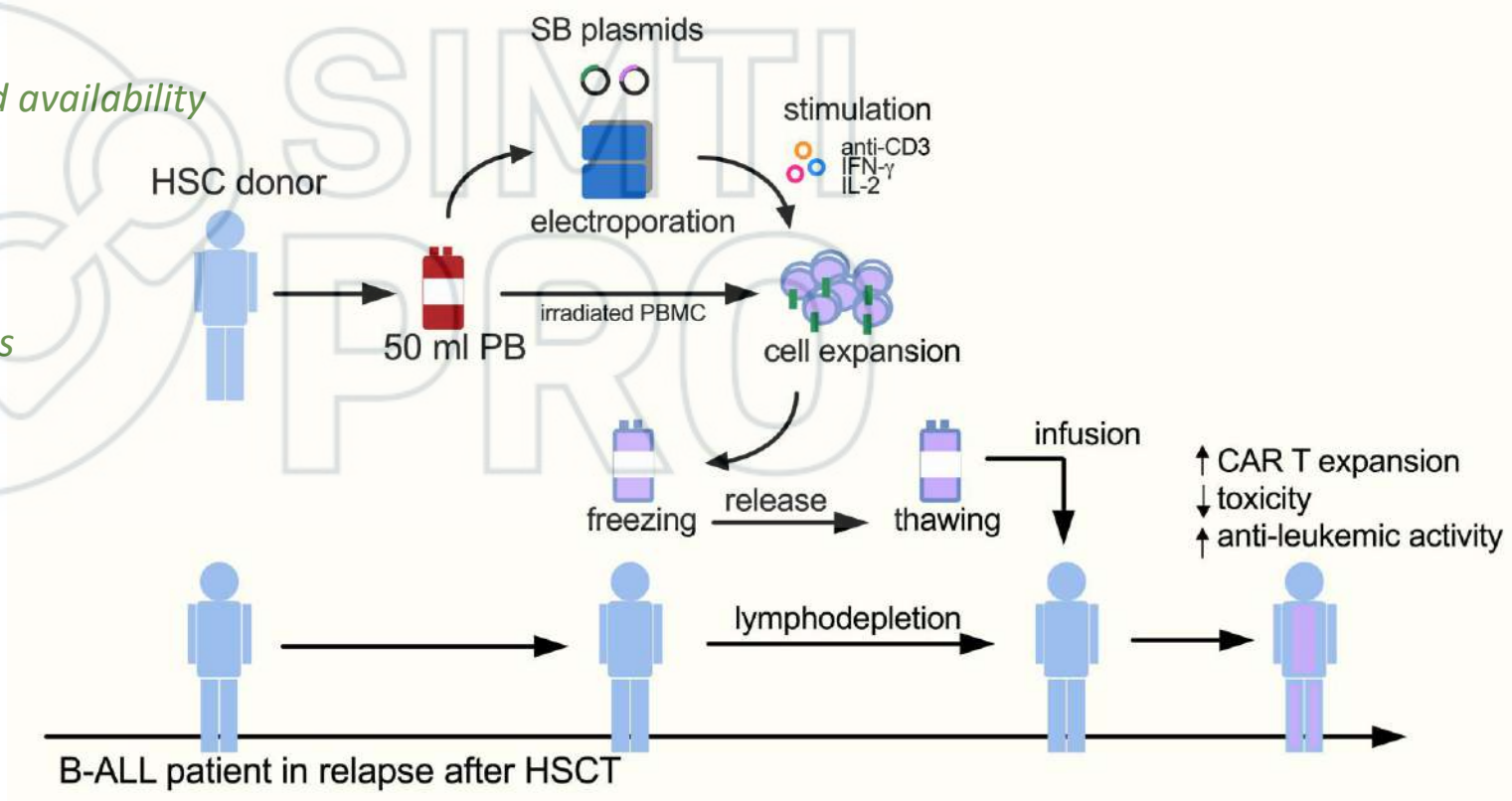
Academic Multicenter Dose Escalation Study (NCT03389035) enrolling adult and pediatric patients
GMP cell product **manufactured in-house** by **non-viral gene transfer** using Sleeping Beauty (SB) transposon
Donor-derived cells differentiated towards Cytokine-Induced Killer (CARCIK-CD19) to prevent GVHD

Non-viral CAR T cell engineering :

- *Simplified process with reduced cost and increased availability*
- *Stable expression*
- *Increased DNA carrying capacity*

Donor-derived CAR T cell engineering:

- *Differentiation towards Cytokine-Induced Killer cells*
- *Low risk of GvHD*



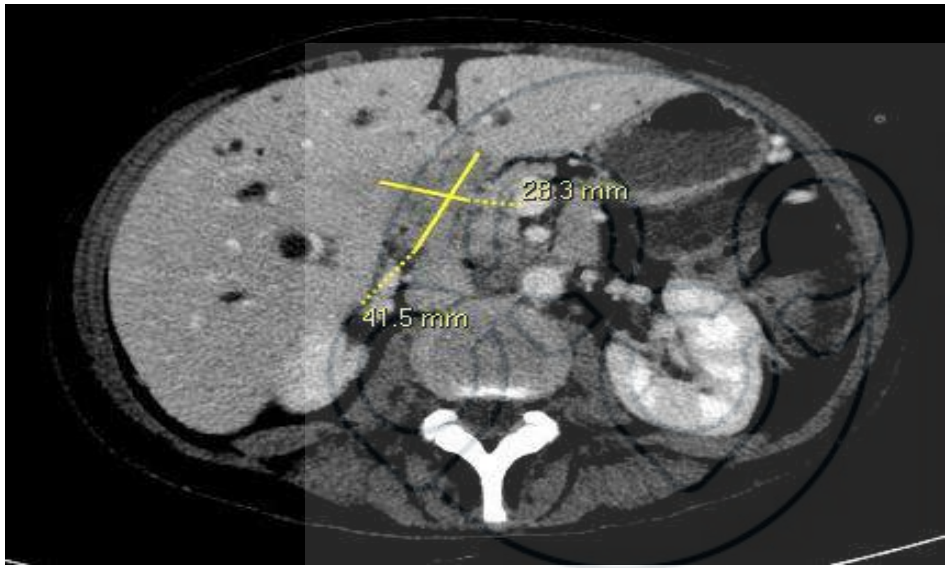
Fondazione IRCCS San Gerardo dei Tintori
Sistema Socio Sanitario Regione Lombardia

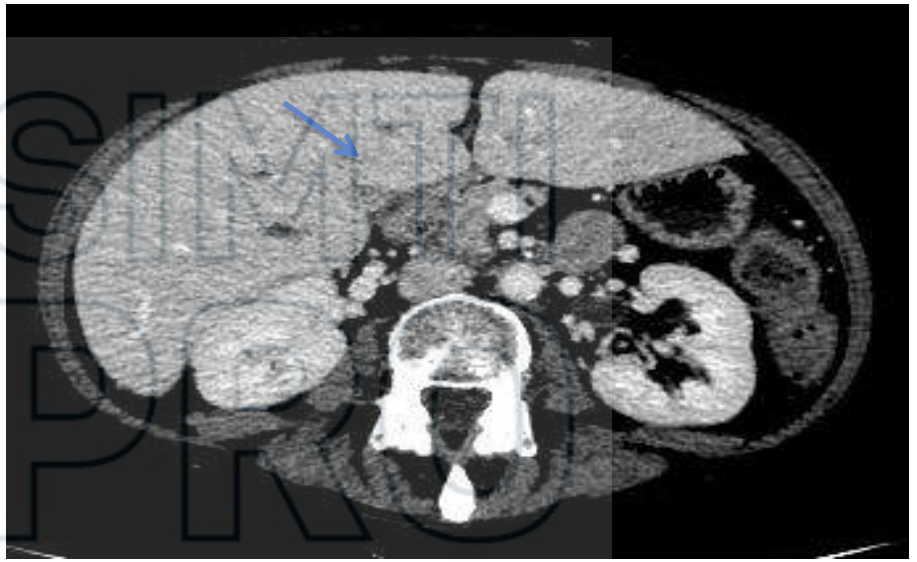
Magnani CF et al. J Clin Invest. 2020 Nov 2; 130(11): 6021–6033
Lussana F. et al ASH 2022

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

CAR-T mediated contraction of extramedullary disease

Patient #21020014: CT scan before and after CARCIK-CD19

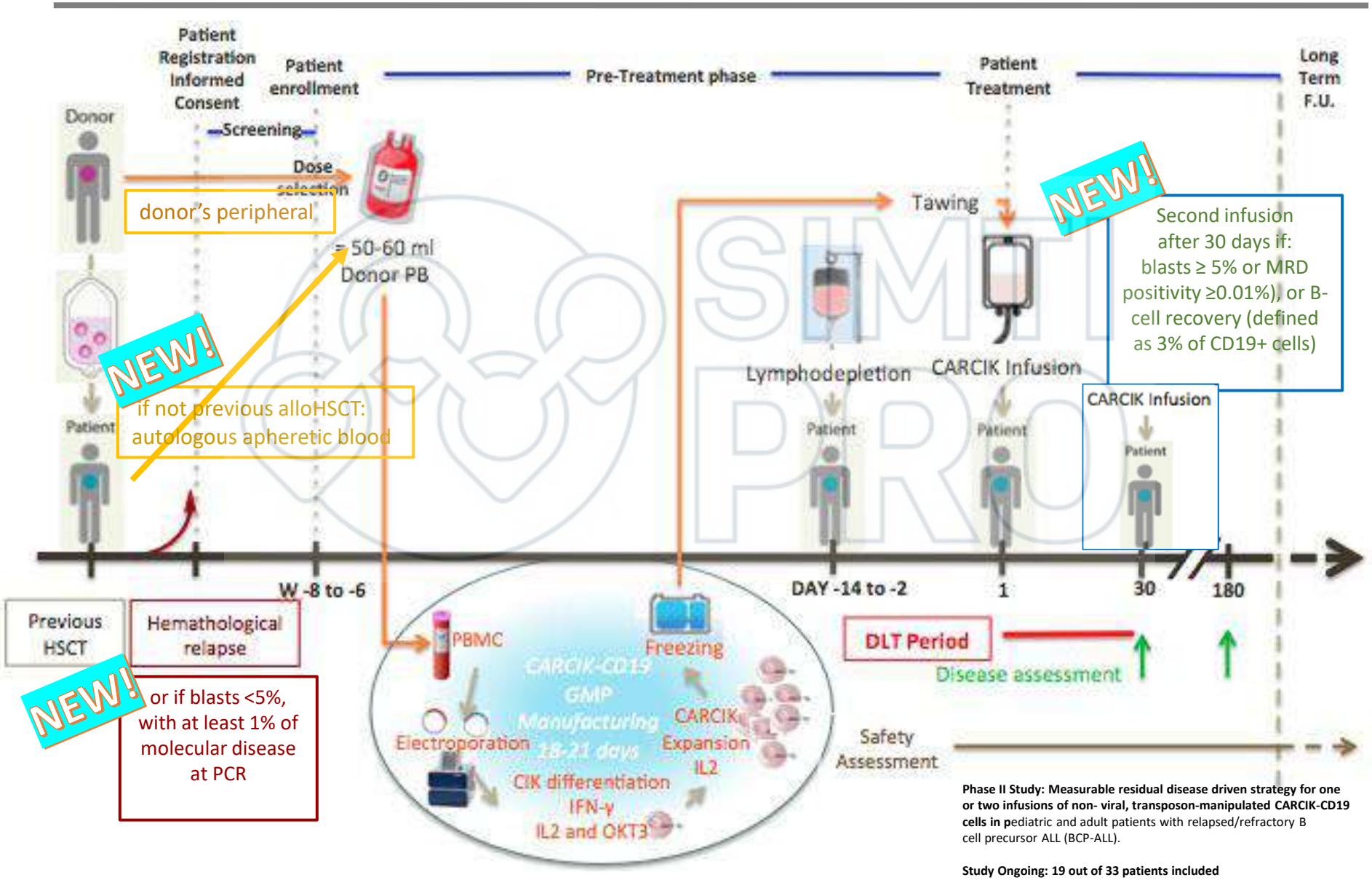




07 June 2019: Relapse post Allo-HSCT presenting liver adenopathy
27 June 2019:
- AST/ALT: 157/287 UI,
- GammaGT: 1183 UI,
- Bil: 18.8 mg/dl

12 September 2019, day +44 after CARCIK-CD19 infusion:
- AST/ALT: 12/58 UI,
- Gamma GT: 82 UI,
- Bil 0,8 mg/dl

FT03CARCIK Phase 2: Flow-chart



Phase II Study: Measurable residual disease driven strategy for one or two infusions of non- viral, transposon-manipulated CARCIK-CD19 cells in pediatric and adult patients with relapsed/refractory B cell precursor ALL (BCP-ALL).

Study Ongoing: 19 out of 33 patients included

Update FT03 Phase II (retreatment) Study (March 13th, 2024)

- A total of thirty-three patients, 3 pediatric and 30 adult were screened.
- 32 out of 33 patients enrolled (1 screening failure).
- 7 patients not infused for early death or Progressive Disease; 4 patients waiting to be infused.
- 21 patients infused so far: 20 with a single dose of CAR-T cells of 15x10^6/Kg; one patient with extramedullary disease at day 28, received a second infusion of CAR-T cells of 15x10^6/Kg.
- 18 out of 20 patients (90%), achieved CR at day 28. Sixteen out of 18 patients in CR were also minimal residual disease (MRD)-negative. Three patients too early to be evaluated at day 28.
- The patient with extramedullary disease at day 28, achieved CR after the second infusion.
- CARCIK-CD19 showed a high profile of safety in all treated patients.
- Data with 15x10^6/Kg are confirming results obtained in the previous Phase I/II study

Protocol FT04CARCIK

EU CT Number	2023-505511-20-00
Short title	Allogeneic CARCIK-CD19 in adults/pediatric B-cell NHL or CLL
Sponsor	Fondazione Tettamanti
Clinical Phase	Phase I/II
Investigational Product	CD19.CAR transfected CIK cells by non-viral Sleeping Beauty (SB) transposon platform (CARCIK-CD19)
Study type	Interventional
Target population	Adult and pediatric patients with B-cell NHL and CLL. Patients are either refractory or relapsed after at least 2 lines of standard and second line treatments and with no available treatment options that are expected to prolong survival (e.g. chemotherapy or high-dose chemotherapy/stem cell transplantation, commercial CART cell therapy or other standard treatment) or patients refusing such treatments
Study centers/production sites	2 in Italy (Monza: pediatric; Bergamo: adult)
Expected study duration	Enrollment: 36 months, Follow-up after infusion: 12 months

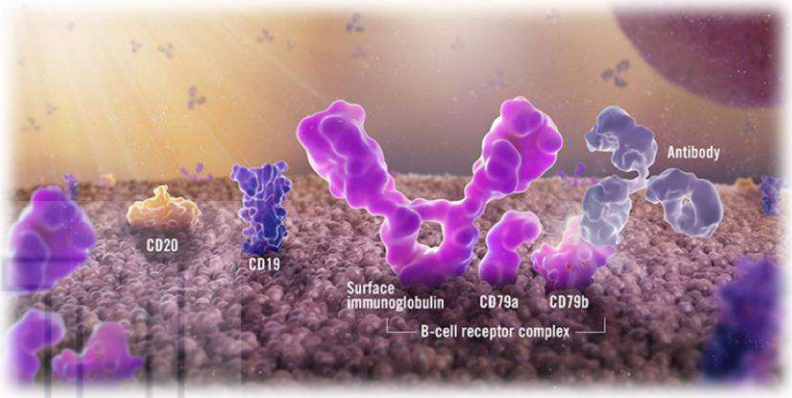
CARCIK-CD19 B-NHL Study: Key Points

- Use of allogeneic cell source vs autologous
  - Improve the product quality and expansion potential
- Not limited to post allogeneic HSCT relapse
  - Extend the patient population
- Explore the partially matched/mismatched setting
  - To promote the *off the shelf* use CARCIK cells (extend treatment to highly aggressive NHL)

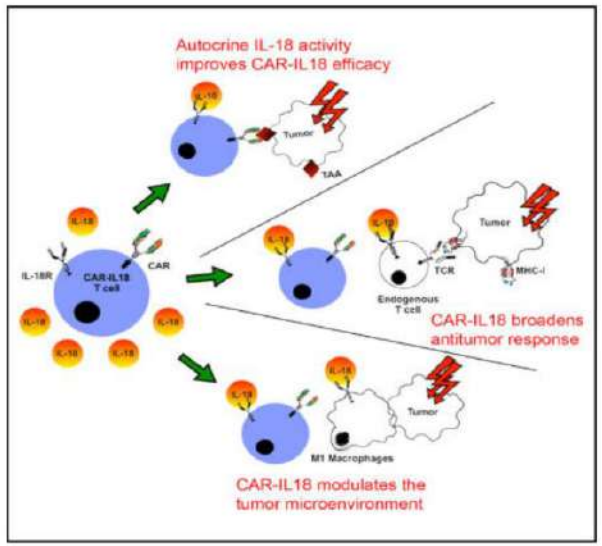
Armored IL-18 CD79b CAR T cells for the treatment of B-cell Lymphomas

Rationale of CD79B targeting:

- B-cell receptor–associated protein;
- Highly expressed in most B-NHL *(Jiang et al., Leukemia 2020)*;
- Still expressed when patients relapse after anti-CD19 and CD22 CAR T cell therapy *(Ormhøj et al., Clin Cancer Res. 2019);*
- The CD79B-targeting antibody–drug conjugate Polatuzumab Vedotin, in combination with Bendamustine and Rituximab shown high remission rates in r/r DLBCL patients *(Sehn et al. J Clin Oncol. 2020)*;



Armored CD79B-IL18 CAR CIK cells to improve anti-tumor efficacy

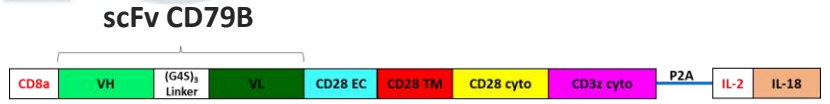


Published in final edited form as:
Cell Rep. 2018 May 15; 23(7): 2130–2141. doi:10.1016/j.celrep.2018.04.051.

Engineered Tumor-Targeted T Cells Mediate Enhanced Anti-Tumor Efficacy Both Directly and through Activation of the Endogenous Immune System

Mauro P. Avanzi[1,4], Oladapo Yeku[1,4,5,*], Xinghuo Li[3], Dinali P. Wijewarnasuriya[3], Dayenne G. van Leeuwen[1], Kenneth Cheung[1], Hyebin Park[1], Terence J. Purdon[1], Anthony F. Daniyan[1], Matthew H. Spitzer[2], and Renier J. Brentjens[1,3,*]

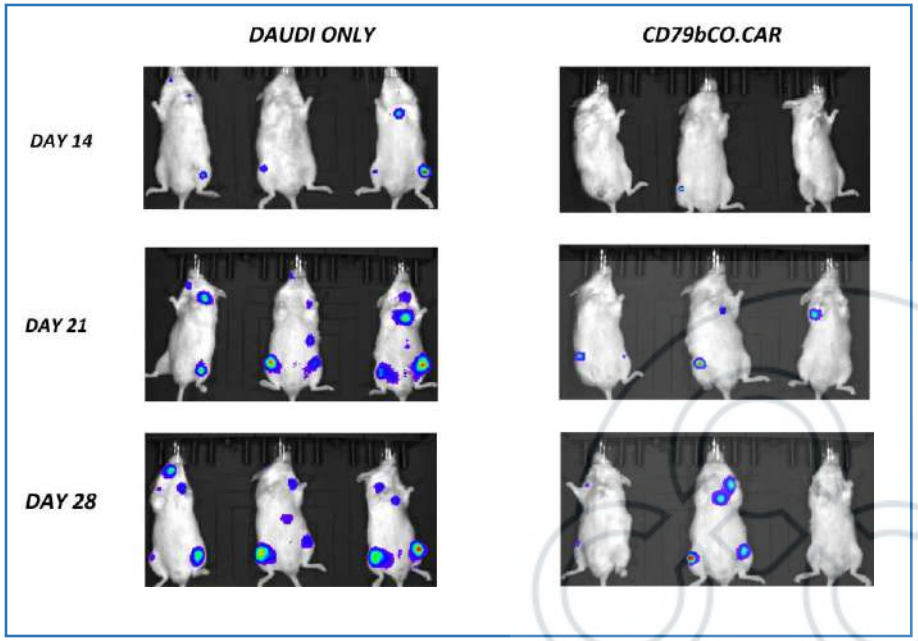
SB-engineered CD79B-IL18 CAR CIK cells

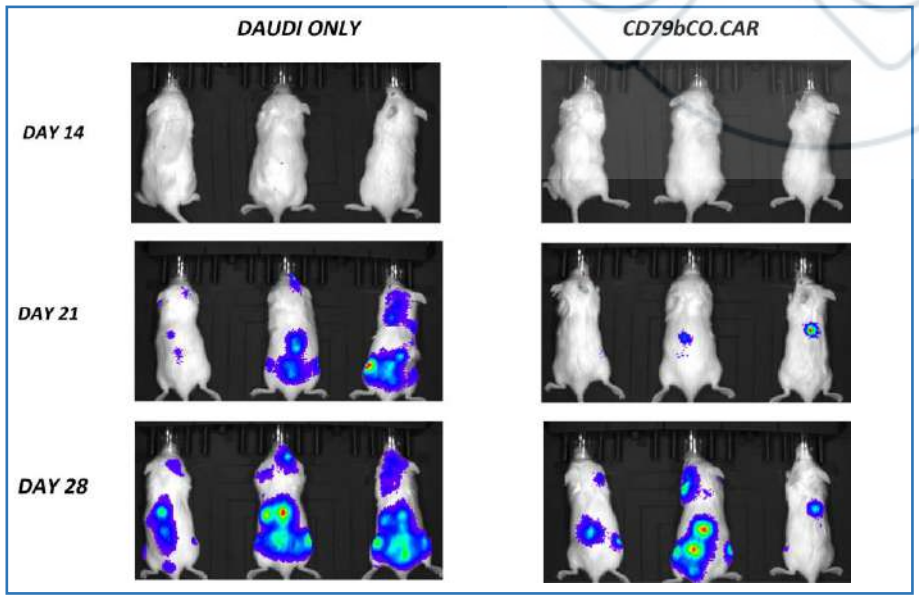


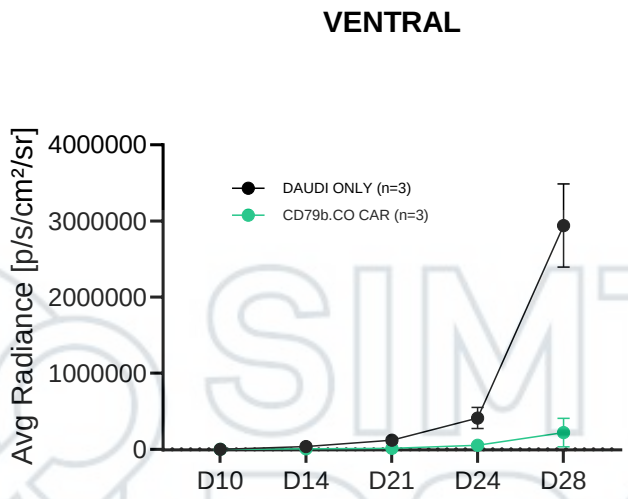
In vitro functional tests
- Cytotoxicity
- Cytokine release
- Proliferation assay

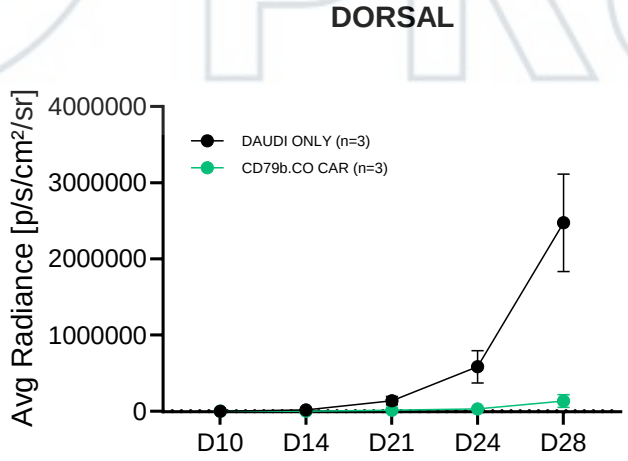
In vivo anti-tumor efficacy and safety

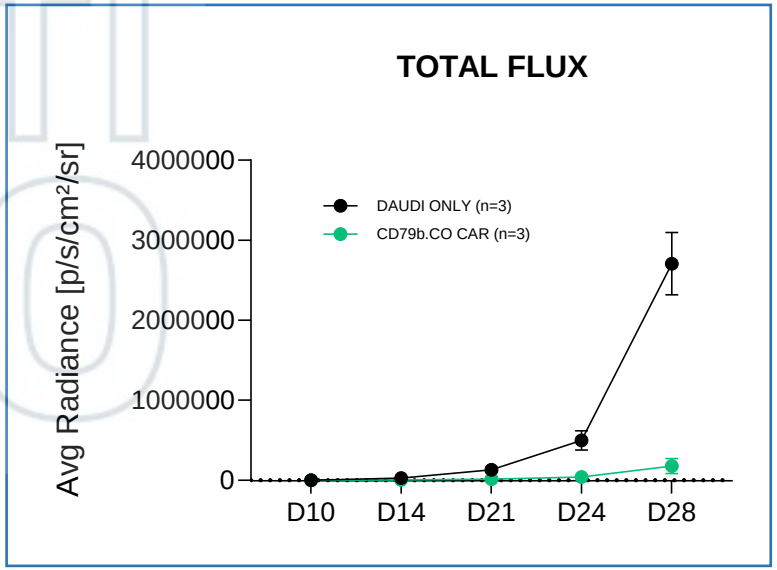
CD79bCO.CAR CIK cells Control Lymphoma progression *In Vivo*











Implementation of CAR-CIK cell platform

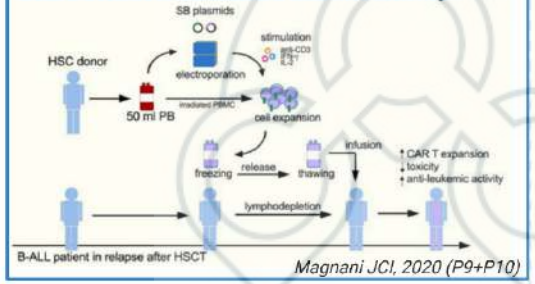
CAR-CIK cell platform

1 Implementation of Non-viral CAR.CIK cell production

2 Allogeneic T cell source & Next generation CARs

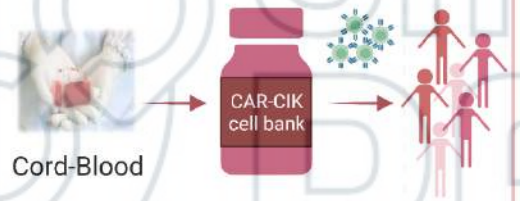
3 CAR & Tumor microenvironment

CARCIK-CD19 Phase I/IIa study

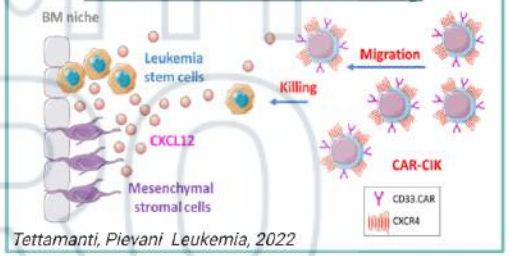


Magnani JCI, 2020 (P9+P10)

Off-the-shelf CAR-CIK therapy

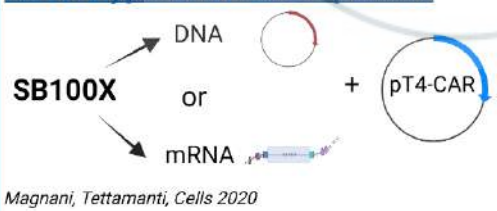


Armored CAR-CIK to enhance TME homing



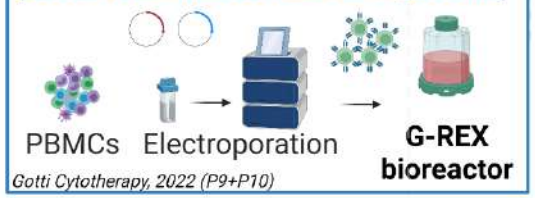
Tettamanti, Pievani Leukemia, 2022

Novel hyperactive transposase



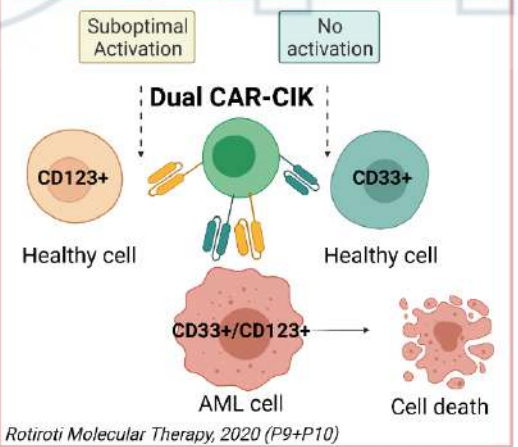
Magnani, Tettamanti, Cells 2020

CAR-CIK culture in closed systems



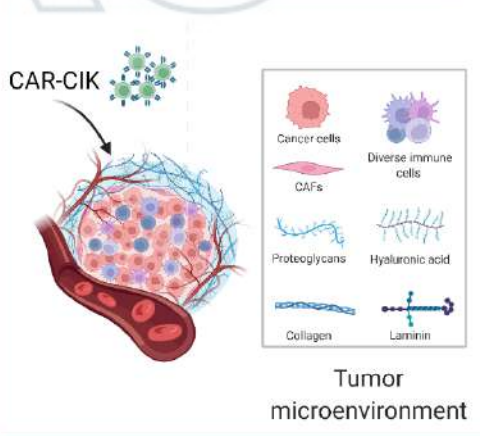
Gotti Cytotherapy, 2022 (P9+P10)

Trans-signaling Dual CAR for AML



Rotiroti Molecular Therapy, 2020 (P9+P10)

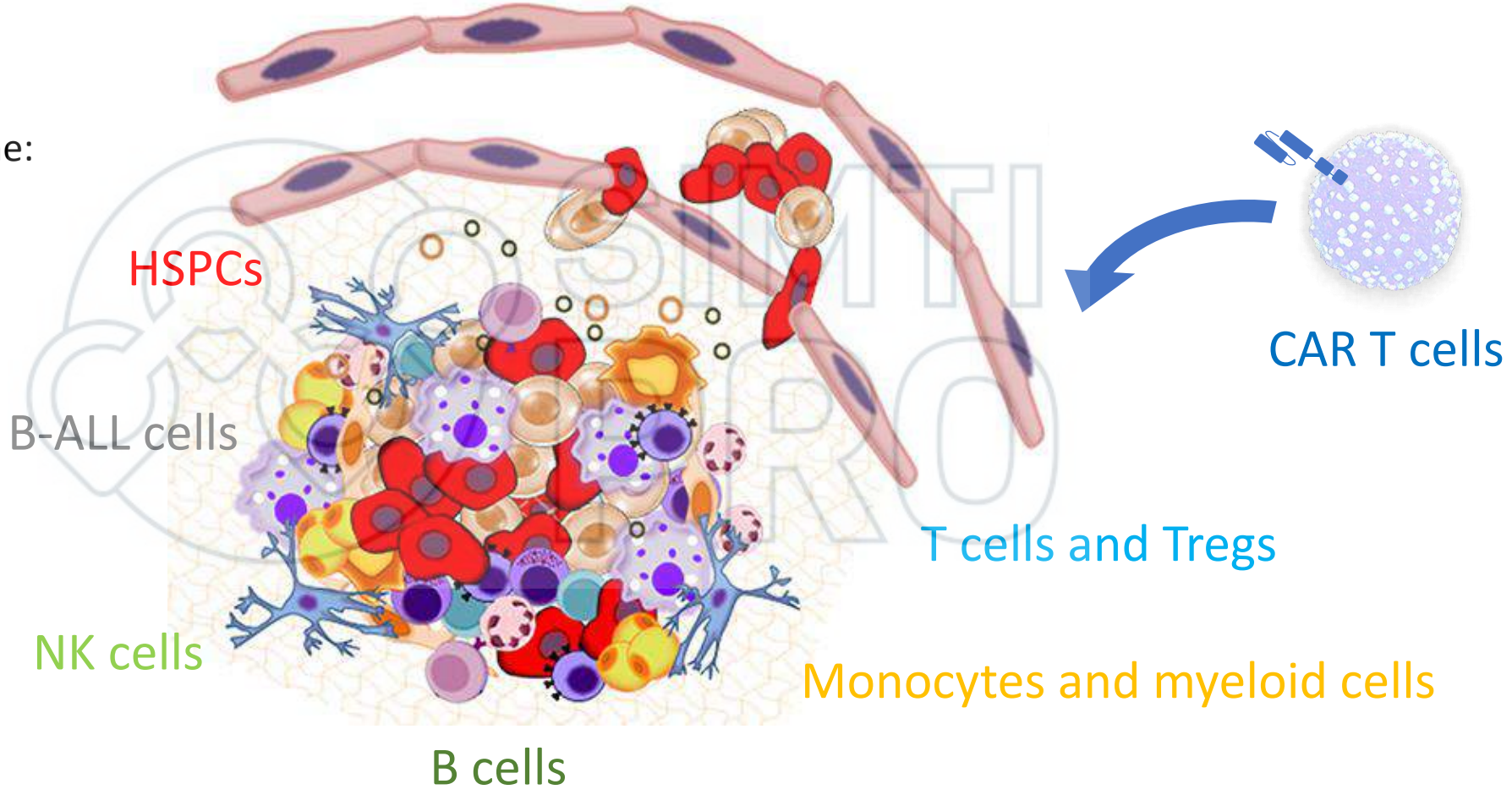
CAR-CIK cells for solid tumors



Contribution of the microenvironment on CAR T cells and endogenous immunity fate is still unclear

The development of leukemia impacts the bone marrow (BM) micro-environment

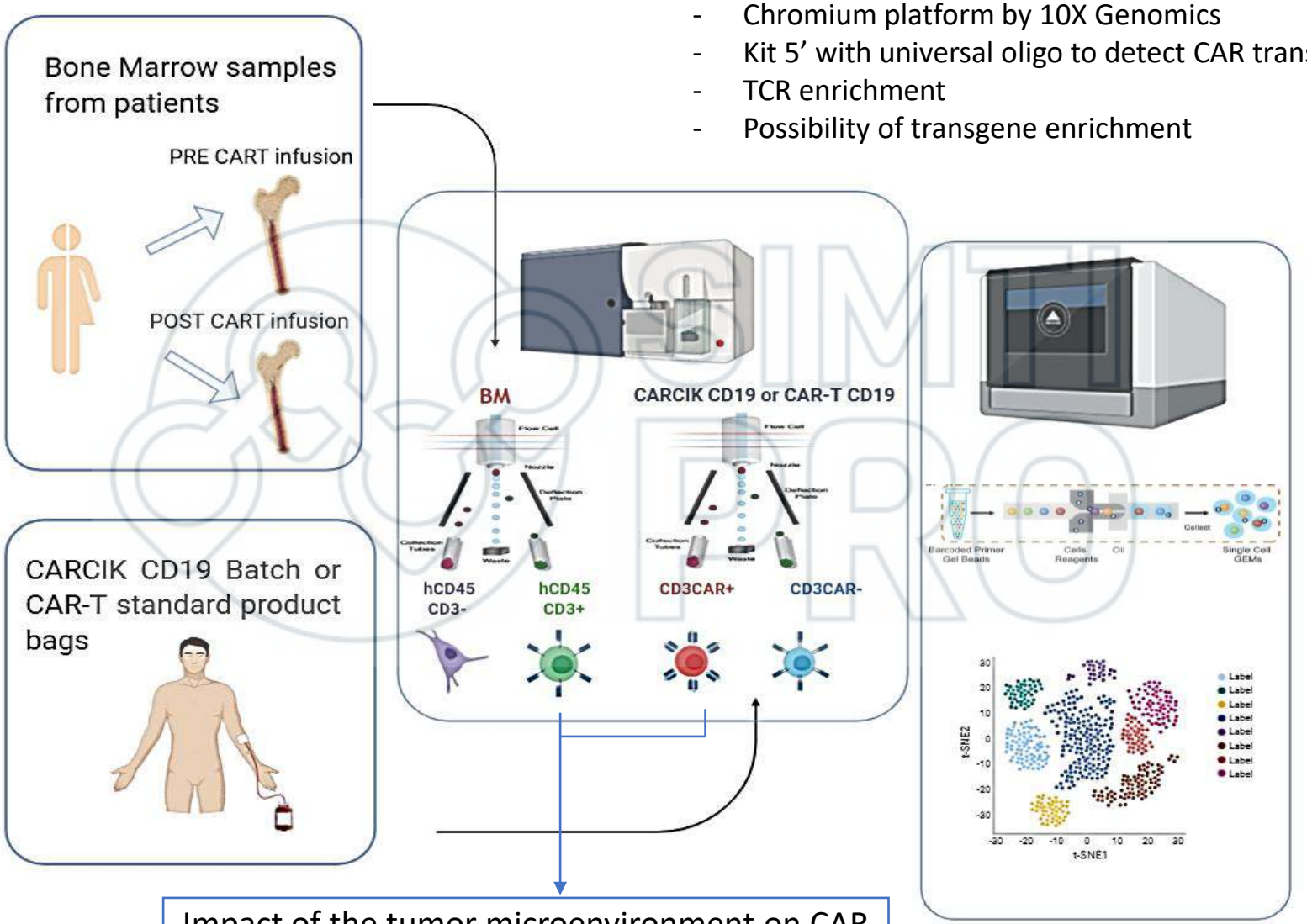
The immune niche:



B-ALL BM microenvironment provides factors that protect leukemic cells and suppress effector T cells

Experimental design to study the interaction between CAR T cells and leukemia microenvironment

- Chromium platform by 10X Genomics
- Kit 5’ with universal oligo to detect CAR transgene and CD19 isoforms
- TCR enrichment
- Possibility of transgene enrichment



Impact of the tumor microenvironment on CAR T cells and the shape of the immune response

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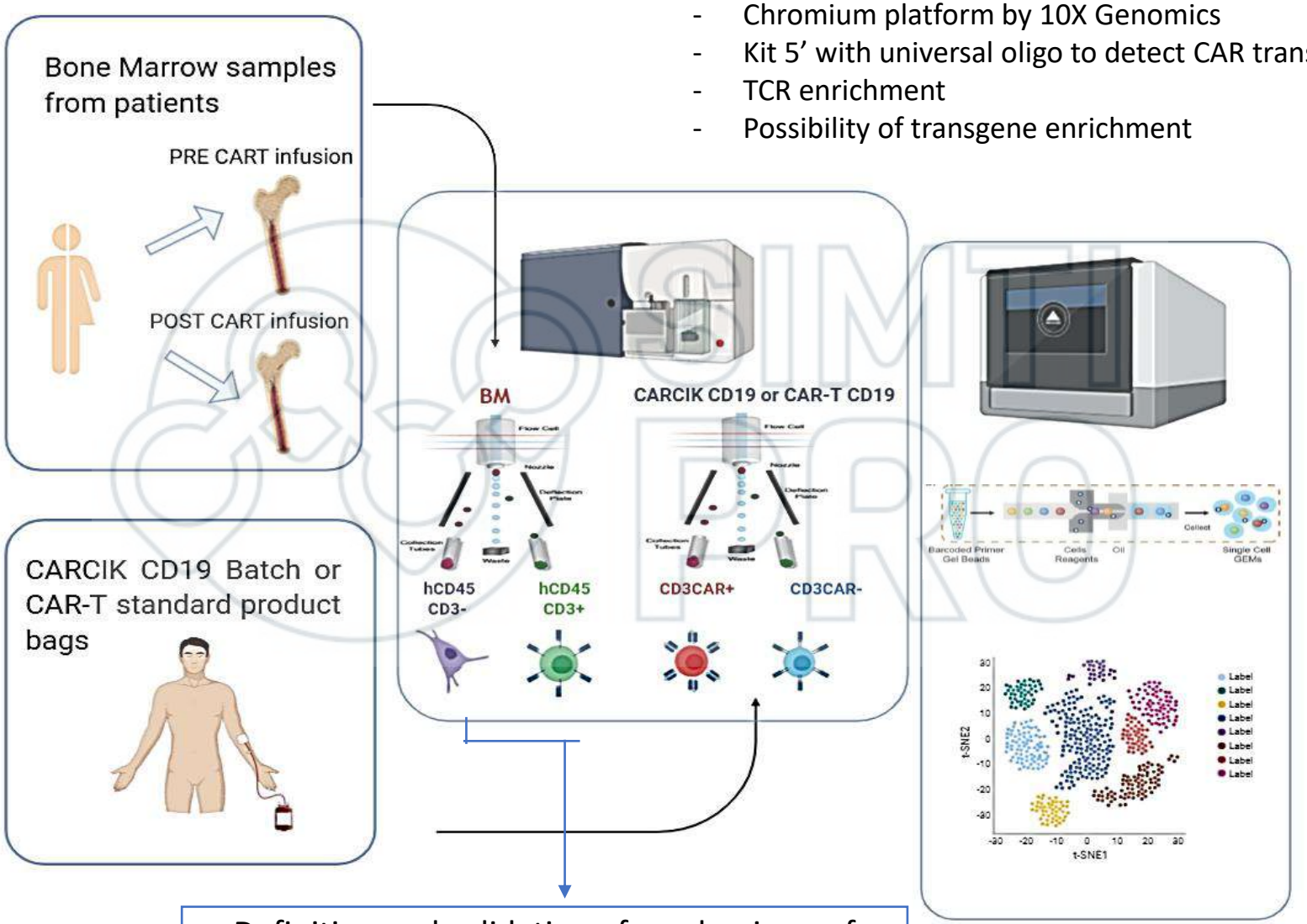
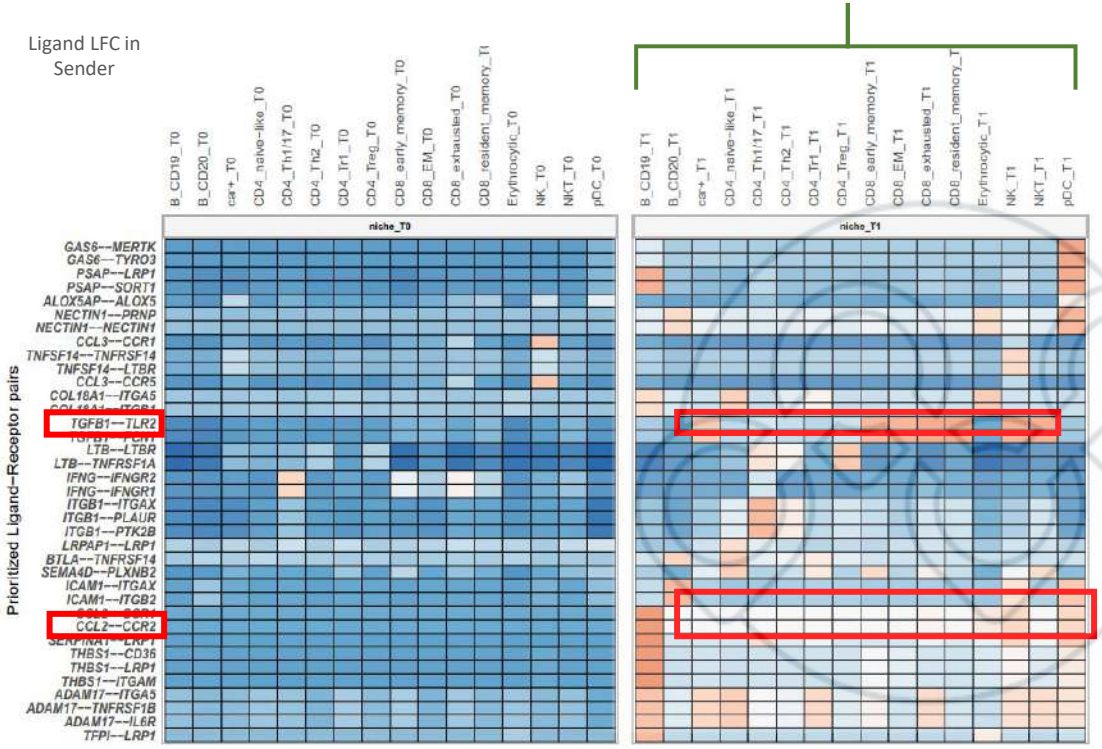
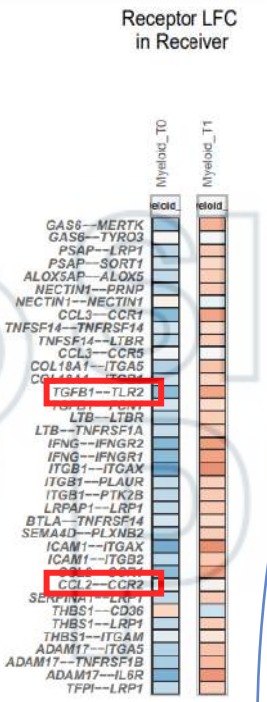


Definition and validation of mechanisms of immune escape by the TME

Modeling intercellular communication suggests induction of HIF1α and immunosuppressive genes in myeloid cells by CAR T cells and endogenous T cells

Sender: all niche

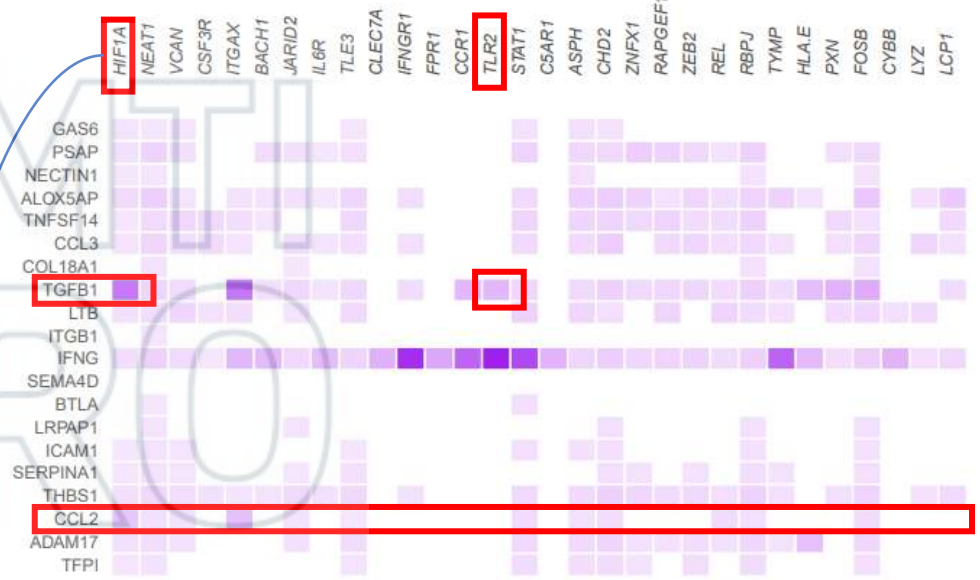


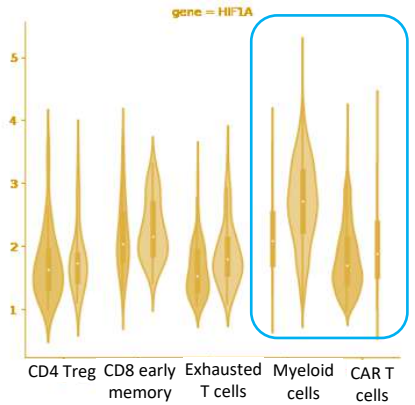


LFC: log fold change in respect to the whole niche at T0

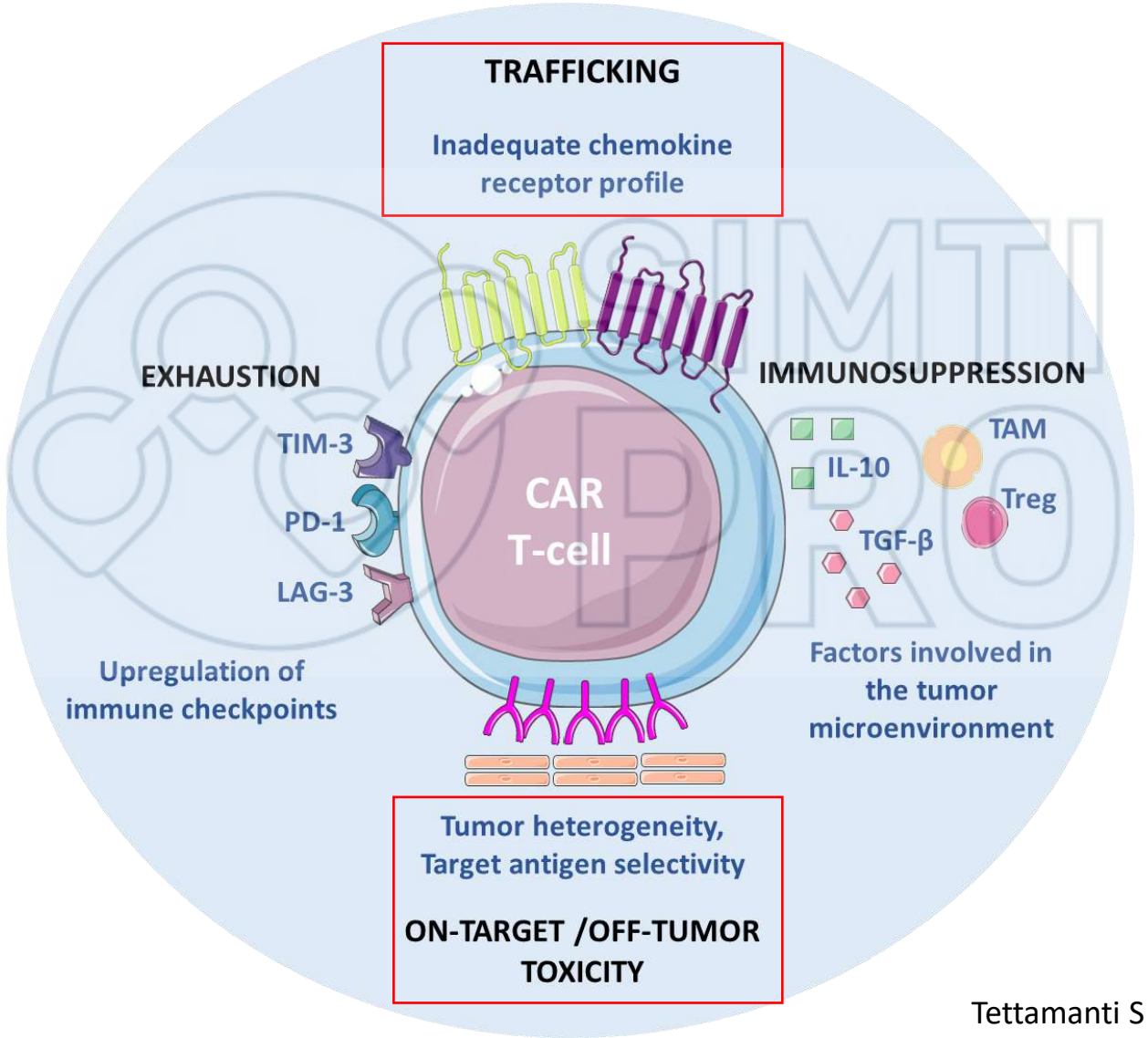
Receiver: myeloid cells

Predicted target gens





AML challenges to CAR T-cell therapy



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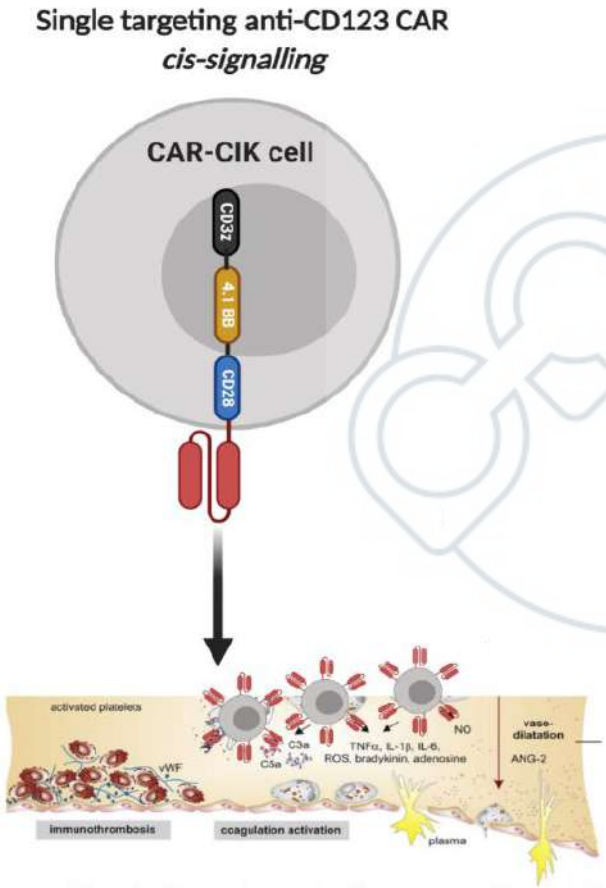
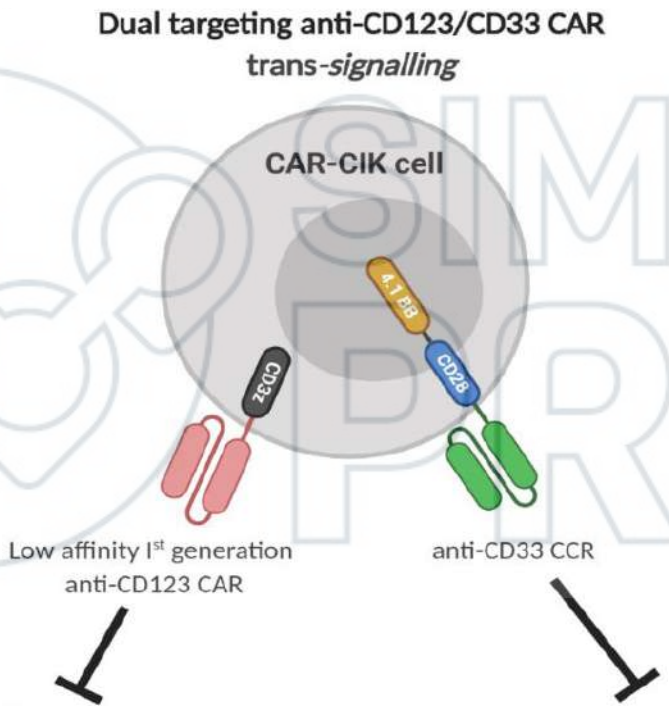
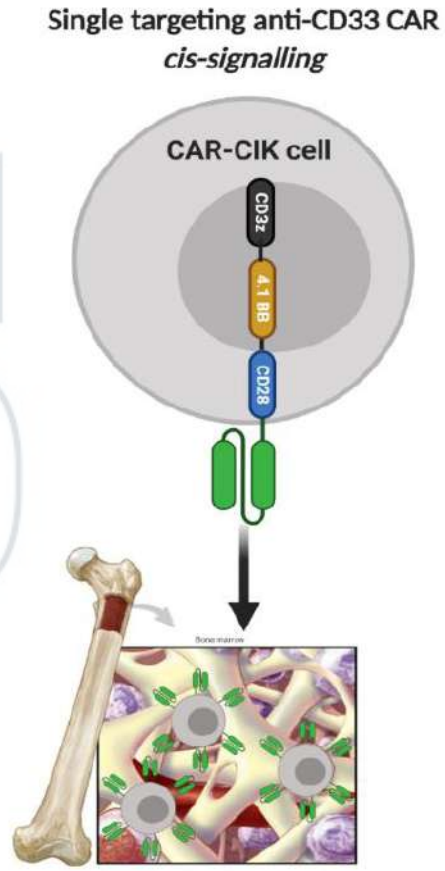
Preclinical developments in AML: Trans-signaling Dual CAR CIK cells



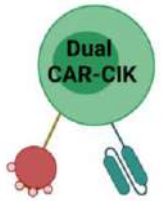
Capillary leak syndrome for "on-target off-tumor" toxicity on CD123 ^{low} endothelial cells





Prolonged myelotoxicity for "on-target off-tumor" effect on CD33 hematopoietic stem progenitor cells

A phase I/II single-arm, clinical trial to evaluate the safety and preliminary efficacy of CD123/CD33 dual CARCIK cell for relapsed/refractory acute myeloid leukemia

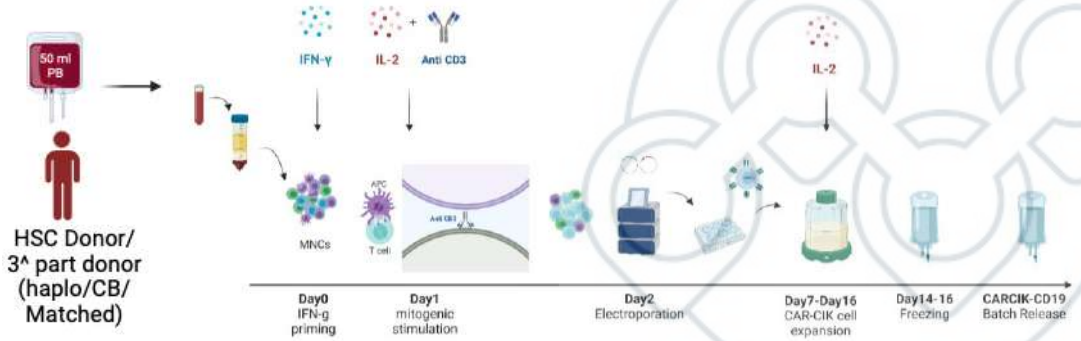


Lack of AML-specific antigens: CD123 expressed on LSC

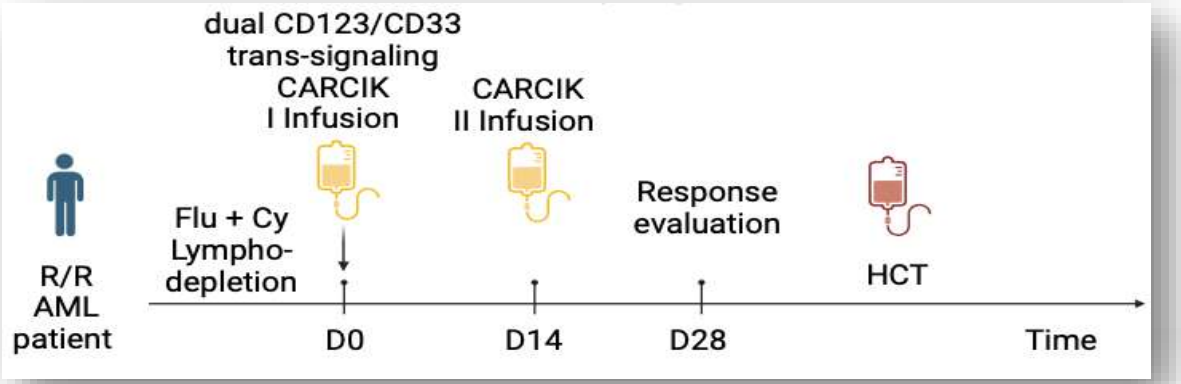
"On target off-tumor" toxicities & Target downmodulation: Transignaling Dual CAR

Exhausted Autologous source: Use allogeneic source with CIK, low risk of GvHD

AML induced immunosuppression: Use allogeneic source with CIK and Lymphodepletion to inhibit Treg



- Expression of CD123 and CD33 on AML blasts at the time of screening
- HCT donor availability
- Fit for HCT
- Patents with relapse after allogeneic stem cell transplant (allo-SCT) will be eligible; need to be off of all immunosuppression for ≥6 weeks, with no more than grade 1 chronic graft-versus host disease (cGVHD)

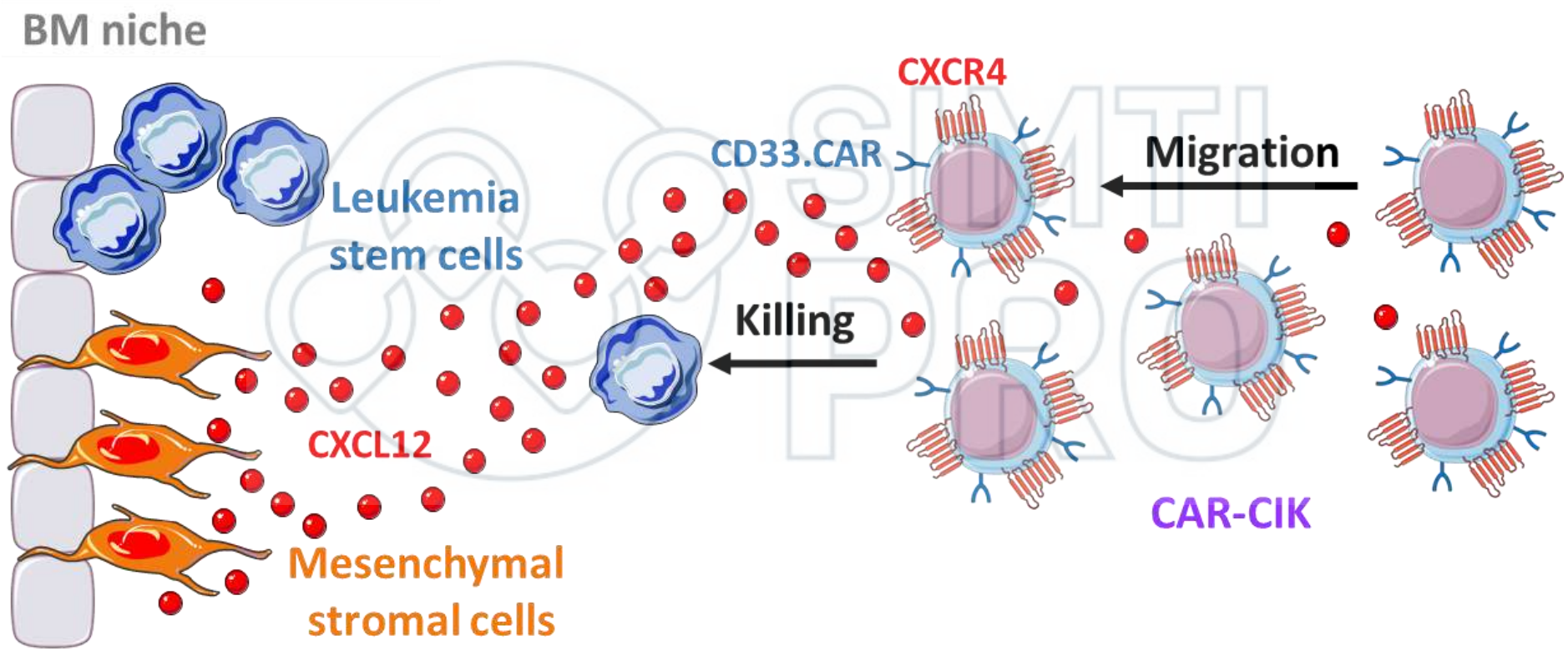


Primary study objective

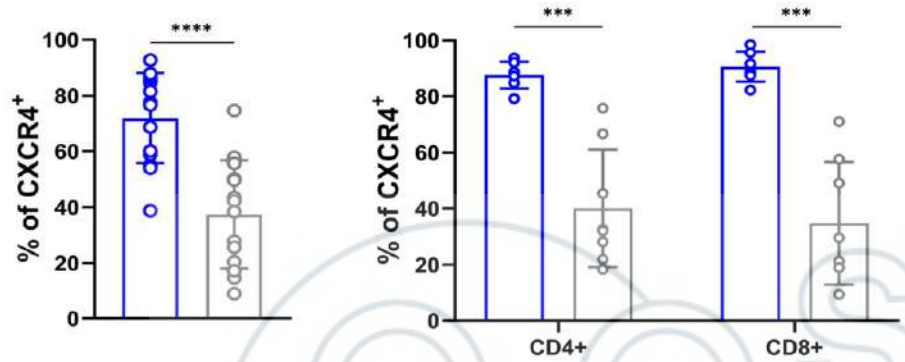
Part A – Dose escalation (Phase Ia)
To determine the maximun tolerated dose (MTD) and recommended phase 2 dose (RP2D) and safety profile of CD123/CD33 dual CARCIK therapy

Part B – Dose Expansion (Phase II)
To determine the efficacy of CD123/CD33 dual CARCIK therapy in terms of complete response rate

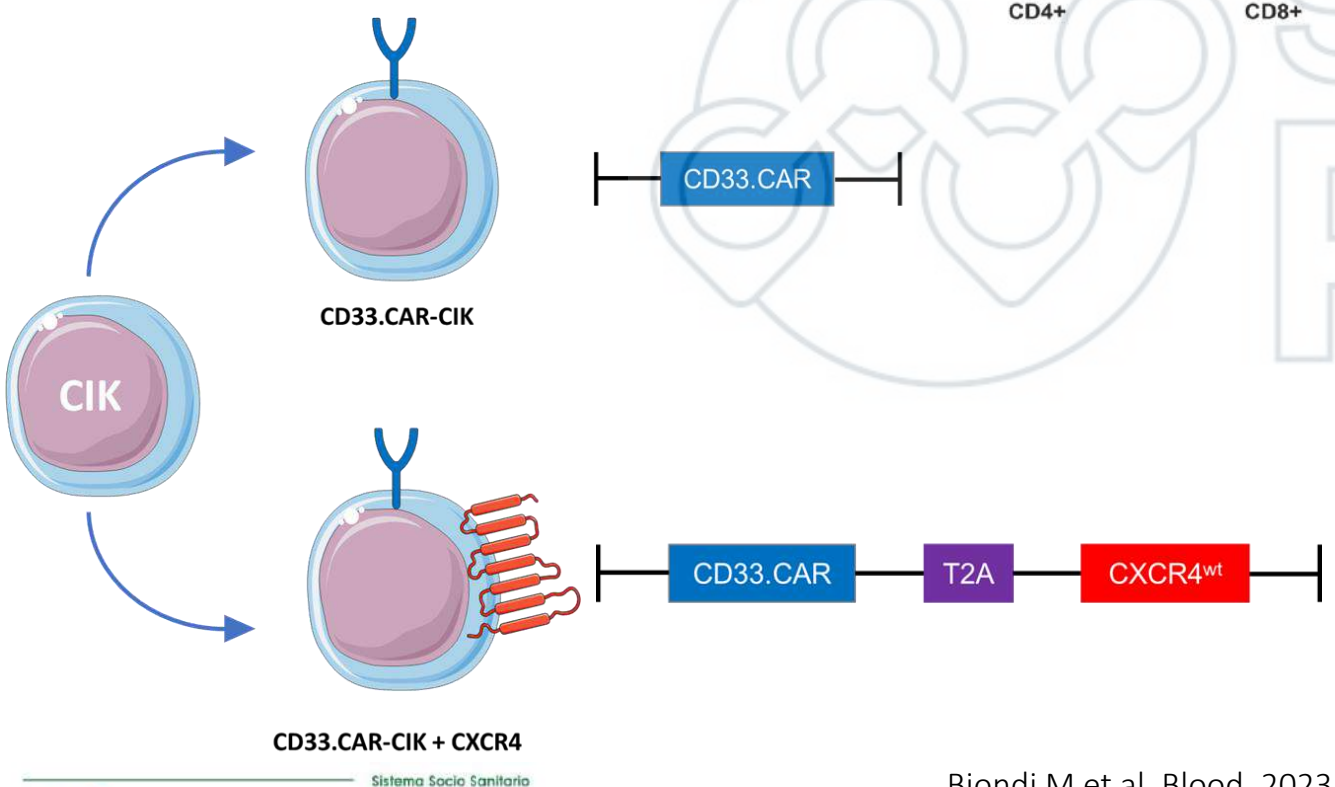
Preclinical development in AML:
Armored CAR-CIK CXCR4-modified CD33.CAR-CIK with enhanced BM homing

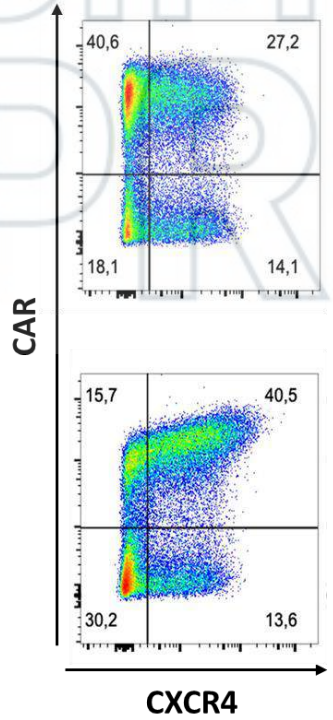


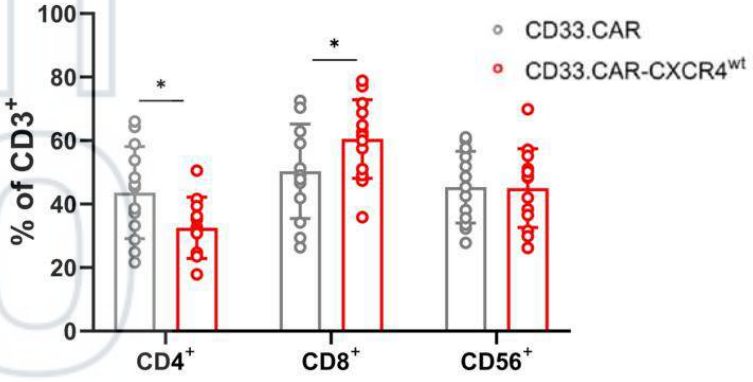
Engineering CXCR4-modified CD33.CAR-CIK cells

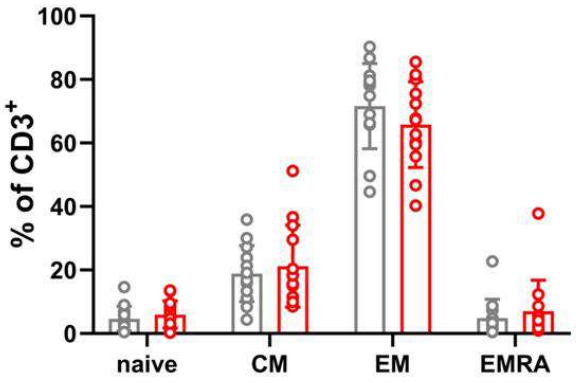


CXCR4 expression drops during CAR-CIKs expansion









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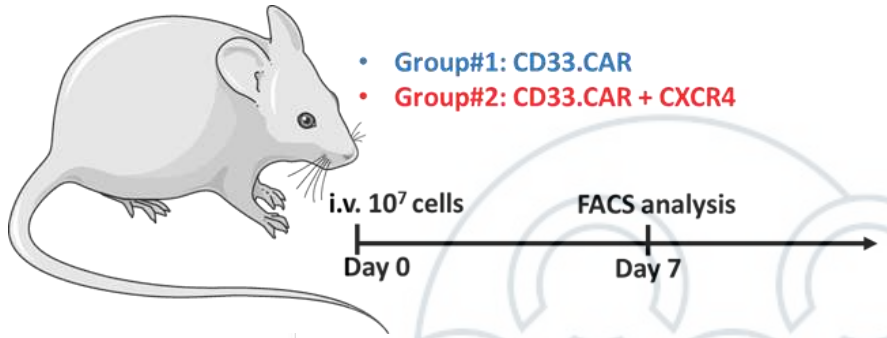
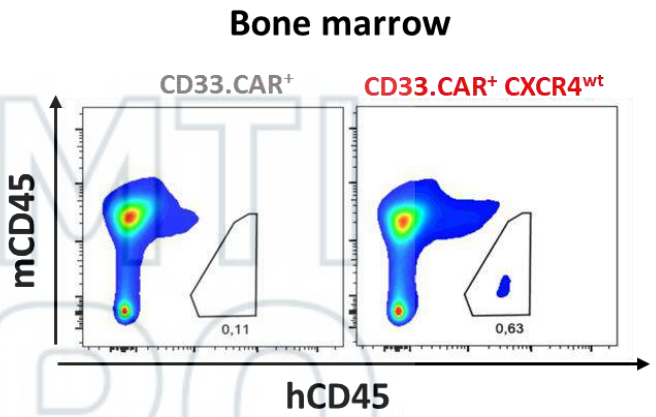
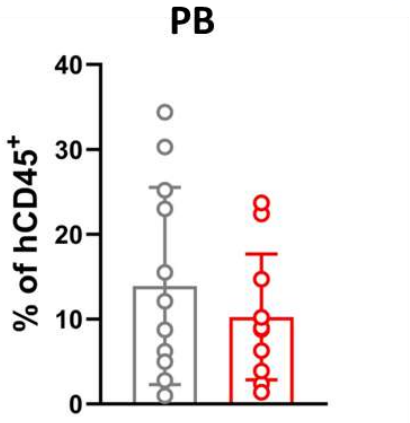
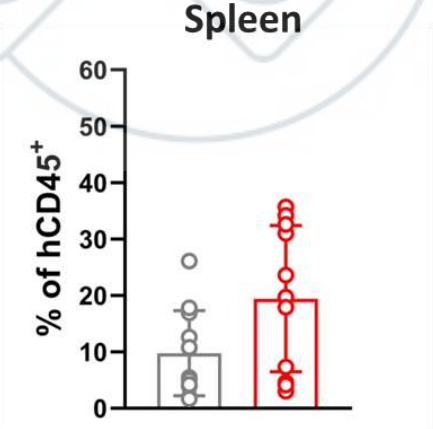
Università degli Studi di Milano Bicocca

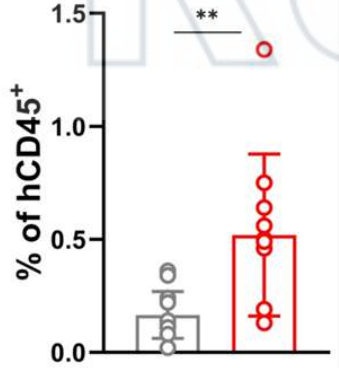
CXCR4-modified CD33.CAR-CIK: *in vivo* homing to the bone marrow

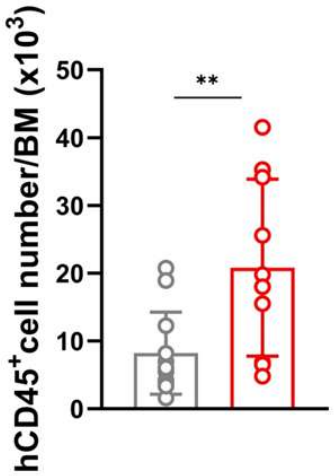




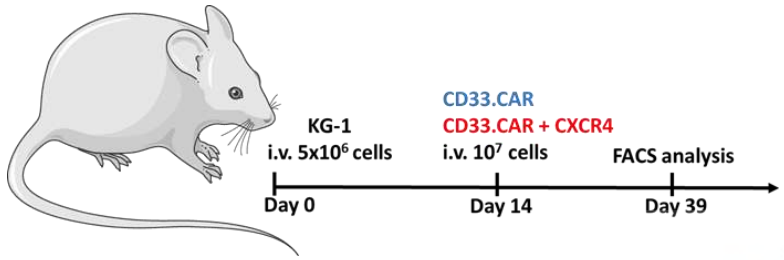


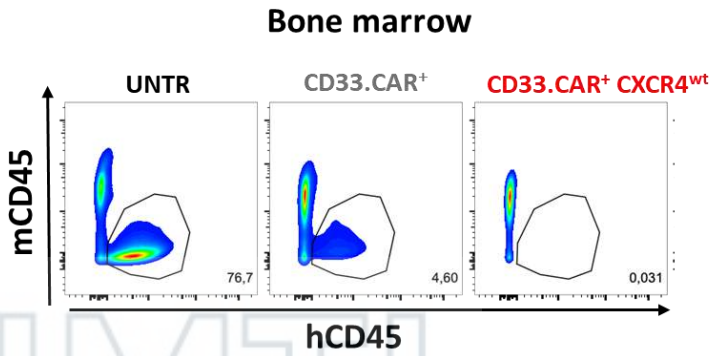


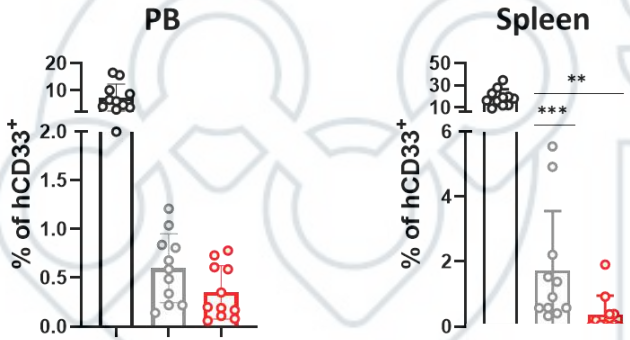


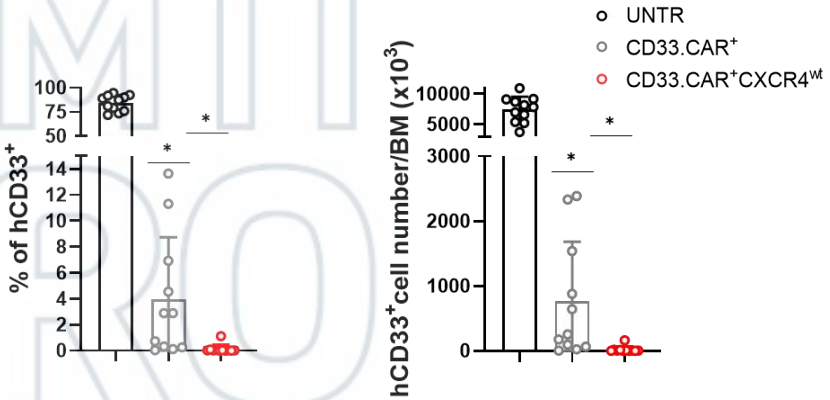


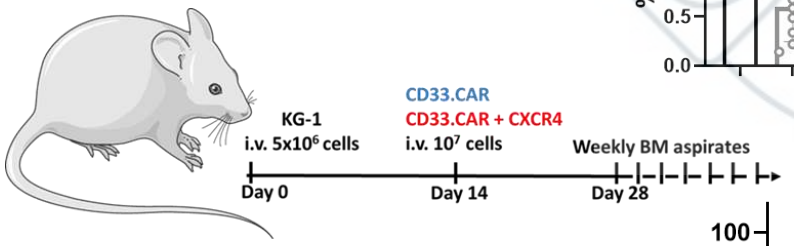
CXCR4-modified CD33.CAR-CIK: *in vivo* antileukemic activity

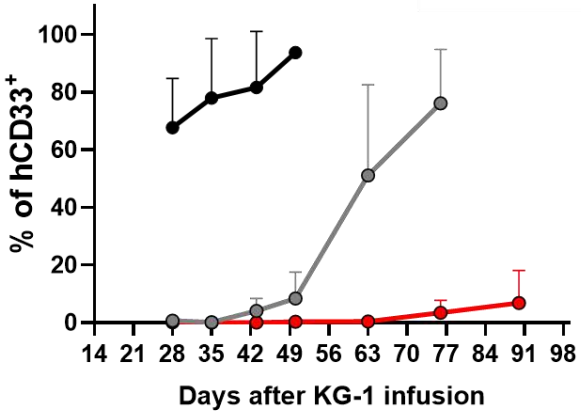


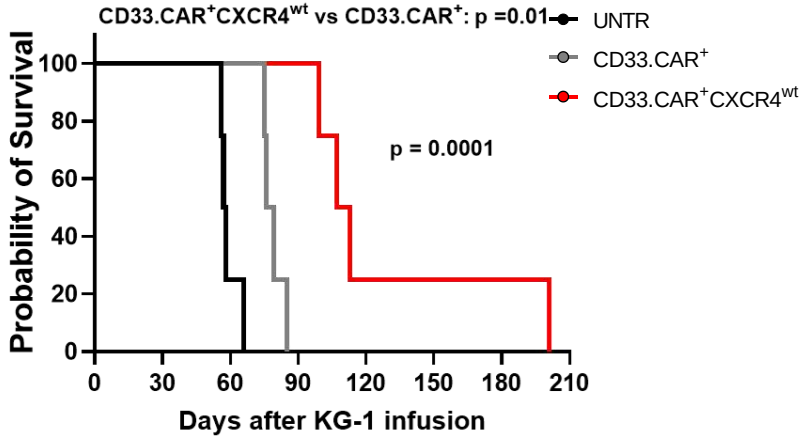












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Conclusions and future perspectives

- The non-viral Sleeping Beauty-derived CAR CIK cells are a solid versatile CAR-T platform alternative to viral vectors, with reduced CoG and simplified production processes→ to be further implemented with mRNA SB100X transposase and more closed systems
- The non viral SB platform can be adopted to derive “off-the-shelf” Cord Blood derived CAR CIK cells
- Cord-blood derived CD19CAR-CIK cells showed *in vitro* and *in vivo* potent antileukemic activity
- The non-viral CAR CIK cell platform can be exploited to generate next-generation CARs, such as Dual CARs or Armored CARs
- Dual CD123/CD33 CAR-CIK cells mediate high anti-leukemic efficacy through trans-acting costimulation
- Arming anti-AML CAR-CIK cells with CXCR4 represents a promising strategy to increase CAR therapeutic potential
- The non-viral CAR CIK cell platform could be used to generate CAR CIK cells against solid tumors.

Aknowledgements

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