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

Convegno Educazionale GITMO

LE TERAPIE CELLULARI IN EMATOLOGIA
TRA PASSATO, PRESENTE E FUTURO

Brescia, 28-29 novembre 2025

Centro Paolo VI

La terapia con cellule indotte da citochine con recettore chimerico: le CARCIK

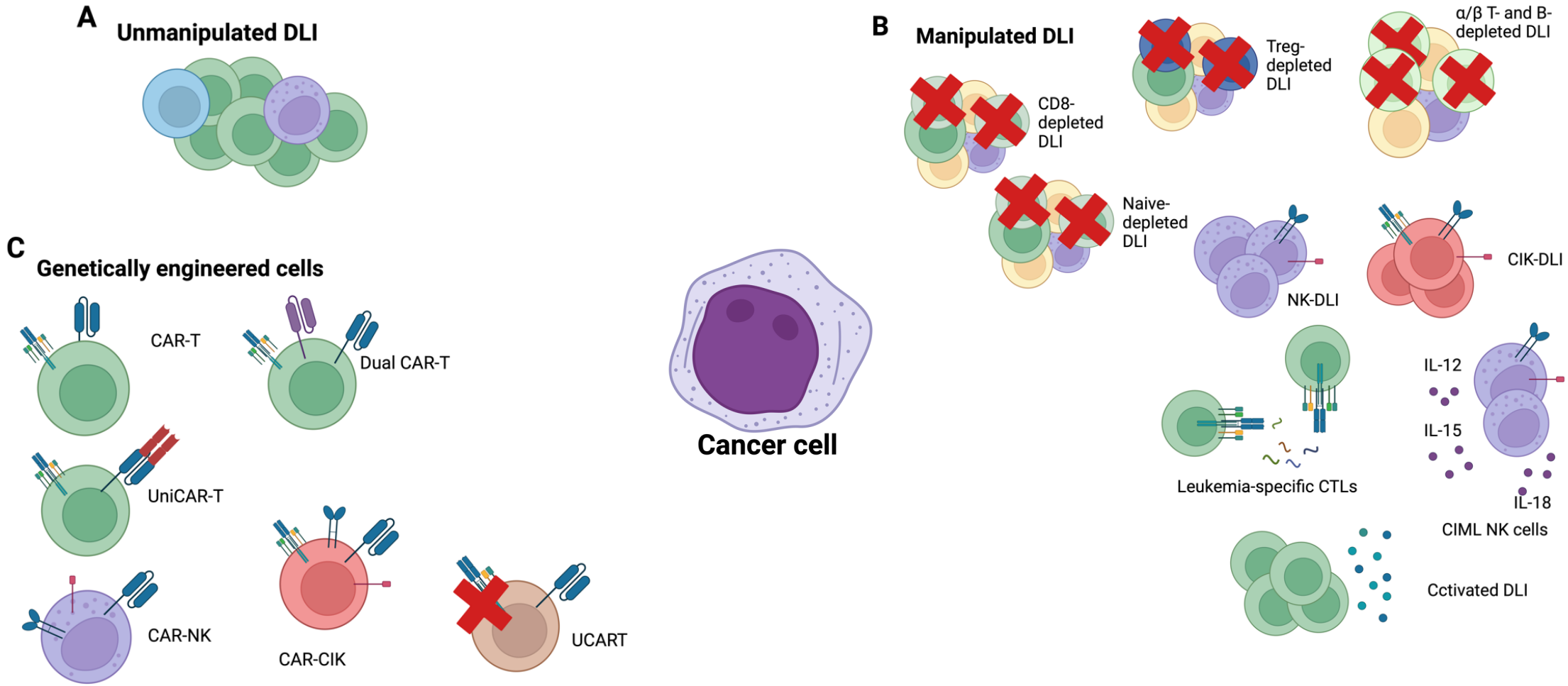
Brescia, 29 Novembre 2025
Benedetta Rambaldi, MD, PhD
ASST Papa Giovanni XXIII, Bergamo

Azienda Ospedaliera
Papa Giovanni XXIII
Bergamo

Disclosures of Benedetta Rambaldi

I have nothing to disclose

Possible adoptive cellular therapy strategies in the post-HCT setting



Rambaldi B et al., *Front Immunol* 2024

DLI: Bergamo experience

Patients treated with DLI between 2015-2025 in Bergamo
N= 133 (21% of all HCT performed)

DLI doses:

- **MRD**

5 - 5 - 10 x 10^6/Kg

- **MUD/MMUD**

1 - 5 - 10 x 10^6/Kg

- **Haplo**

0.5/1 - 5 - 10 x 10^6/Kg

	N (%)
Donor	
MMR	32 (24.1)
MUD	68 (51.1)
MMUD	24 (18)
Haplo	9 (6.8)
Disease	
AML	71 (53.4)
ALL	14 (10.5)
MDS	9 (6.8)
NHL/CLL/HL	11 (8.3)
MF	20 (15)
Other (CML, MDS/MPD, AA)	8 (6)
DLI Indication	
Chimerism induction	61 (45.9)
Minimal residual disease relapse	35 (26.3)
Hematological relapse	37 (27.8)
Median N° DLI infusion	3 (1-7)
Median Time (days) between HCT and 1° DLI	83 (36-963)

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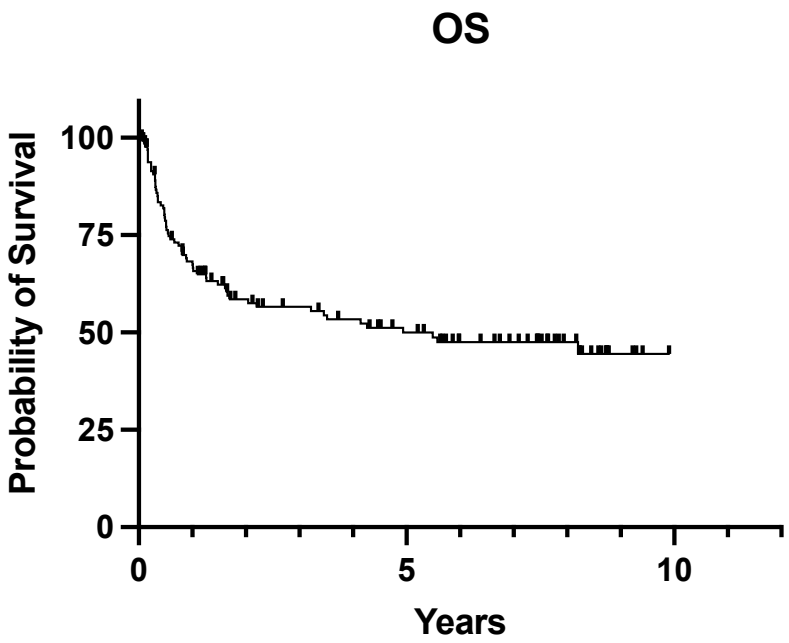
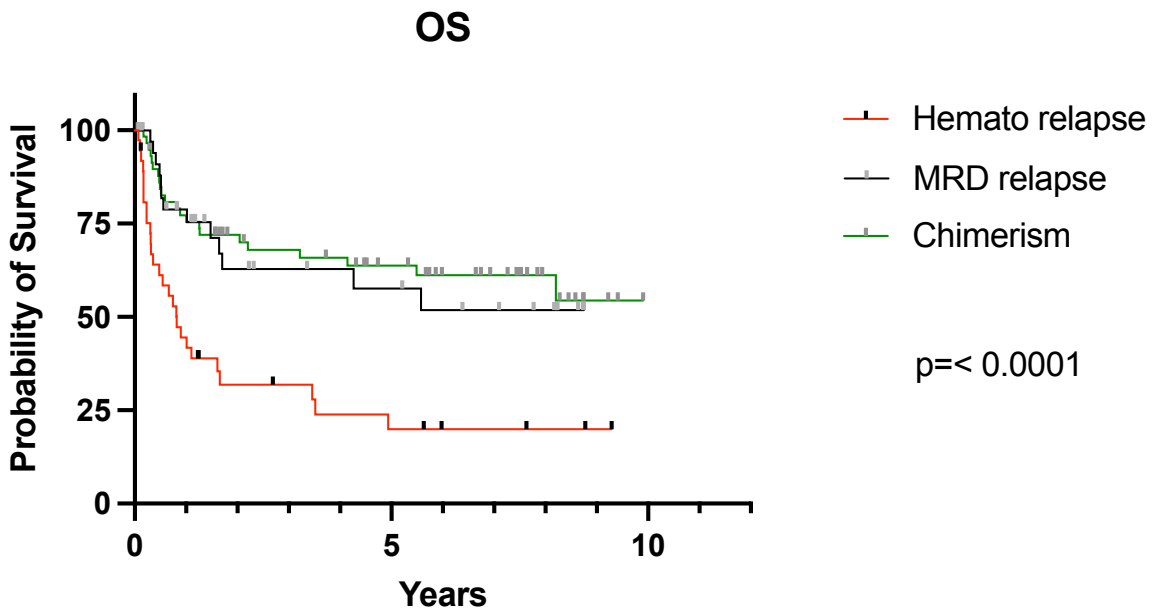
Data unpublished, please do not post

Survival and GVHD incidence in DLI treated patients



Median Os 5.5 yrs



aGVHD all grade 29 (21.8%) with a median time of 57 (7-189 days)
cGVHD all grade 41 (30%) with a median time of 119 (41-602 days)
TRM post DLI 4 (3%)

Median OS 9,6 months Hemato relapse
Median OS not reached MRD relapse
Median OS not reached Chimerism

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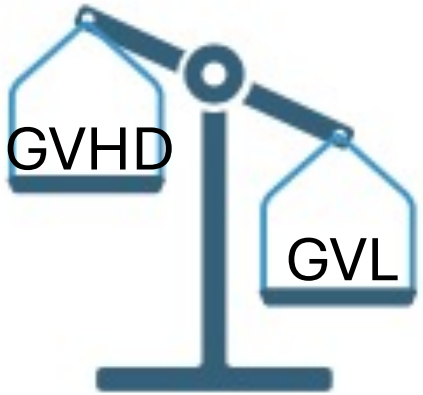
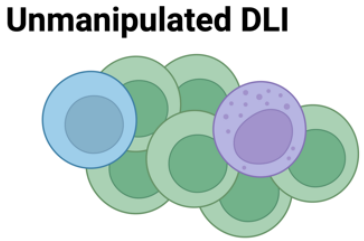
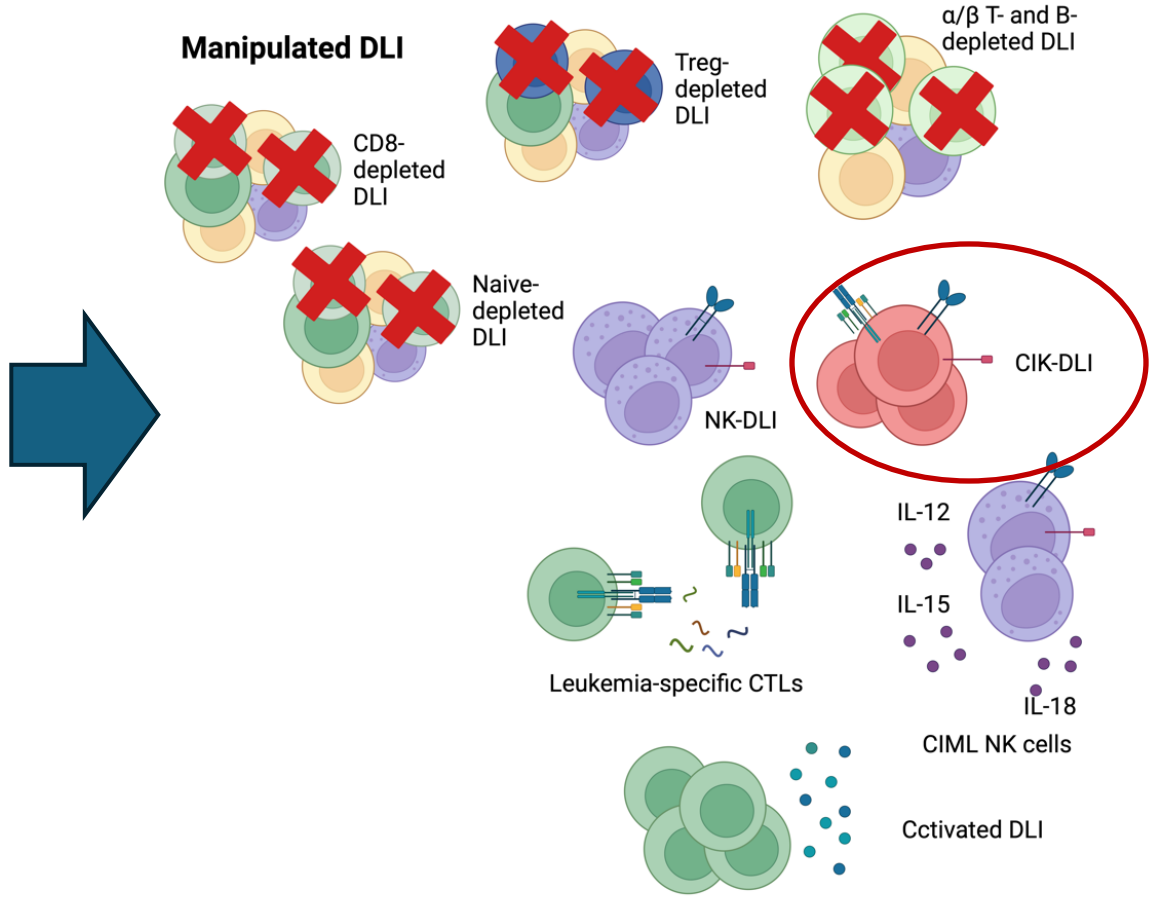
Data unpublished, please do not post

Enhancing GVL activity and hampering GVHD

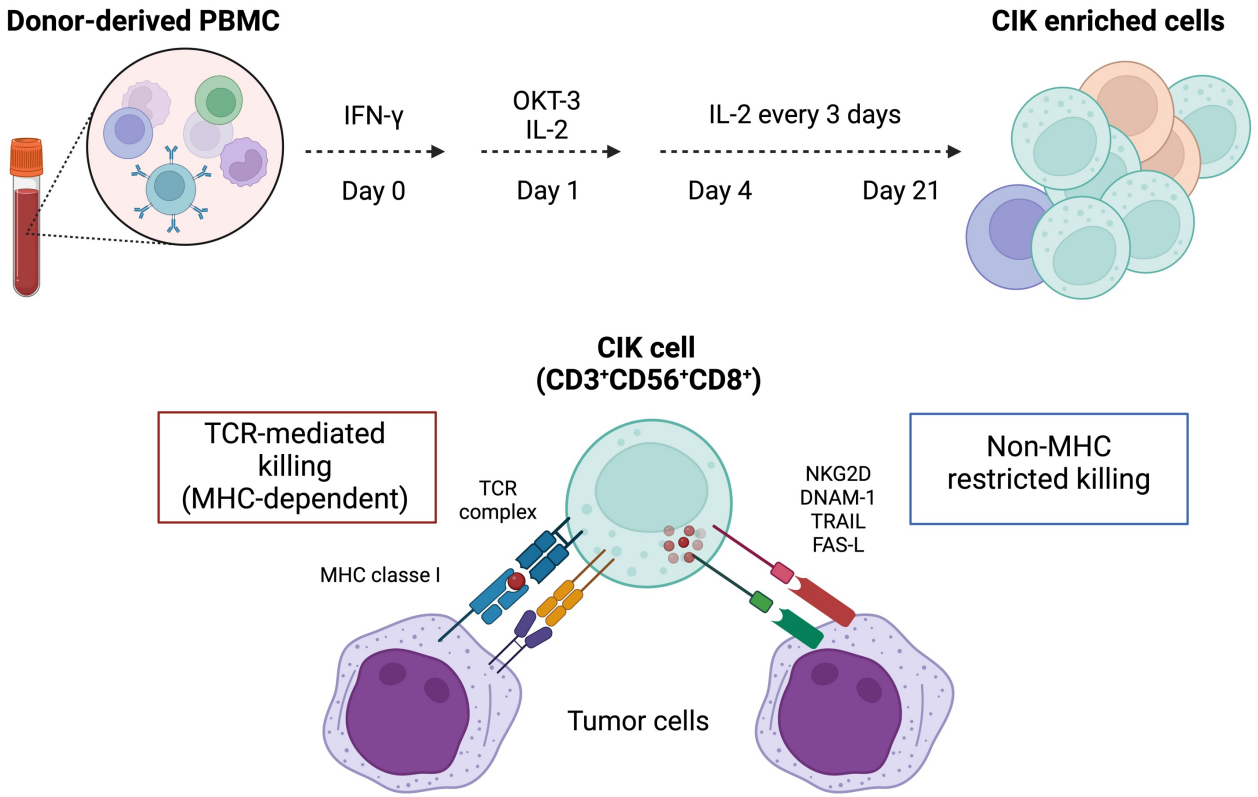


enhance GvL hampering GvHD effect



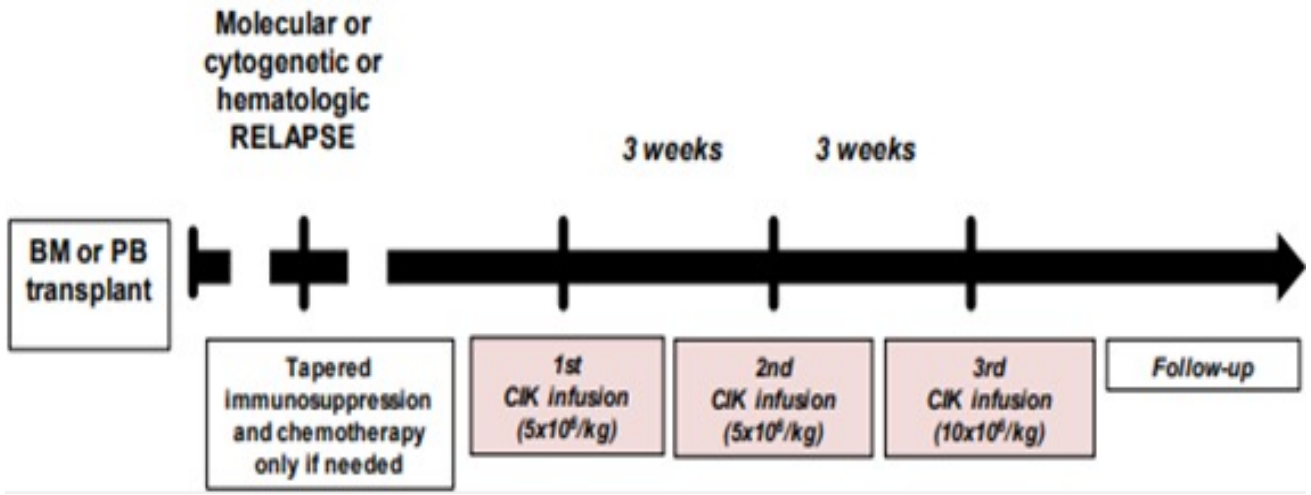


Allogeneic CIK cells enhance GvL hampering GvHD effect



1 Introna M, et al. Bone Marrow Transplant. 2006; 2 Pievani et al, Blood, 2011; 3 Linn et al. Journal of Biomed and Biotech 2010; 4 Sangiolo et al. Journal of Cancer 2011; 5 Introna et al, Haematologica 2007; 6 Rambaldi A (2015) Leukemia 29(1):1-10; 7 Introna M (2017) Biol Blood Marrow Transplant. 23(12):2070-8; 8 Golay J etal.: Cytotherapy. 2018 Aug;20(8):1077-1088 ; 9 Magnani C et al.: Journal of Clinical Investigation 2020

Haplo-CIK Trial and Compassionate use



- Phase I/II study on HaploCIK (EudraCT number *2018-000716-24)* → *enrolled N=19* → *treated N=13*

- Compassionate Use →enrolled N=26 → *treated N=18*
  - *To be treated N=2*
  - *On hold due to GVHD or toxicity N=4*
  - *Dead for disease progression before infusion N=2*

  → *Evaluable day 21 N=15*

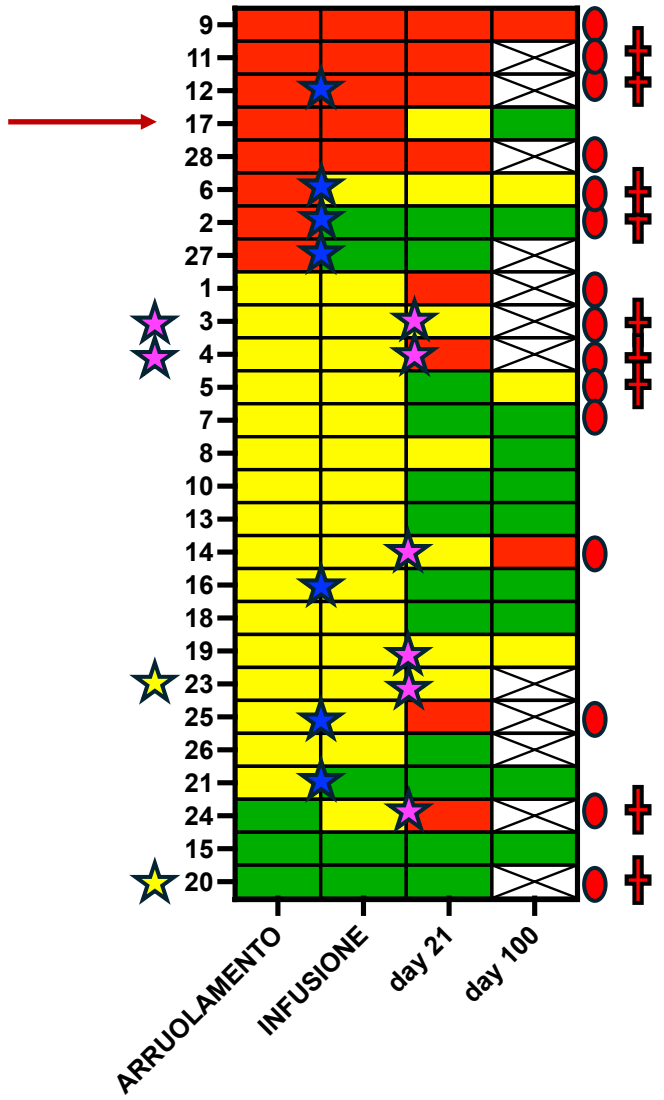
Scannella A and Lussana F SIE 2024

Haplo-CIK Trial and Compassionate use

Evaluable patients 28 (Phase I/II study
N=13, Compassionate Use Study N=15)

10/28 Alive in CR (35%)

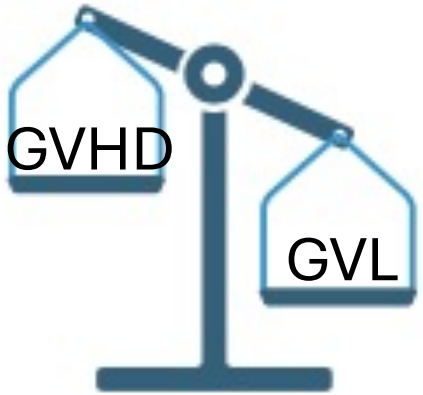
- Complete Response MRD neg and full donor Chimerism
- MRD Relapse or Mixed Chimerism
- Hematological Relapse
- Bridging Therapy
- Concomitant Therapy
- Therapy after relapse, but before CIK request
- Relapse/Progression
- Dead



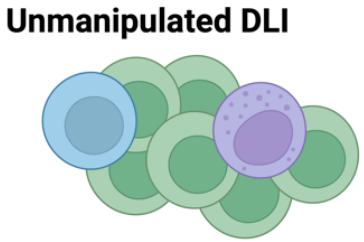
1 aGVHD G1
1 cGVHD lieve

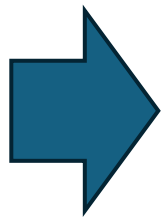
Update will be presented at ASH 2025 as poster presentation

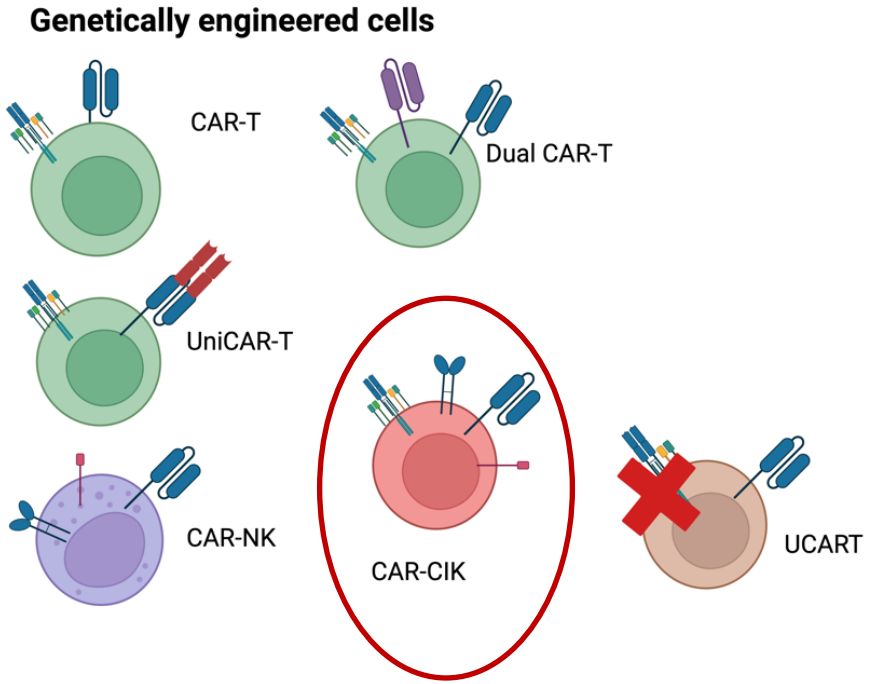
Enhancing GVL activity and hampering GVHD



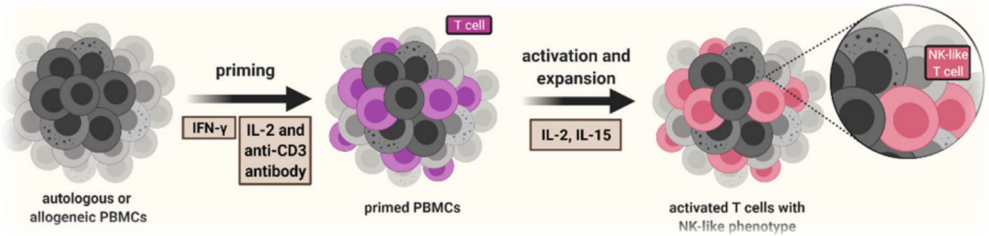
enhance GvL hampering GvHD effect



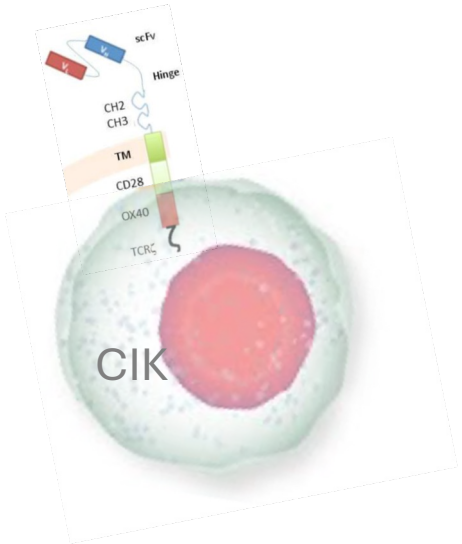


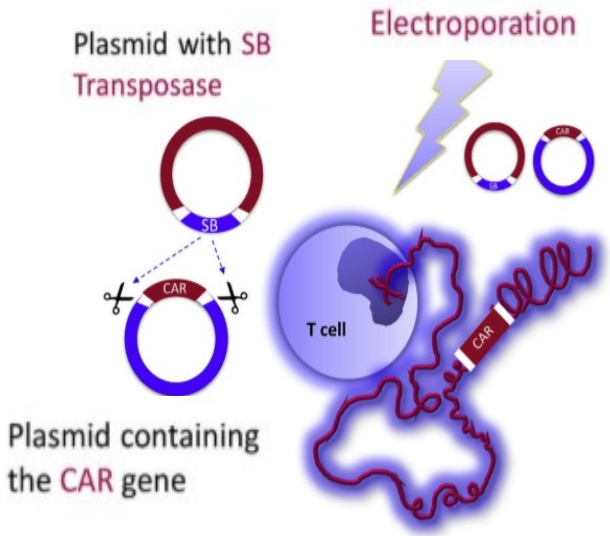


Our approach: Non-Viral Sleeping Beauty Transposon system and allogeneic CARCIK



Cytokine-Induced Killer cells as *effector cells*





Sleeping Beauty

Non-viral *gene transfer manufacturing*

Viral vectors

Efficient /standardized but time-consuming, expensive, non random integration

TRANSPOSONS

Mobile DNA elements naturally occurring in the genome

Easy to purify, non-immunogenic, random pattern of integration

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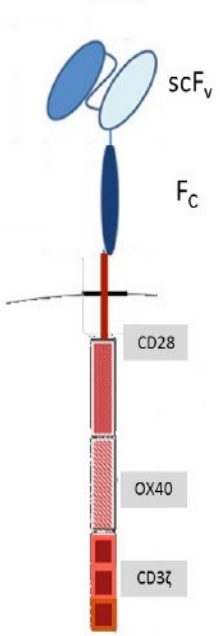
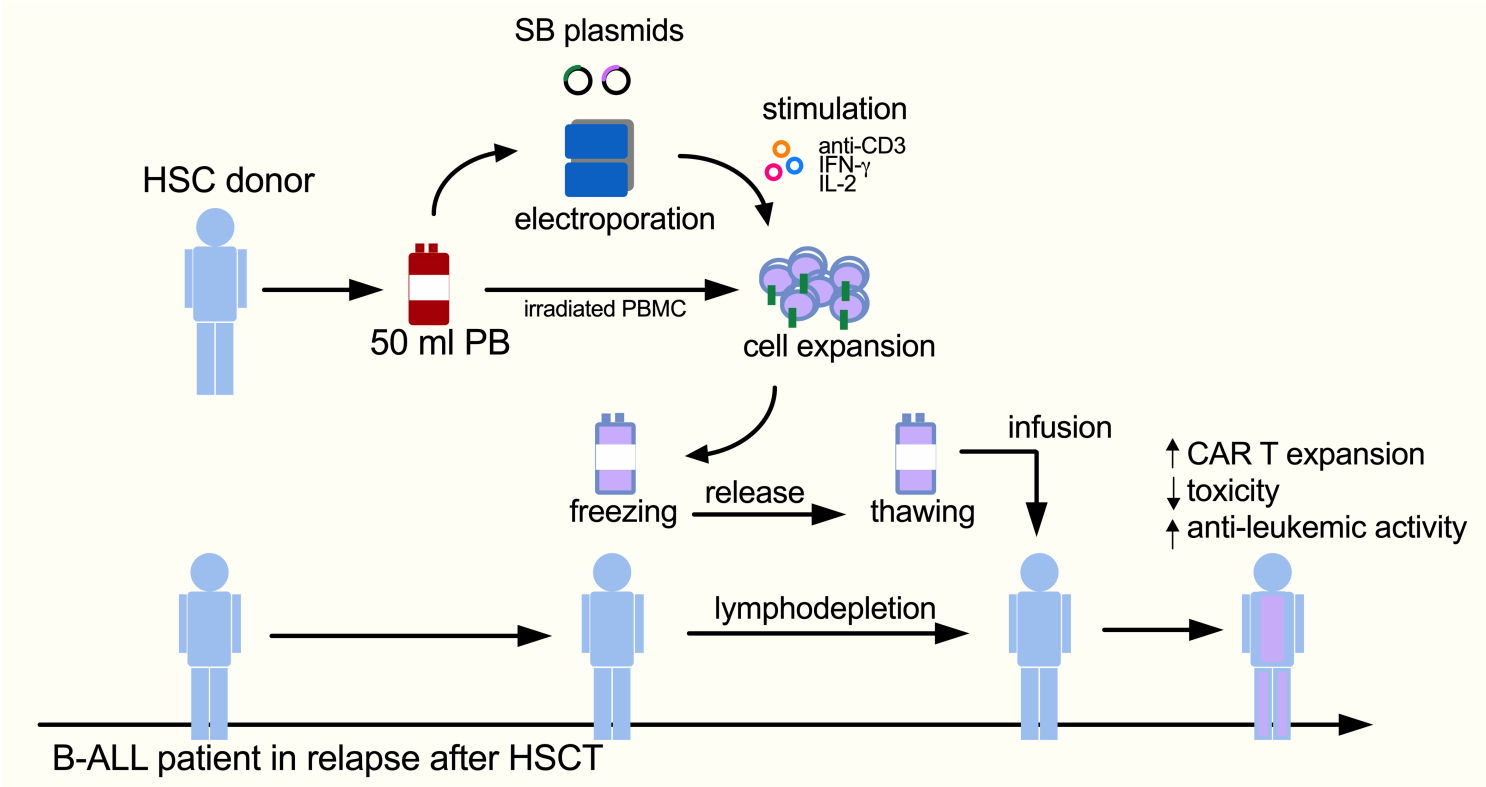
Magnani C et al. Journal of Clinical Investigation; 2020
Lussana F et al. Blood Cancer journal; 2025

Sleeping beauty-engineered CARCIK-CD19 cells achieve anti-leukemic activity without severe toxicities



Third generation CAR



Clinical Trials:

- **FT01** NCT03389035
- **FT03** NCT05252403

Magnani C et al. Journal of Clinical Investigation; 2020
Lussana F et al. Blood Cancer journal; 2025

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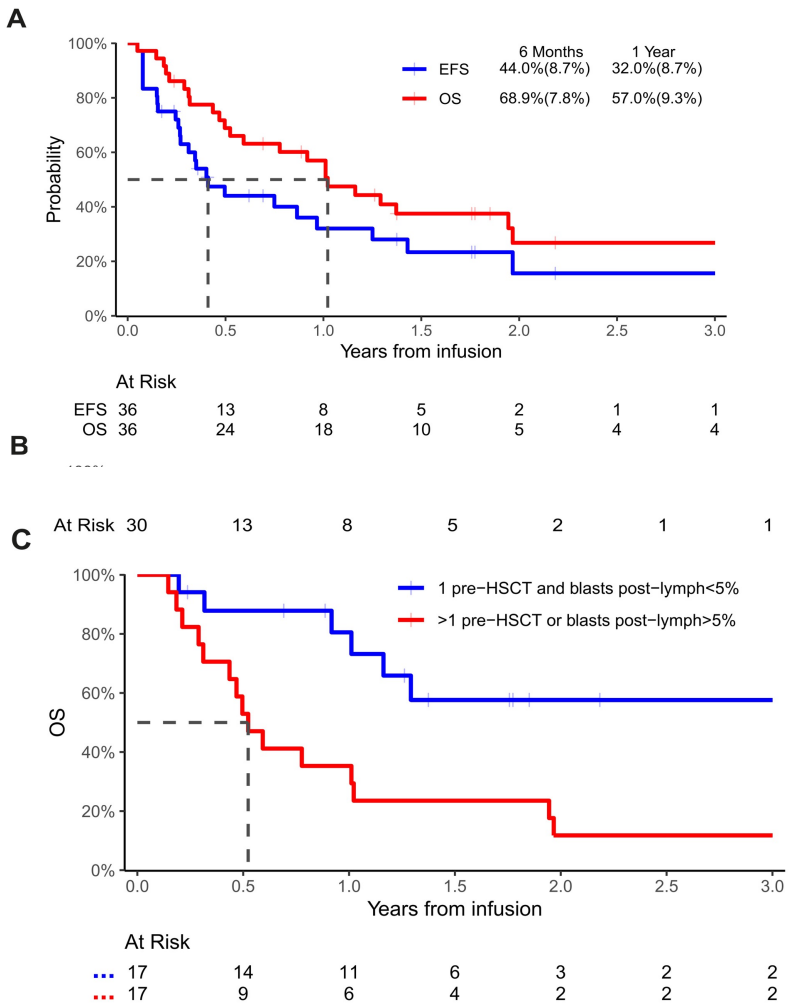
Efficacy of allogeneic CARCIK-CD19

Efficacy

- Median follow-up 2.2 years
- 30/36 reached CR (83%)
- 86% were MRD-negative
- 12 patients (33%) did not experience a relapse:
  - 3 patients (25%) underwent consolidation with a second alloHSCT
  - 6 patients (17%) are still disease-free without additional therapies (1 with CAR-T circulating after 40 months)
  - 3 (25%) died in CR (1 due to sepsis, 1 due to hyporexia and ascites, and 1 due to epilepsy with SNC negativity for disease)



Lussana F et al. Blood Cancer journal; 2025

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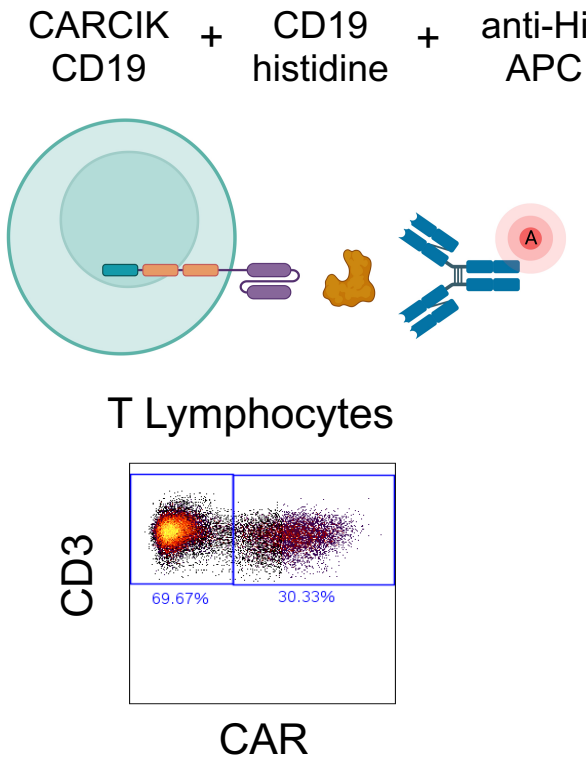
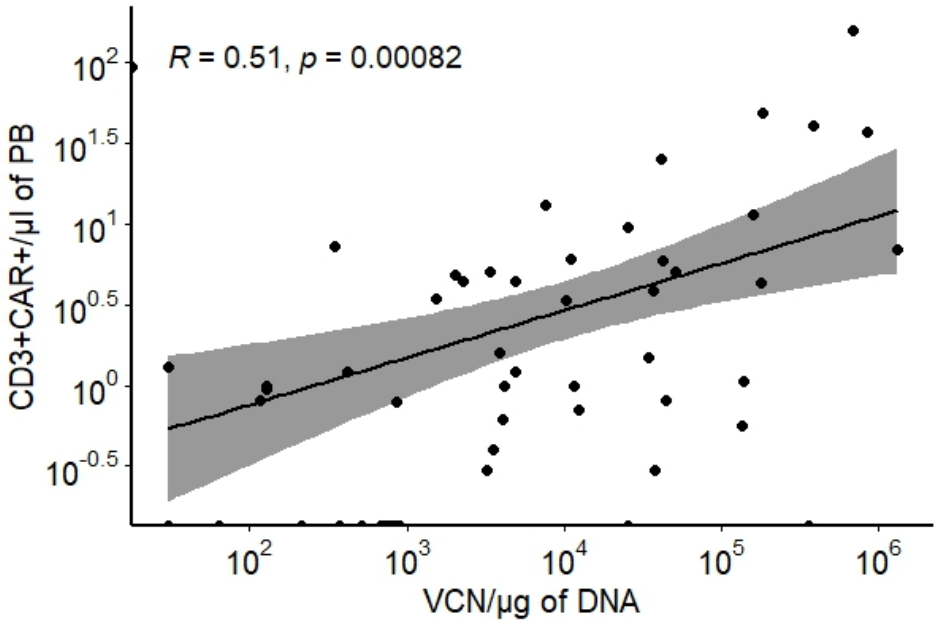
CARCIK-CD19 *in vivo* monitoring using flow cytometry





Advantages:

- Rapid information from bench to bed side
- High sensibility (> 0,1%)
- High specificity (VCN correlation)
- Study of multiple sites (blood, BM, liquor, pleural effusion)

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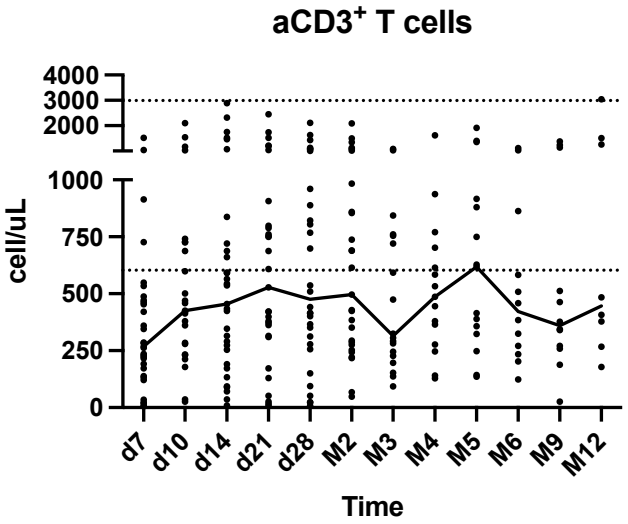
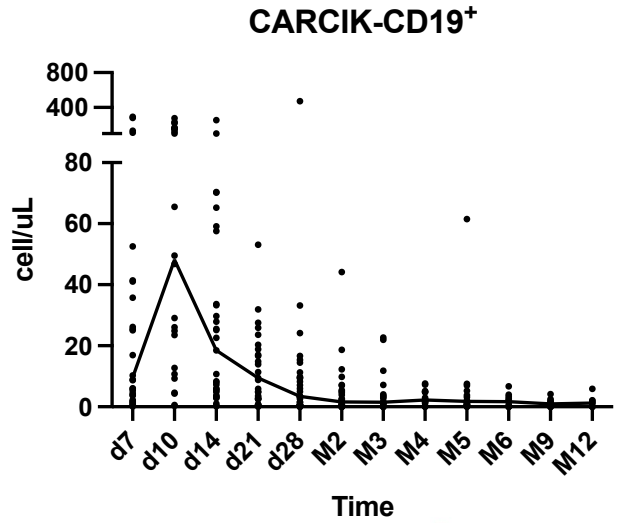
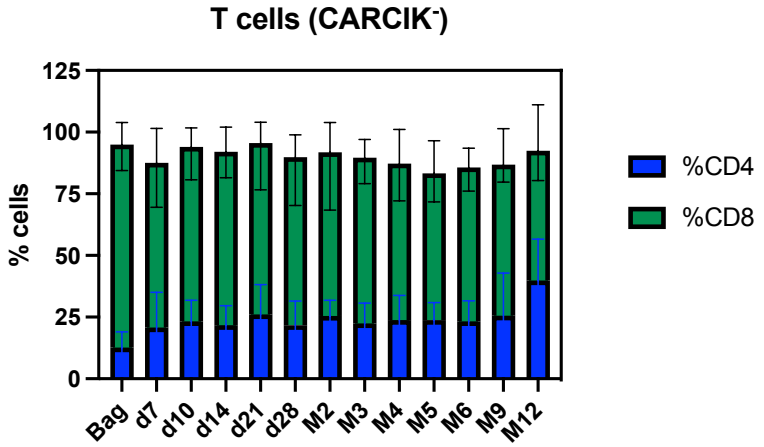
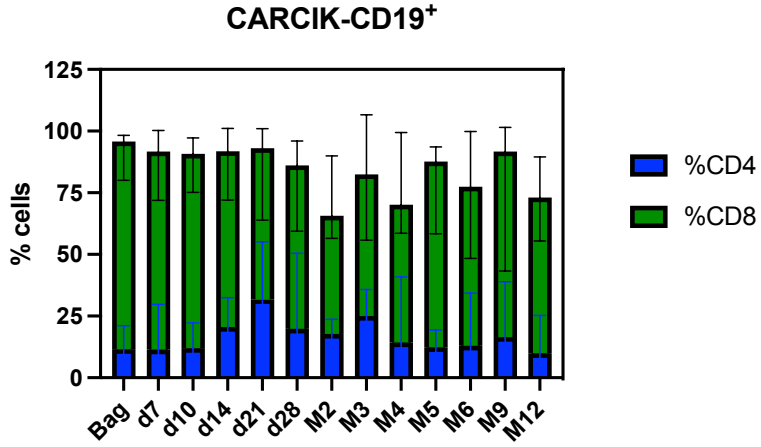
Zaninelli S and Rambaldi B, Cytometry. 2023

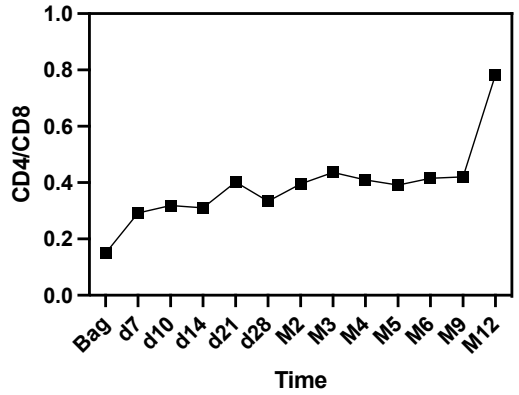
CD3⁺ T cells and CARCIK-CD19 reconstitution kinetics

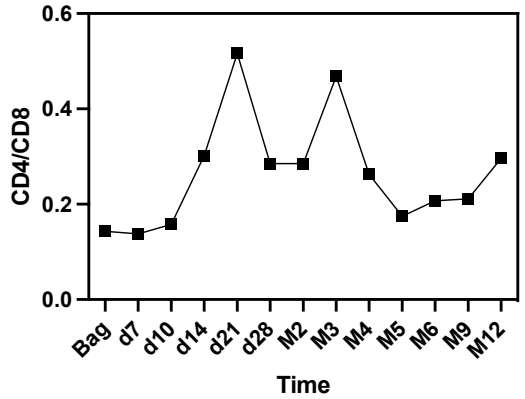












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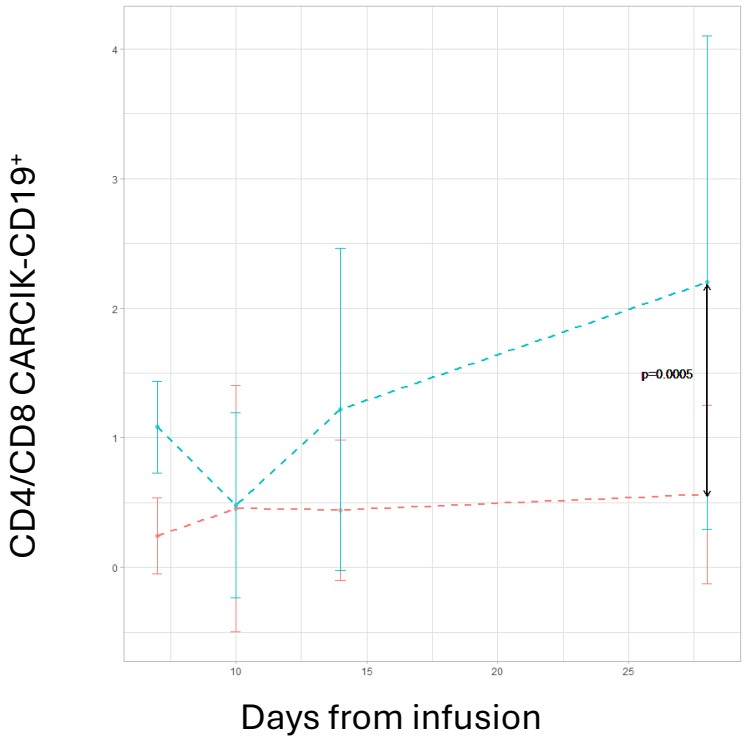
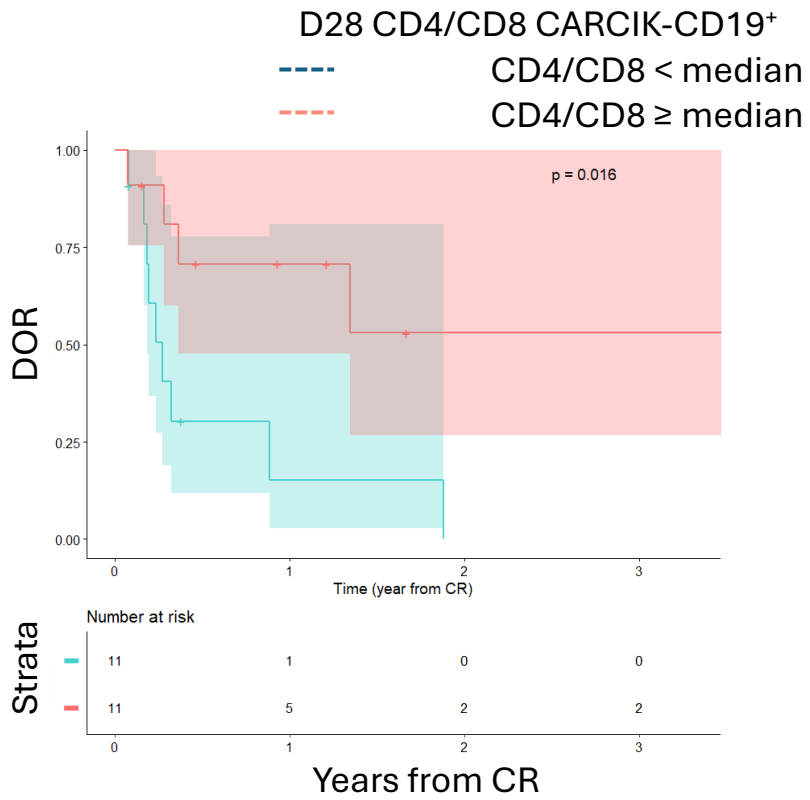
N=33

Rambaldi B, EBMT, Oral comunication, 2024

CD4/CD8 CARCIK-CD19+ ratio and treatment response



CR d28
NR
CR



- A lower CD4/CD8 CARCIK-CD19 at early time points was associated with CR d28
- A higher CD4/CD8 ratio after day 28 correlates with DOR

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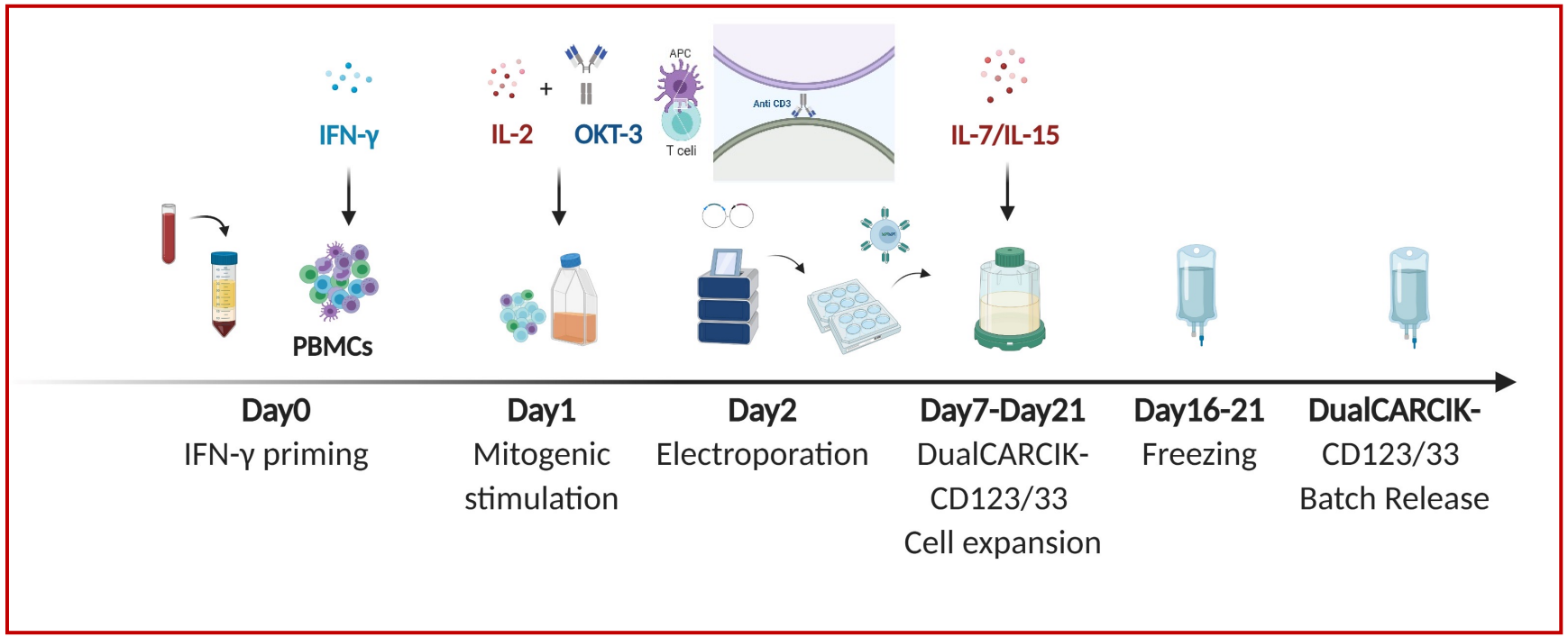
N= 33

Rambaldi B, EBMT, Oral comunication, 2024

Manufacturing Implementation



Implementations:

- feeder-free method
- use of the G-REX bioreactor

Already in place for Phase I-II trial to determine the safety of allogeneic CARCIK-CD19 for R/R B-NHL and B-CLL (**FT04** NCT05869279)

Pisani I, et al. *J Transl Med*. 2025
Gotti E…Golay J et al.: *Cytotherapy*. 2018

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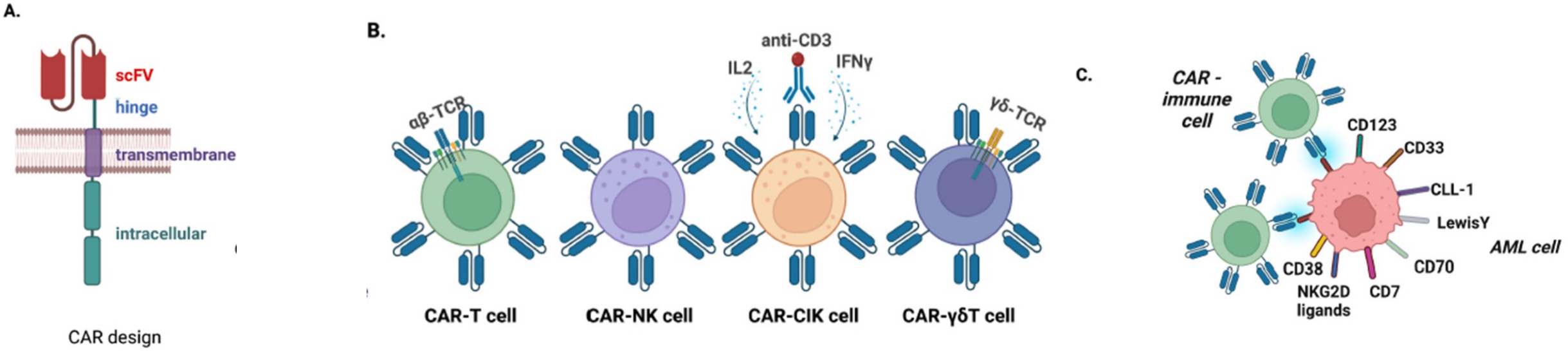
Chimeric Antigen Receptor (CAR) cells for AML

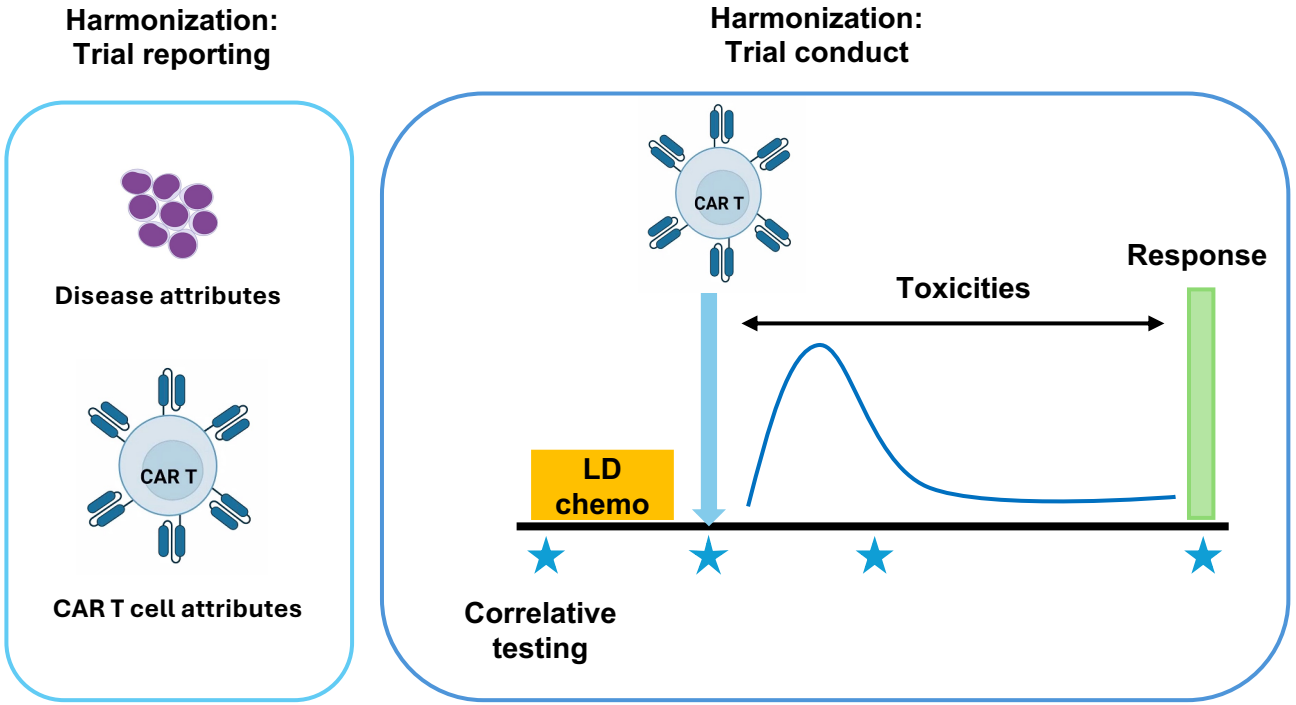


Major barriers in CAR T cell therapy in AML

1. On-target off-tumor toxicity
2. Unsatisfactory clinical results (global ORR 30%)
3. Quality and quantity of autologous T or NK cell at leukapheresis
4. Aggressiveness of R/R AML requires fast manufacturing
5. Low in vivo persistence of CAR-T cells

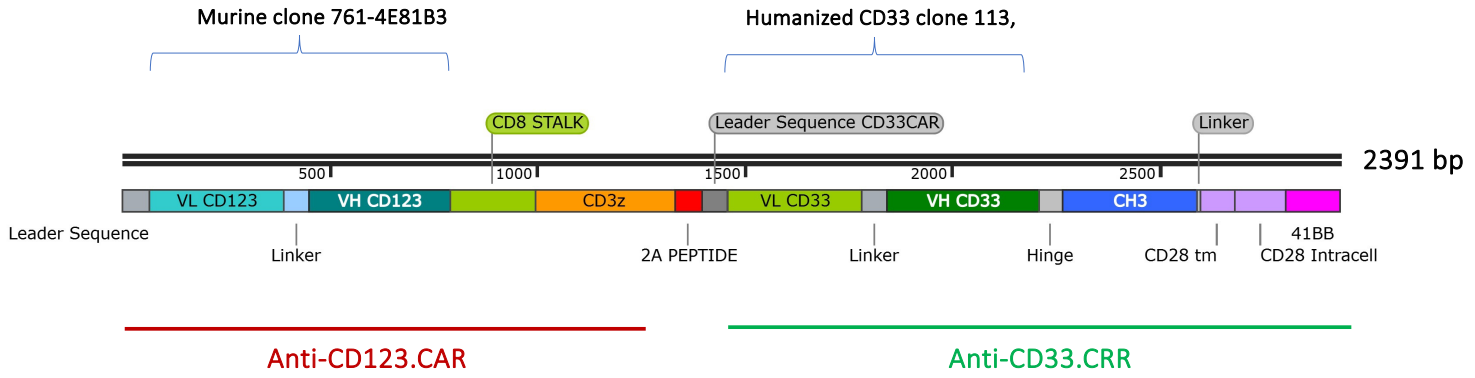
International Consensus Guidelines for the Conduct and Reporting of CAR T Cell Clinical Trials in AML

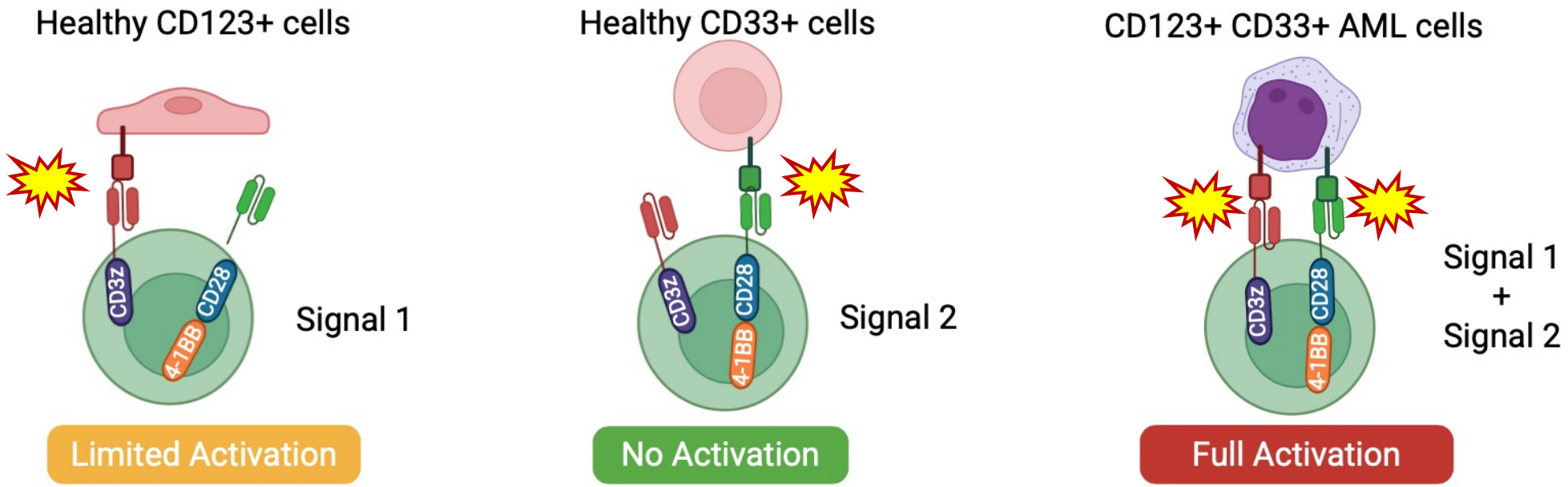
Swati Naik[1], Richard Aplenc[2], Susanne Baumeister[3-6], Marco Becilli[7], Anand S. Bhagwat[2,8], Challice L. Bonifant[9], Elizabeth Budde[10], Christopher D. Chien[11], Kevin J. Curran[12], Anthony F. Daniyan[13], Alexandra Dreyzin[11], Rebecca A. Gardner[14], Sara Ghorashian[15], Stephen Gottschalk[1], LaQuisa C. Hill[16-18], M. Eric Kohler[19], Adam Lamble[20], Franco Locatelli[7], Maksim Mamonkin[16,18], Bilal Omer[16,18], Jae Park[21], Concetta Quintarelli[7,22], **Benedetta Rambaldi[23]**, Rebecca M. Richards[24], David Sallman[25], Tim Sauer[26], Nirali N. Shah[11], Marion Subklewe[27-28], Corinne Summers[20], Sarah K. Tasian[2,29,30], Naomi Taylor[11], **Sarah Tettamanti[31]**, Michael R. Verneris[19], M. Paulina Velasquez[1], Saar I. Gill[32]



Naik S et al. *Blood Adv* 2025

Targeting Strategy: Dual CD123/33.CAR transfected CIK cells by non-viral Sleeping Beauty (SB) transposon platform





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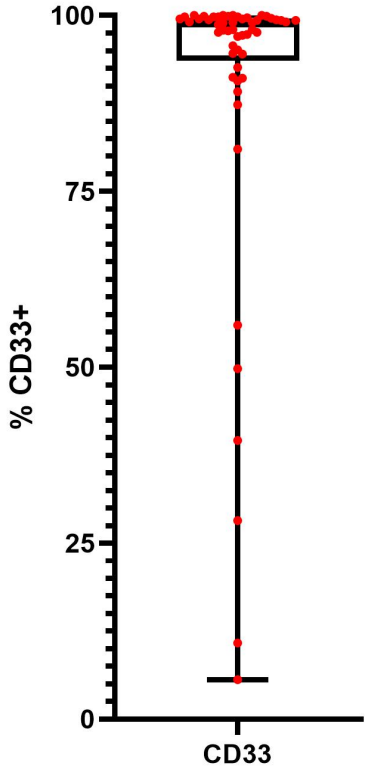
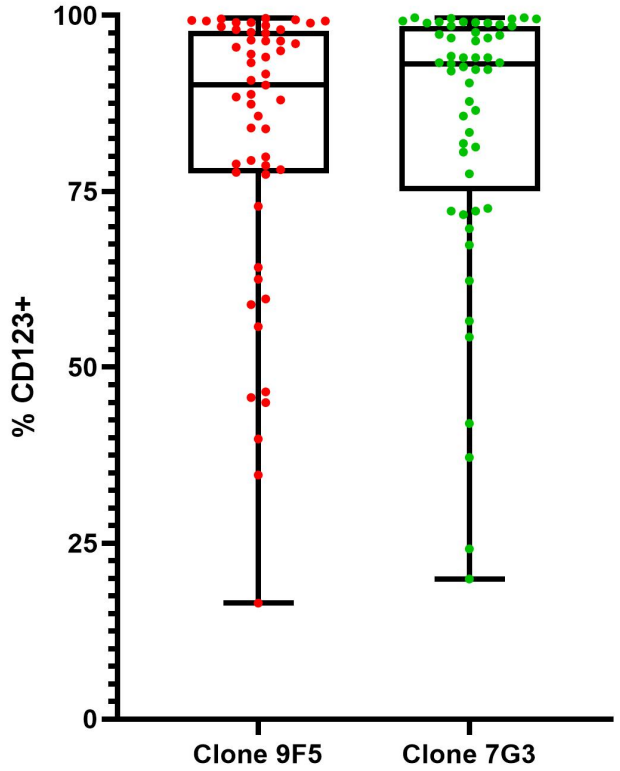
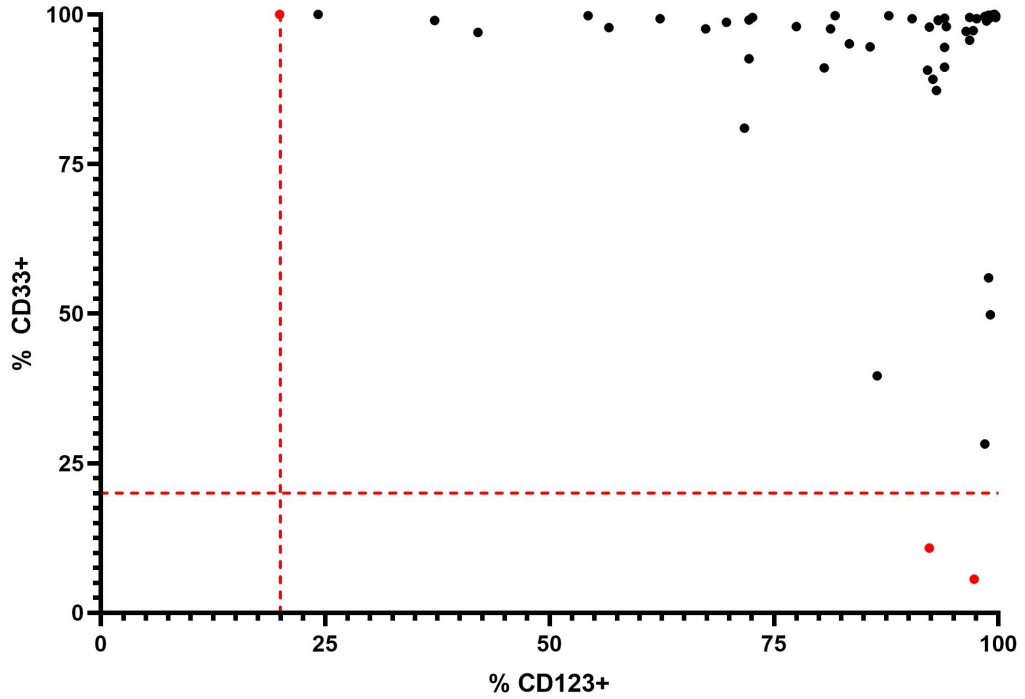
Perriello V, Rotiroti MC,….Tettamanti S, Blood Advances 2022

CD123 and CD33 expression in the AML Bergamo Cohort





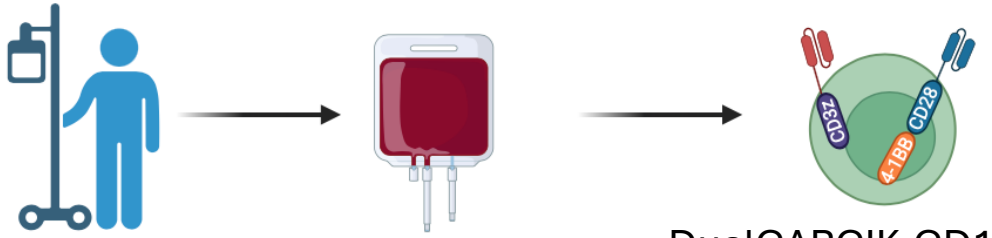


N=53

Dr. Cristian Meli and Alice Arnoldi (student)

Anti-CD123/CD33 Chimeric Antigen Receptor Cytokine-Induced Killer (DualCARCIK-CD123/33) Cells for Acute Myeloid Leukemia

Manufactory



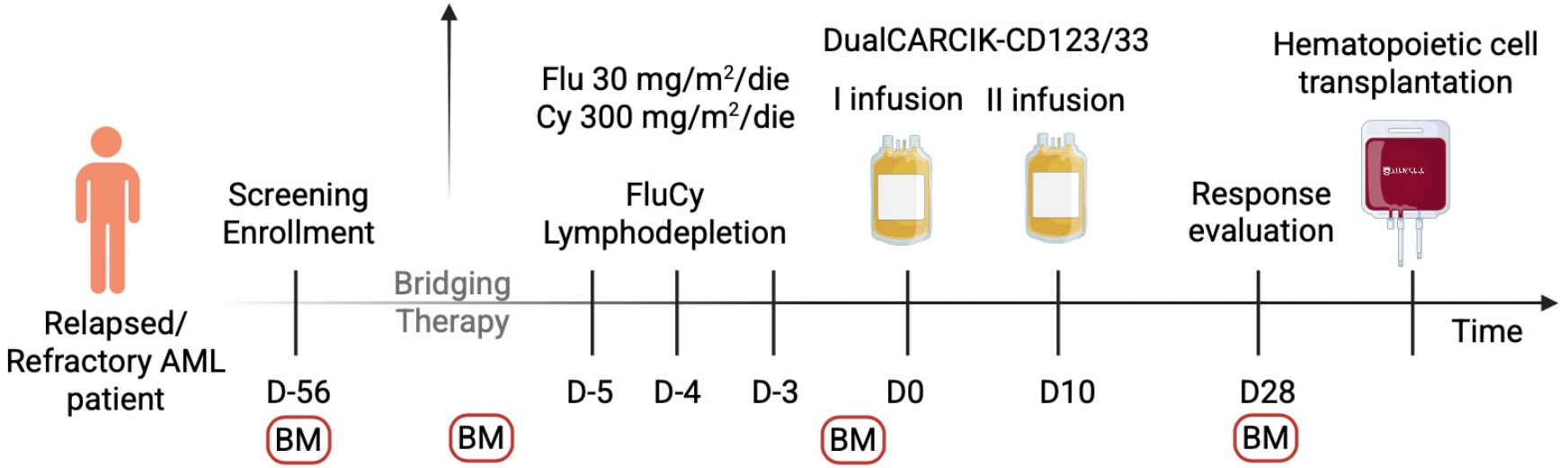
Healthy donor (haploidentical or matched)

50-100 ml of Peripheral blood

DualCARCIK-CD123/33 Generation, using viral-free Sleeping Beauty transposon system

Clinical Trial

A phase I single-arm, multicenter clinical trial (Bergamo and Monza)



CARCIK monitoring in Flow and PCR (VCN)

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Key Inclusion Criteria

1. Morphologically confirmed (bone marrow blasts > 5%) diagnosis of AML or quantifiable MRD positivity with >1% blasts by flow cytometry, if relapse after HCT

2. Confirmed expression of CD33 and CD123 on leukemic blasts by flow cytometry

3. Availability of an at least haploidentical (i.e. 4/8 HLA matched by allele typing) familial donor willing to and eligible for blood donation for the CARCIKCD123/33 cell production

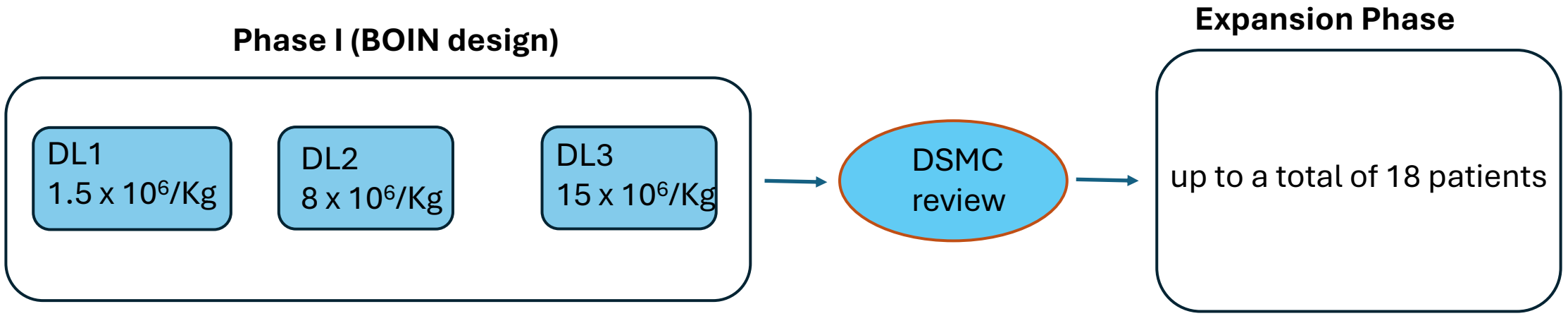
4. Eligibility to proceed to a HCT, different donor is allowed

5. Age limits: children (6 months - 17 years old) and adults (≥18 years old)

Key Exclusion Criteria

1. Rapidly progressive disease not compatible with the time schedule of the protocol
2. Uncontrollable CNS leukemia
3. Allogeneic HCT within 3 months prior to Dual CARCIK infusion
4. Active GvHD Grades II-IV or moderate/severe chronic GvHD (for patients who had previously been allotransplanted)
5. Ongoing immunosuppressive therapy including corticosteroid use >/=25 mg/day (> or 0.3 mg/kg for children) of prednisone or equivalent within 1 weeks prior to Dual CARCIK infusion

Dose Escalation Study



Dose Level	Day 0	Day 10 (+/- 3)	Total dose
1 (starting dose)	0.5 x 10^6/Kg	1 x 10^6/Kg	1.5 x 10^6/Kg
2	3 x 10^6/Kg	5 x 10^6/Kg	8 x 10^6/Kg
3	5 x 10^6/Kg	10 x 10^6/Kg	15 x 10^6/Kg

- **Primary Objectives**: the safety and the feasibility (manufacturing process and number of patients infused).
- **Secondary Objective**: the efficacy in terms of hematological response rate at day 28.

Conclusions

- Manipulated approaches, such as CIK cells can enhance GVL while hampering GVHD

- Allogeneic CARCIK-CD19 cells are effective and safe in R/R ALL after HCT and showed long term persistence in responder patients, optimal for minimal residual disease positivity (**FT03** NCT05252403, compassionate program)

- Phase I-II trial to determine the safety of allogeneic CARCIK-CD19 for R/R B-NHL and B-CLL is ongoing (**FT04** NCT05869279)

- Novel strategy are need for R/R AML and DualCARCIK-CD123/33 cells showed favorable pre-clinical safety profile and efficacy, and the upcoming phase I clinical trial will test their safety in patients (**FT05**)

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University of Milano - Bicocca
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