GIORNATE EMATOLOGICHE VICENTINE

XI edizione

9-10 Ottobre 2025
Palazzo Bonin Longare - Vicenza

DLI e terapie cellulari nel post-trapianto allogenico

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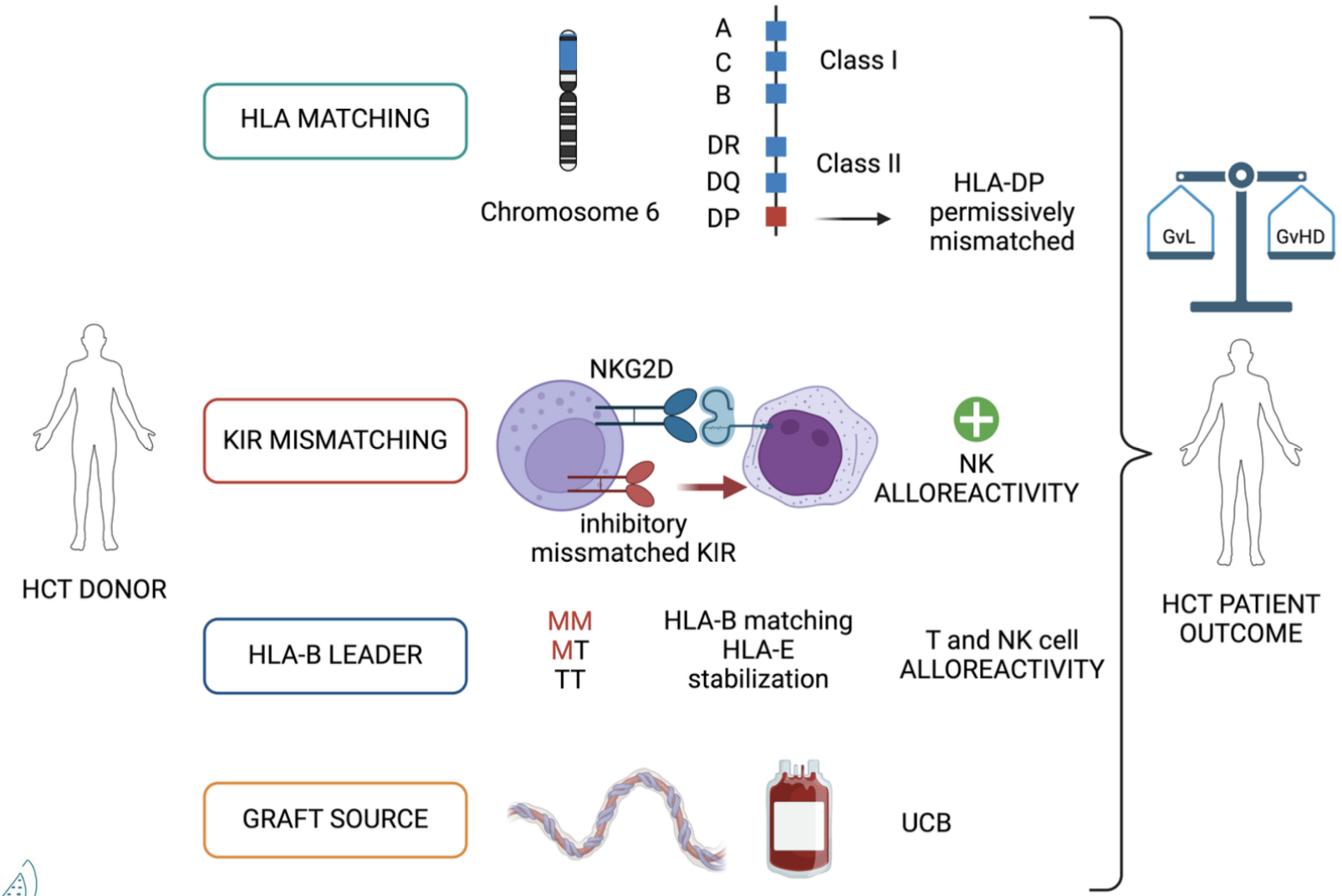
Azienda Ospedaliera
Papa Giovanni XXIII
Bergamo

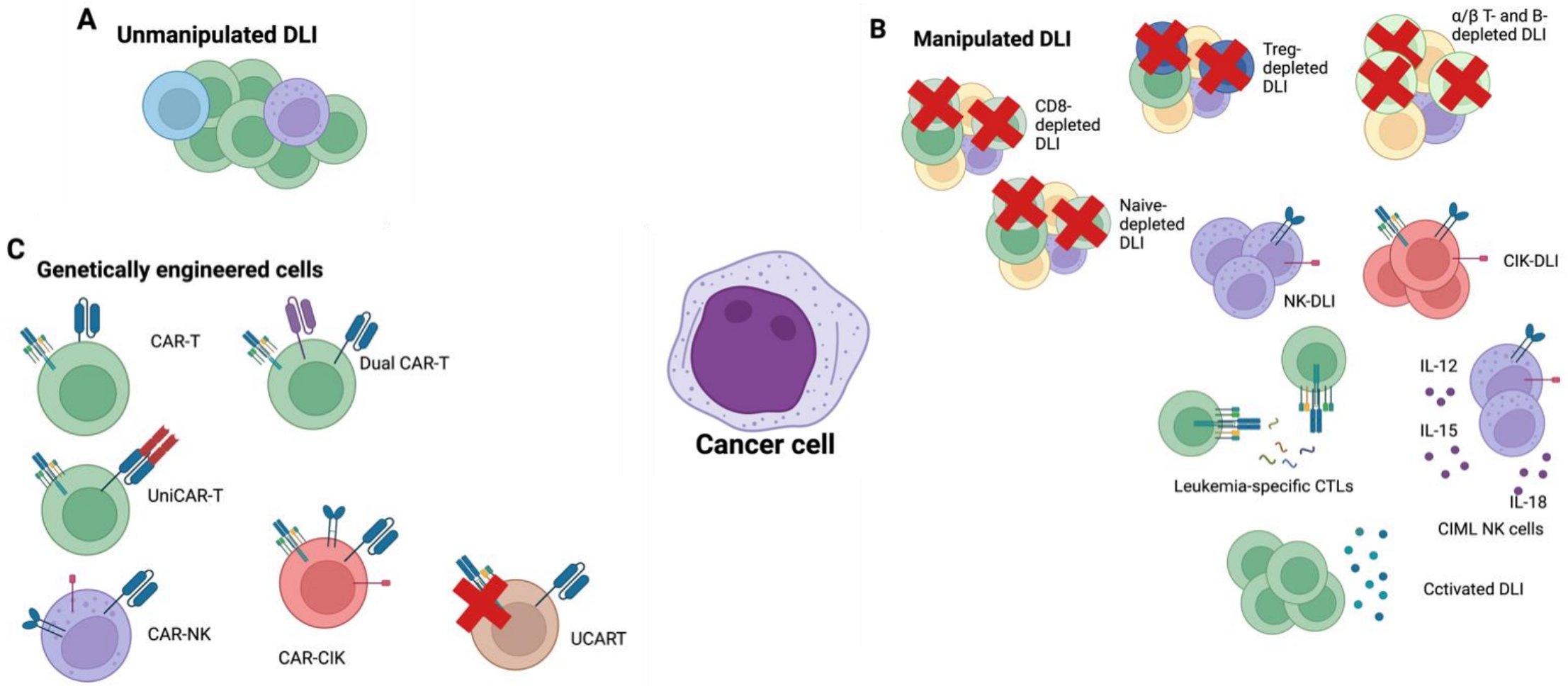
Non ho conflitti di interesse da dichiarare

Graft versus Leukemia (GvL) immunological basis



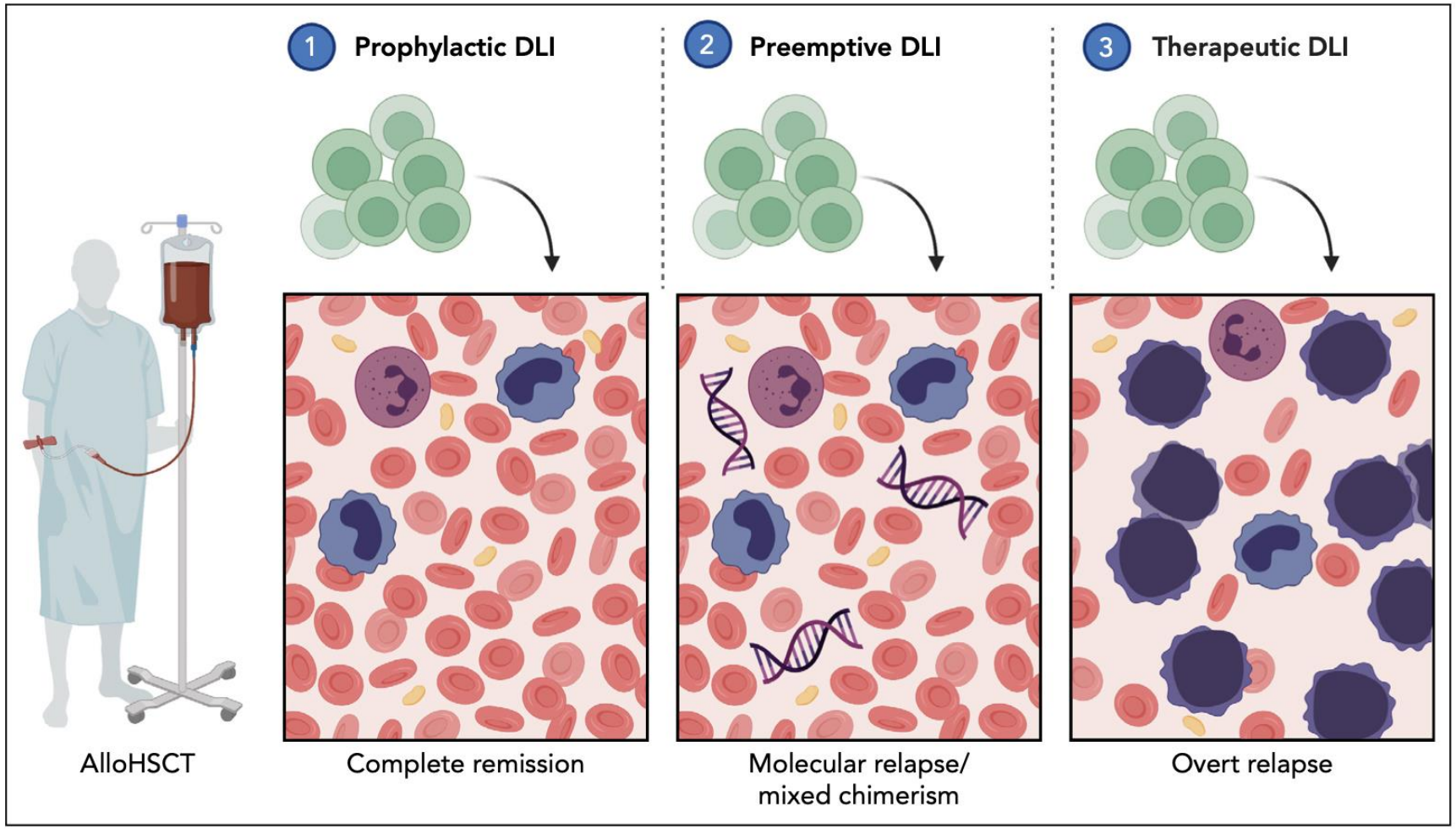
Rambaldi B et al., *Front Immunol* 2024

Possible adoptive cellular therapy strategies in the post-HCT setting



Rambaldi B et al., *Front Immunol* 2024

DLI indications



Biederstädt A and Rezvani K *Blood* 2023

DLI: EBMT recommendations

	Recommended DLI dose for matched related donor	Recommended DLI dose for matched unrelated donor	Recommended DLI dose for mismatched unrelated donor or haploidentical donor	Number of DLIs
Prophylactic[48–50]				
3 months (ex-vivo TCD: any risk; no ex-vivo TCD: high risk or refractory disease)	1×10^5 cells/kg	1×10^5 cells/kg	1×10^5 cells/kg*	1–3†
6 months‡	1×10^6 cells/kg	1×10^6 cells/kg	5×10^5 cells/kg	1–3†
Pre-emptive[51,52]				
3 months	$1–5 \times 10^5$ cells/kg	1×10^5 cells/kg	1×10^5 cells/kg	1–4§
6 months‡	$1–3 \times 10^6$ cells/kg	1×10^6 cells/kg	5×10^5 cells/kg	1–4§
Therapeutic[4,44,46,53]				
After systemic therapy¶	1×10^7 cells/kg	1×10^7 cells/kg	1×10^6 cells/kg	1–4‖

Pagliuca S et al., *Lancet Haematol* 2024

Disease specific sensitivity to DLI

- **high sensitivity** >> chronic myeloid leukaemia, myelofibrosis, low-grade non-Hodgkin lymphoma and multiple myeloma
- **Intermediate sensitivity** >> chronic lymphocytic leukaemia, acute myeloid leukaemia, myelodysplastic syndrome, and Hodgkin lymphoma
- **lower sensitivity** >> acute lymphoblastic leukaemia and diffuse large B-cell lymphoma

Alyea EP et al. Biol Blood Marrow Transplant 2010;
NCI First International Workshop on the biology, prevention and treatment of relapse after allogeneic hematopoietic cell transplantation

Post-DLI GVHD

HLA-matched setting

- **2-4 Acute GVHD** >> **11·9%** (95% CI 8·2–16·3%) median onset day +51
- **Chronic GVHD** >> **30·7%** (24·9–36·6%) (median onset day +135)

No differences between prophylactic and pre-emptive DLI

6% of patients died from DLI-induced GVHD

HLA-haploidentical setting

- prophylactic DLI >> **2–4 acute GVHD 17%** (95% CI 7–27%)
  Chronic GVHD 53% (95% CI 40-67%)
- pre-emptive DLI >> 2–4 acute GVHD 20% (95% CI 2–38%)
  Chronic GVHD 21% (95% CI 3-39%)
- therapeutic DLI >> 2–4 acute GVHD 17% (95% CI 9–24%)
  Chronic GVHD 24% (95% CI 15-33%)

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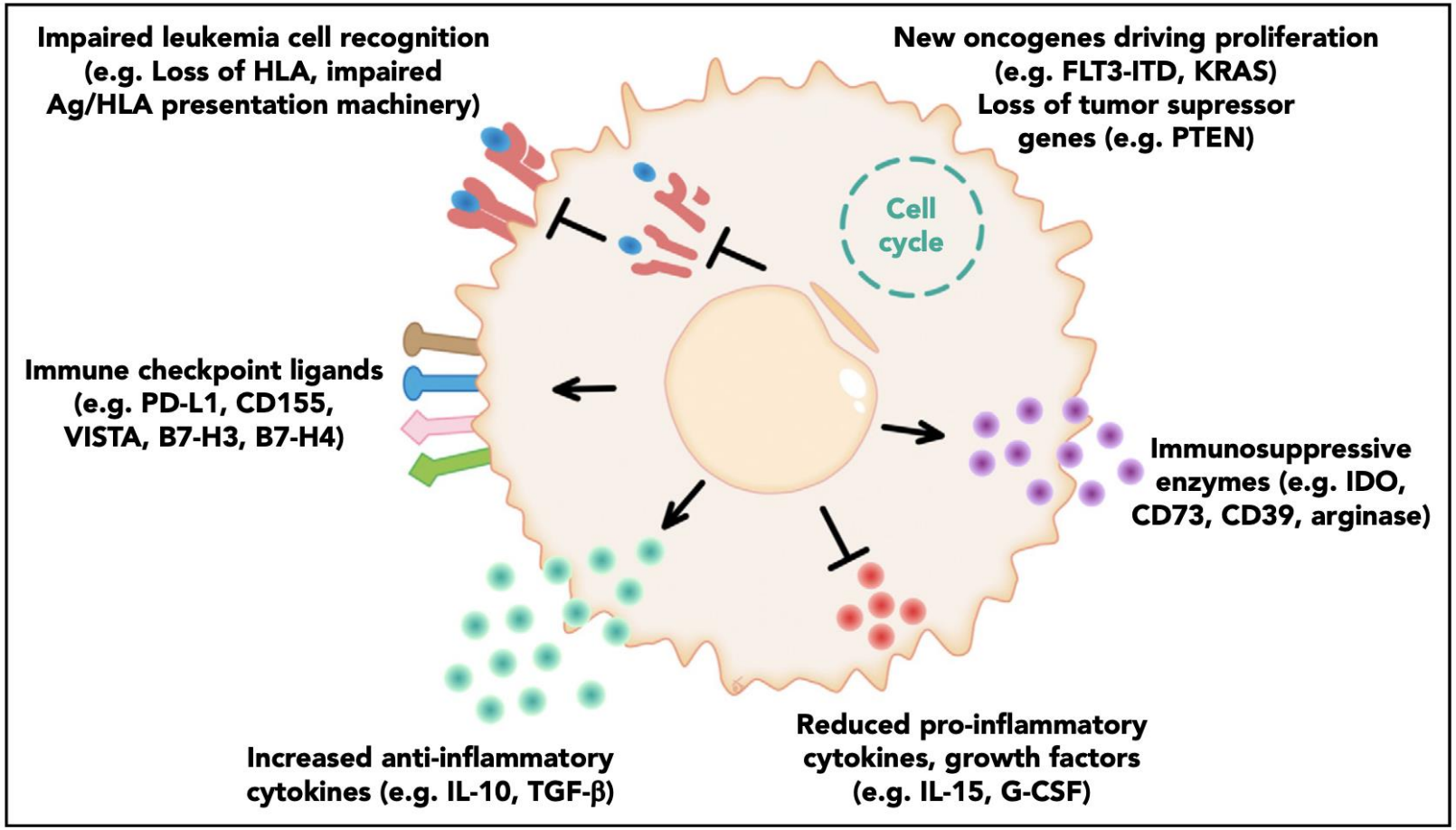
Schmid C et al. *Bone Marrow Transplant* 2022;
Santoro N et al. *Bone Marrow Transplant* 2023;

Mechanisms of tumor escape

Vago L, et al. *N Engl J Med* 2009;
Toffalori C, et al. *Nat Med* 2019;
Christopher MJ, et al. *N Engl J Med* 2018;
Gambacorta V, et al. Cancer Discov 2022;
Pagliuca S, et al. *Nat Commun* 2023;

- **30% of AML relapses are associated with HLA loss**
- **EBMT recommendation is to check for HLA loss in the haplo or mismatched setting, before proceeding with DLI**



Zeiser R and Vago L *Blood* 2019

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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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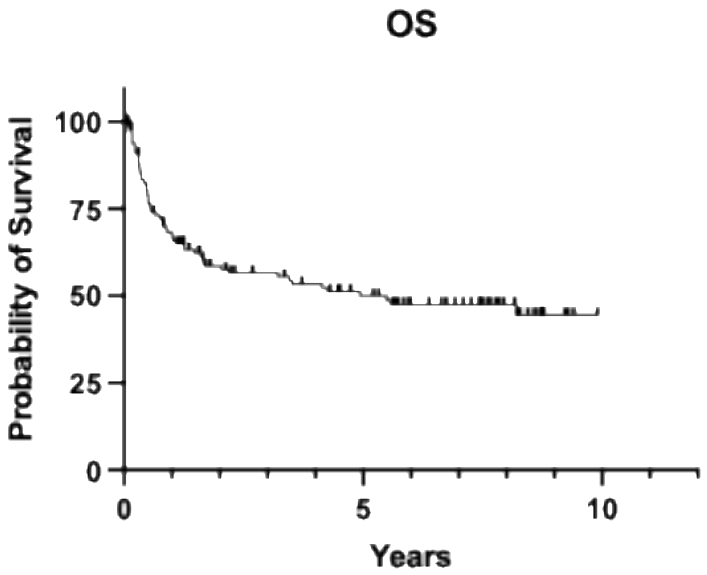
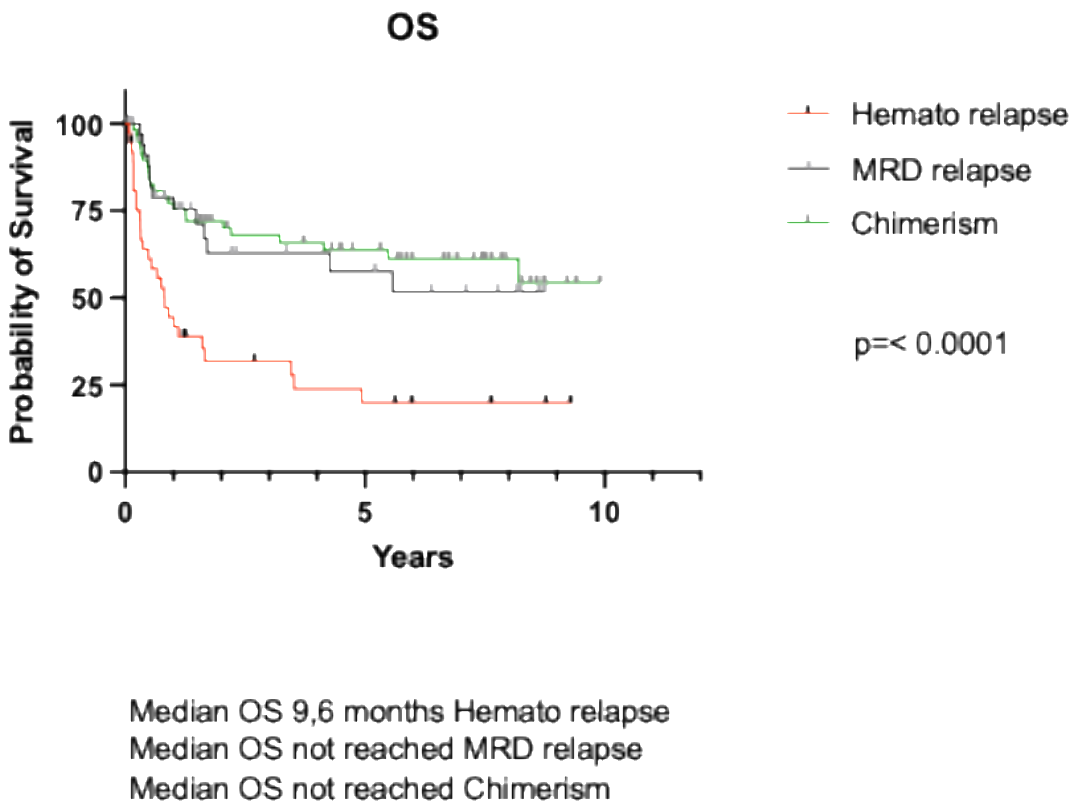
Data unpublished, please do not post

Survival and GVHD incidence in DLI treated patients



Median Os 5.5 yrs



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

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%)

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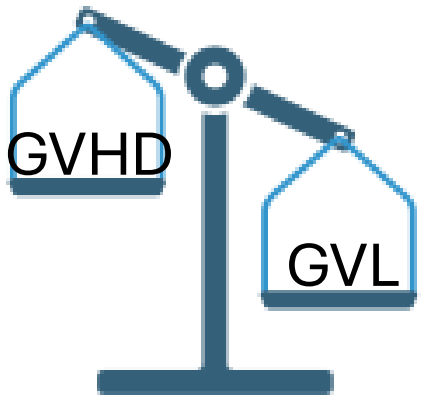
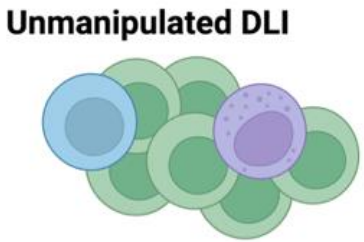
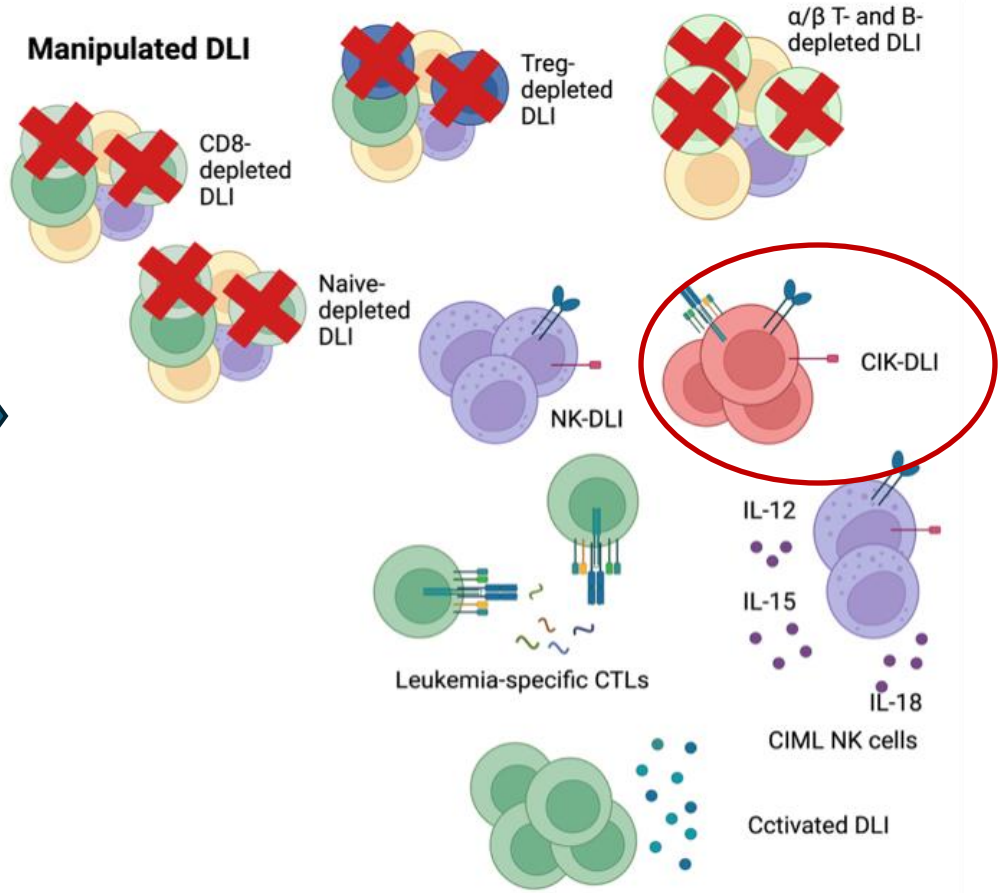
Data unpublished, please do not post

Enhancing GVL activity and hampering GVHD

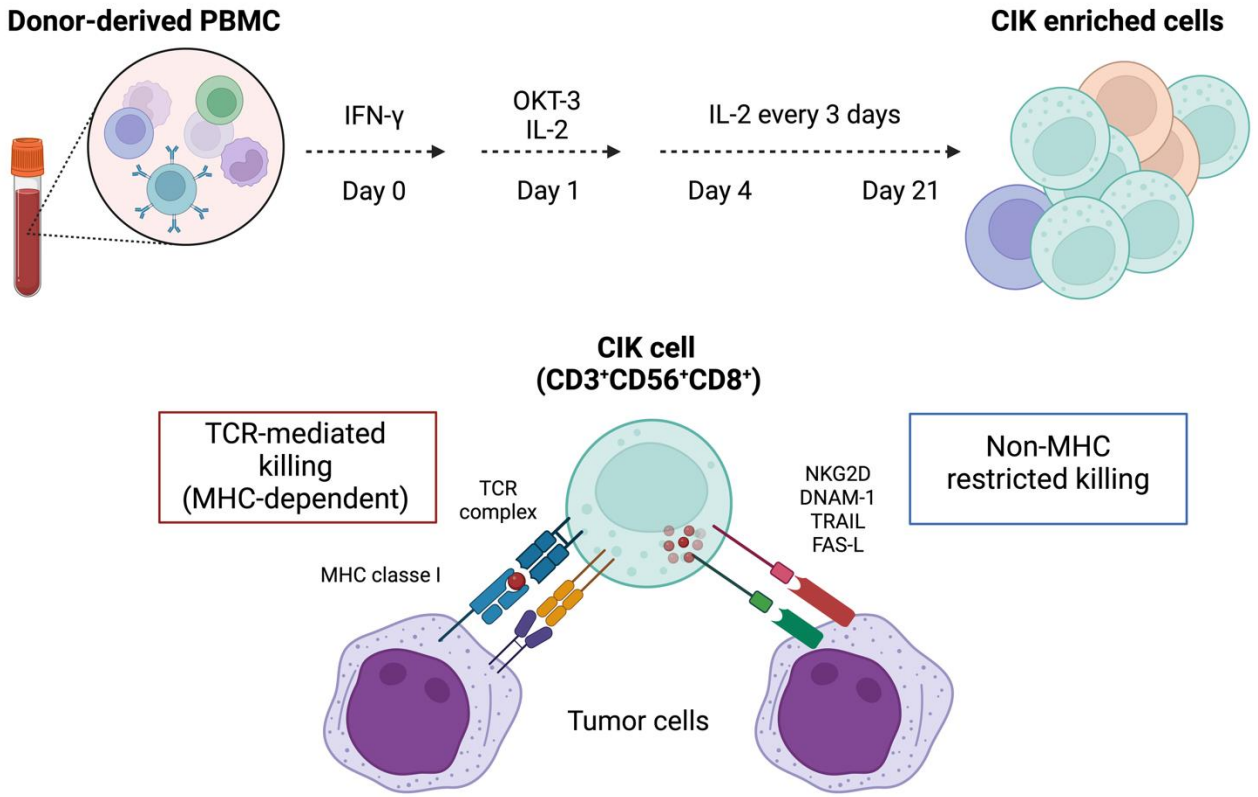


enhance GvL hampering GvHD effect





Allogeneic CIK cells enhance GvL hampering GvHD effect

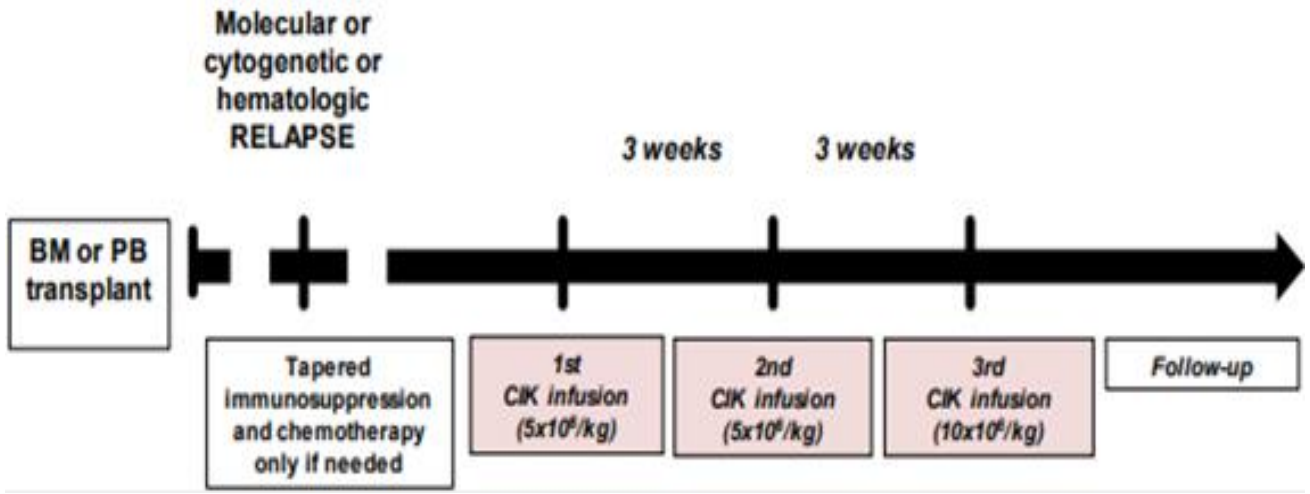


1 Introna M, et al. Bone Marrow Transplant. 2006; 2 Pievani et al, Blood, 2011; 3 Linn et al. Journal of Biomed and Biotech 2010; 4 Sangiolo et al. Journal of Cancer 2011; 5 Introna et al, Haematologica 2007; 6 Rambaldi A (2015) Leukemia 29(1):1-10; 7 Introna M (2017) Biol Blood Marrow Transplant. 23(12):2070-8;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

Published clinical trials using CIK cell therapy

Study	Study design	Pts (N)	Response	GvHD	Outcome
Introna et al Haematologica 2007	Single- center, open-label phase I study	11	1 SD; 1 PR; 3 CR	4 aGvHD (grade I-II) 2 cGvHD	Alive 5/11
Laport et al. BBMT 2011	Single center open-label phase I study	18	2 CR;	2 aGvHD (grade II) 1 limited cGvHD	Alive 10/18
Linn et al. BBMT 2012	Phase I/II clinical study	16	5 ORR	3 aGvHD	2-yrs Alive in CR 2/16
Rettinger et al. Haematologica 2016	Retrospective multicenter study	13	10 CR; 1 PR; 2 NR	6 aGvHD (3 grade I and 3 grade III) 3 cGvHD	OS 69%
Luo et al. Leukemia Research 2016	single- center, open-label phase I study	15	15 ORR	2 aGvHD 1 grade I and I grade III	6 Alive
Introna et al BBMT 2017	Multicenter, open-label phase IIA, study	74	19 CR; 3 PR; 8 SD	12 aGvHD (16%), 5 grade III-IV 11 cGvHD (15%)	3-year PFS 29% OS 40%
Pfeffermann et al. Cytotherapy 2018	Compassionate use study	1	1 CR	None	2-years alive in CR 1
Merker BBMT 2019	Retrospective, single- center, study	36	53% CR	9 aGvHD (25%) 8 grade I-II 1 grade III 2 limited cGvHD (6%)	6-months OS 77% (81% prophylaxis, 75%pre-emptive)
Narayan et al. BBMT 2019	Single- center, open-label phase II study	31	/	1-year aGvHD 25.1%	2-year NRM 6.8% EFS 27.3% OS 50.6%

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*

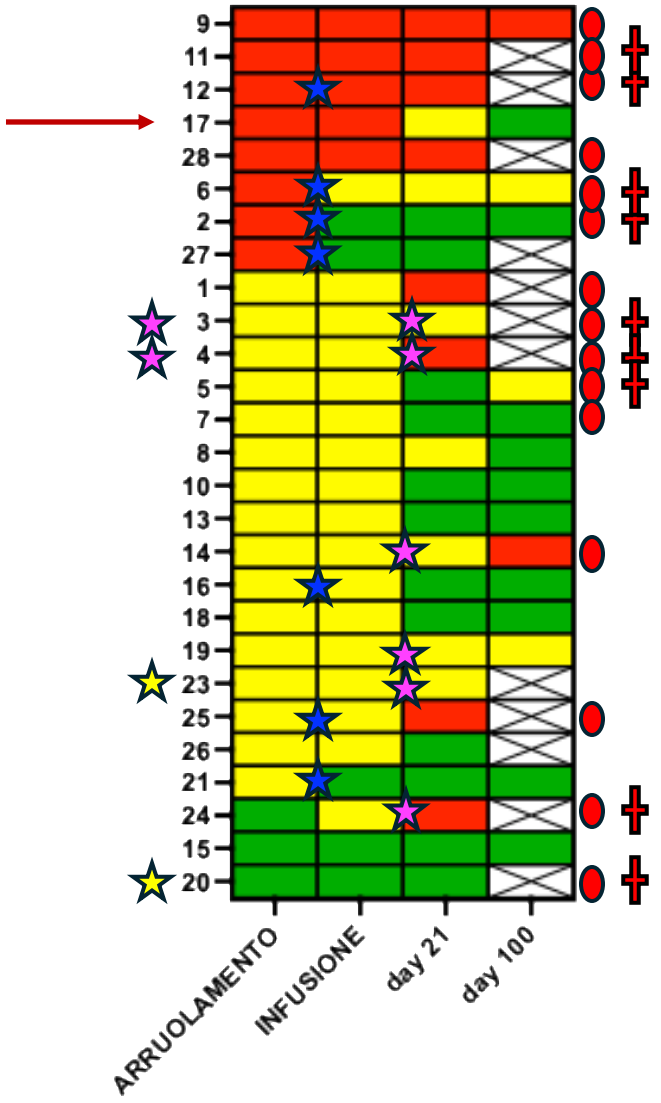
Scannella A and Lussana F SIE 2024

Haplo-CIK Trial and Compassionate use

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

10/28 Alive in CR (35%)

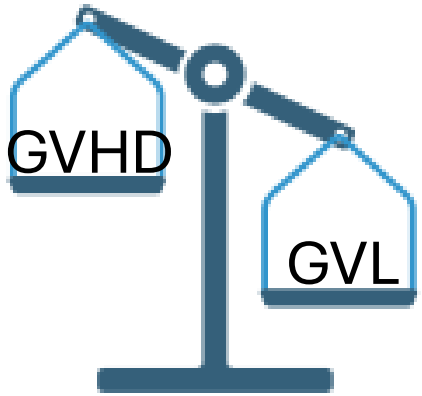
- Complete Response MRD neg and full donor Chimerism
- MRD Relapse or Mixed Chimerism
- Hematological Relapse
- Bridging Therapy
- Concomitant Therapy
- Therapy after relapse, but before CIK request
- Relapse/Progression
- Dead



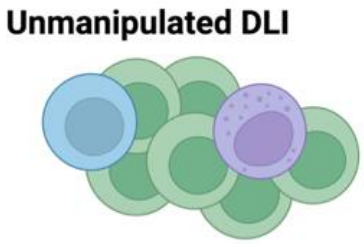
1 aGVHD G1
1 cGVHD lieve

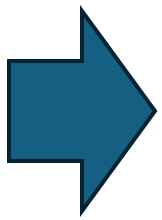
Update will be presented at ASH 2025 as poster presentation

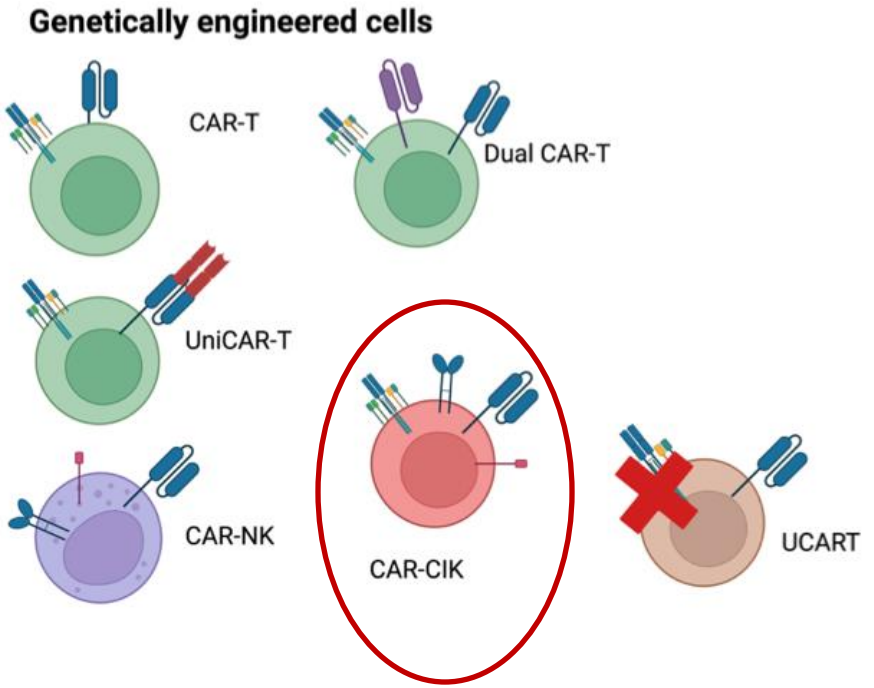
Enhancing GVL activity and hampering GVHD



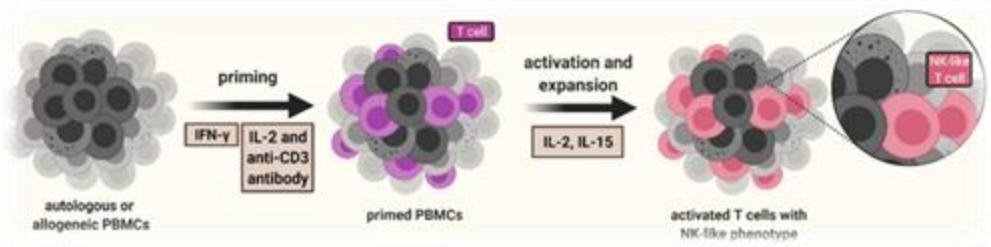
enhance GvL hampering GvHD effect



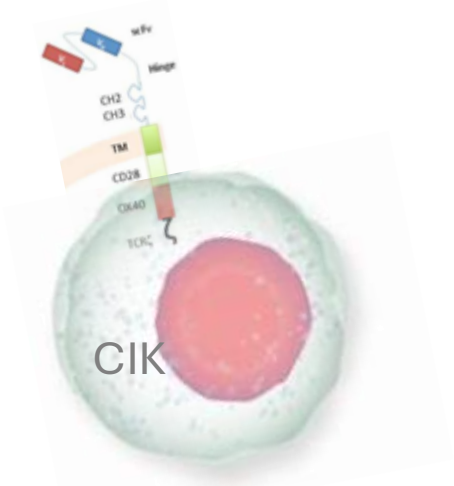


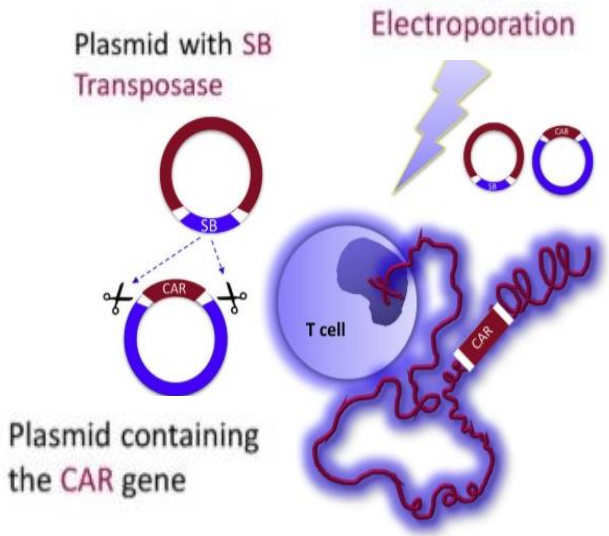


Our approach: Non-Viral Sleeping Beauty Transposon system and allogeneic CARCIK



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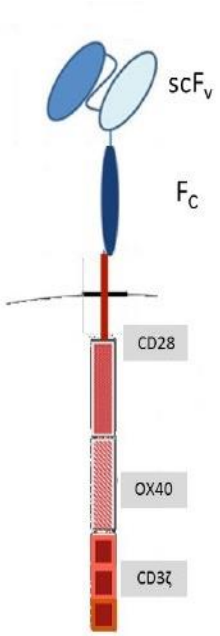
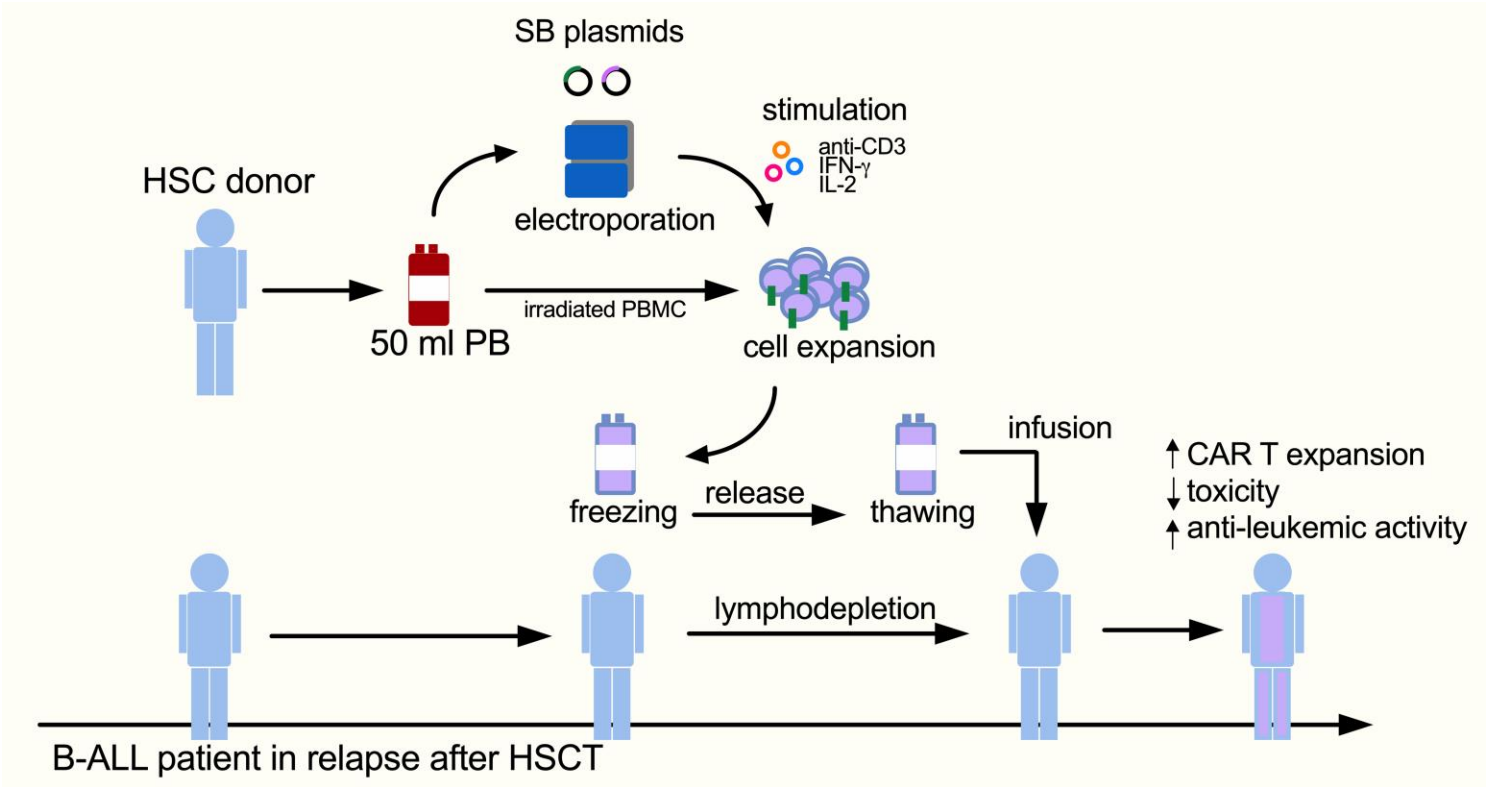
Magnani C et al. Journal of Clinical Investigation; 2020
Lussana F et al. Blood Cancer journal; 2025

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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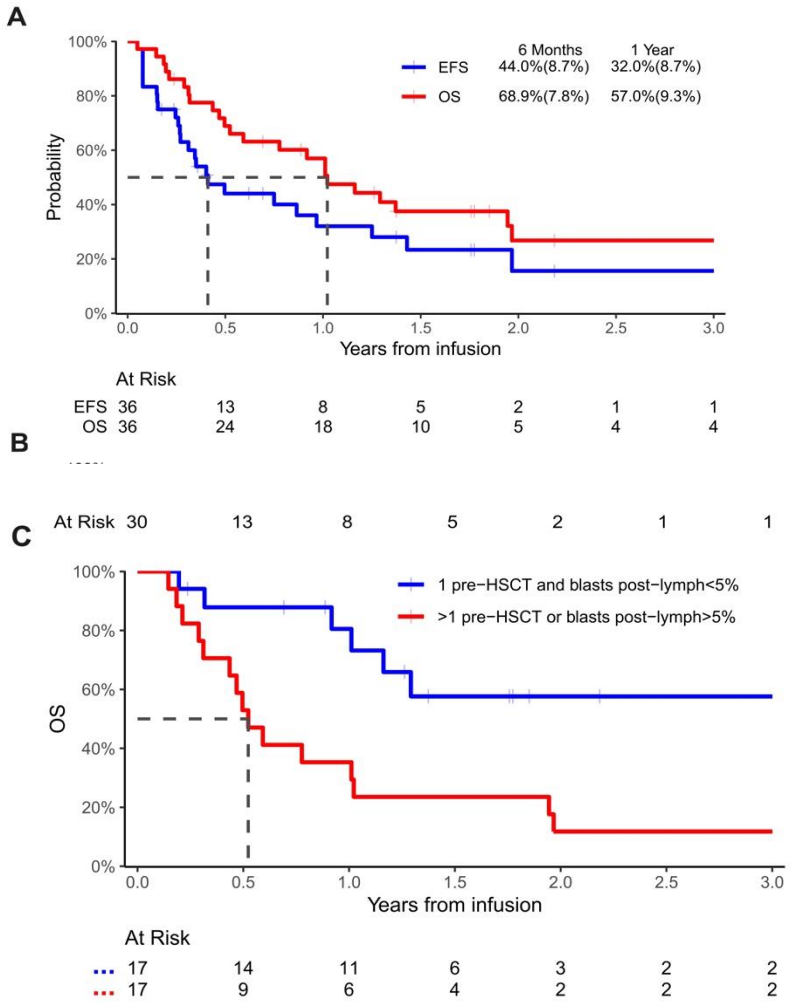
Efficacy of allogeneic CARCIK-CD19

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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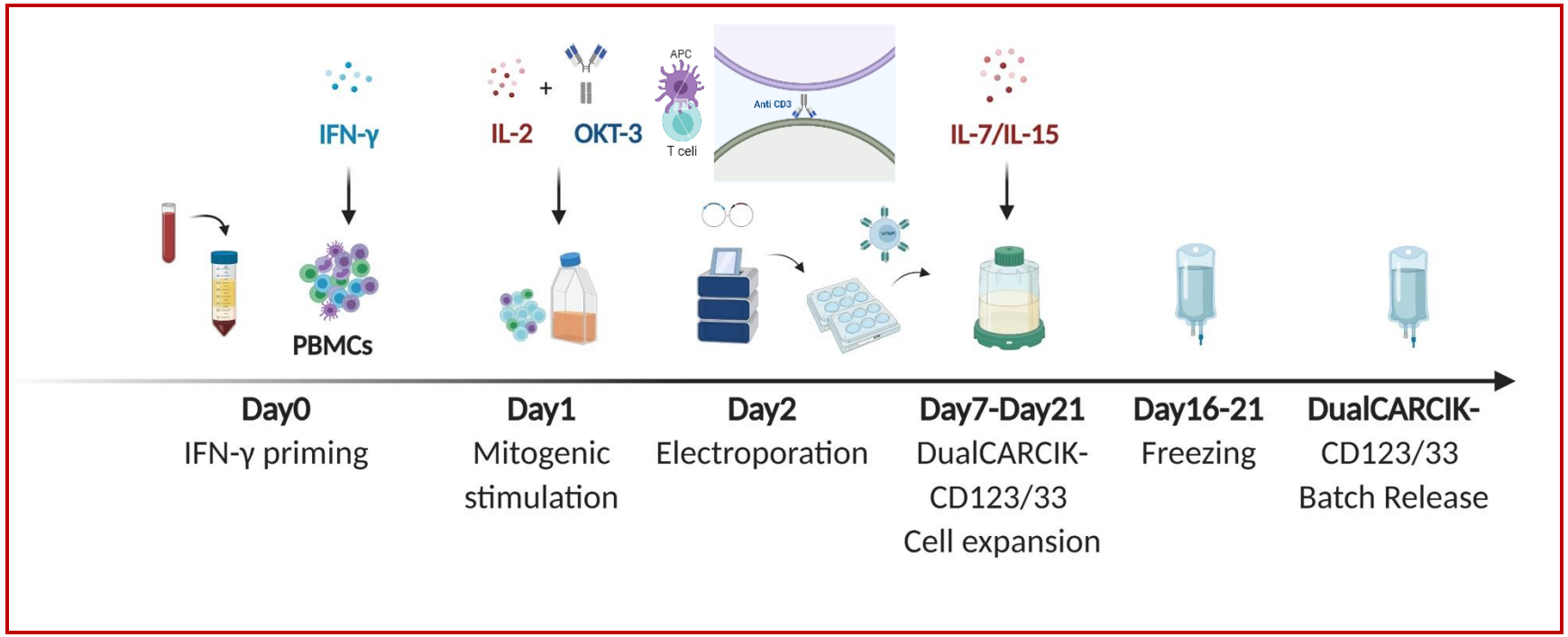
Manufacturing Implementation



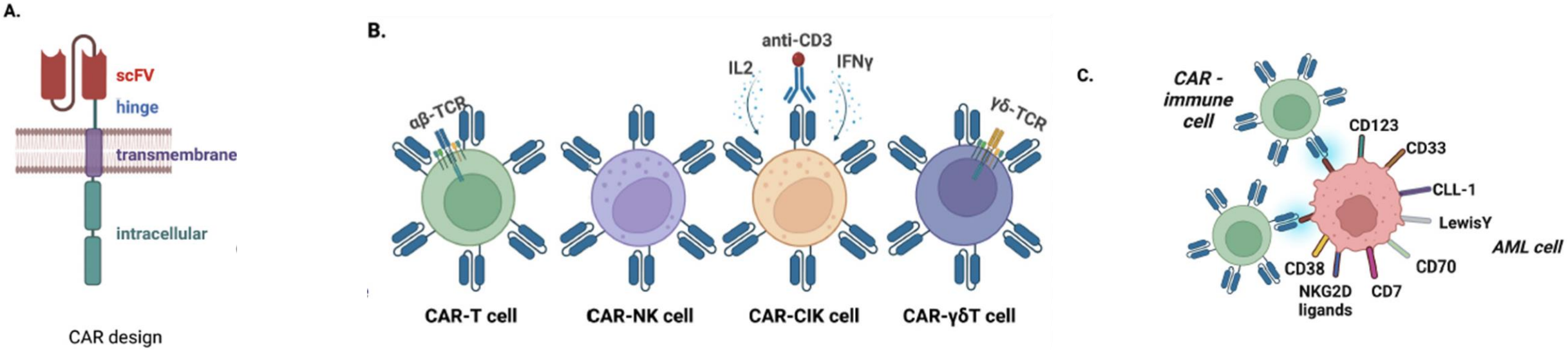
Implementations:

- 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 (**FT04** NCT05869279)

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

Chimeric Antigen Receptor (CAR) cells for AML

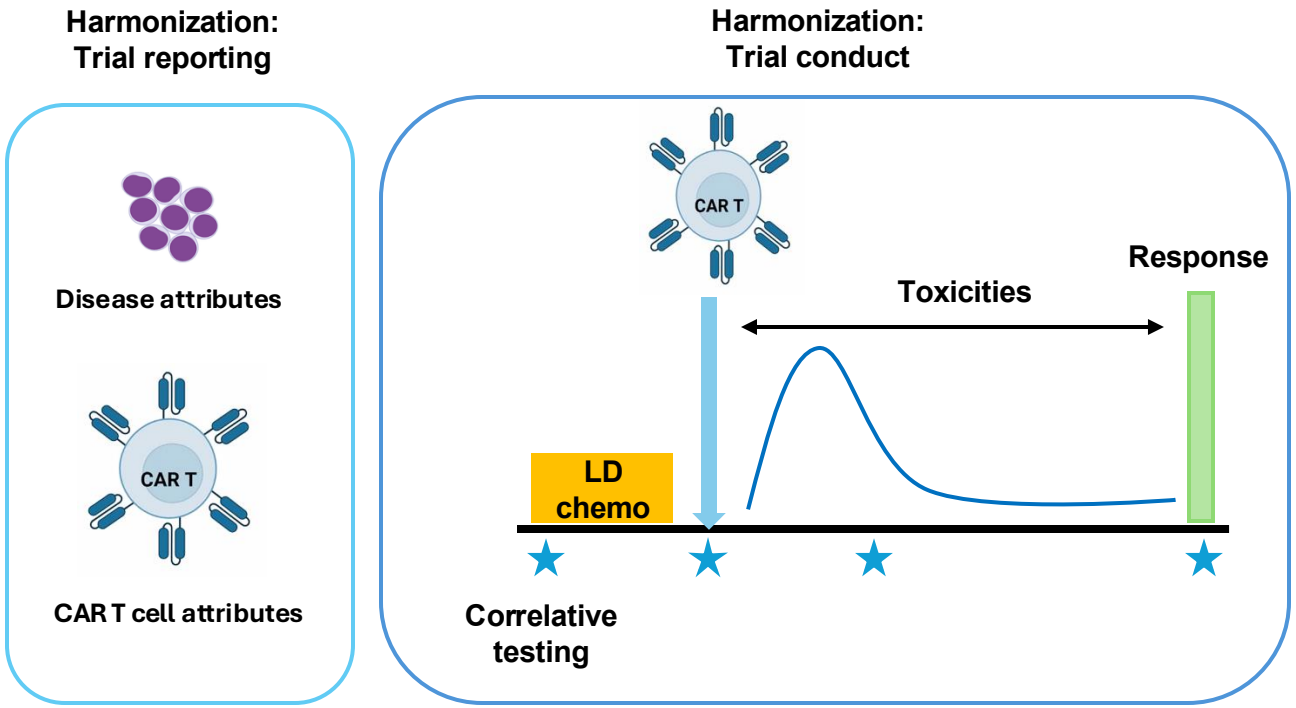


Major barriers in CAR T cell therapy in AML

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

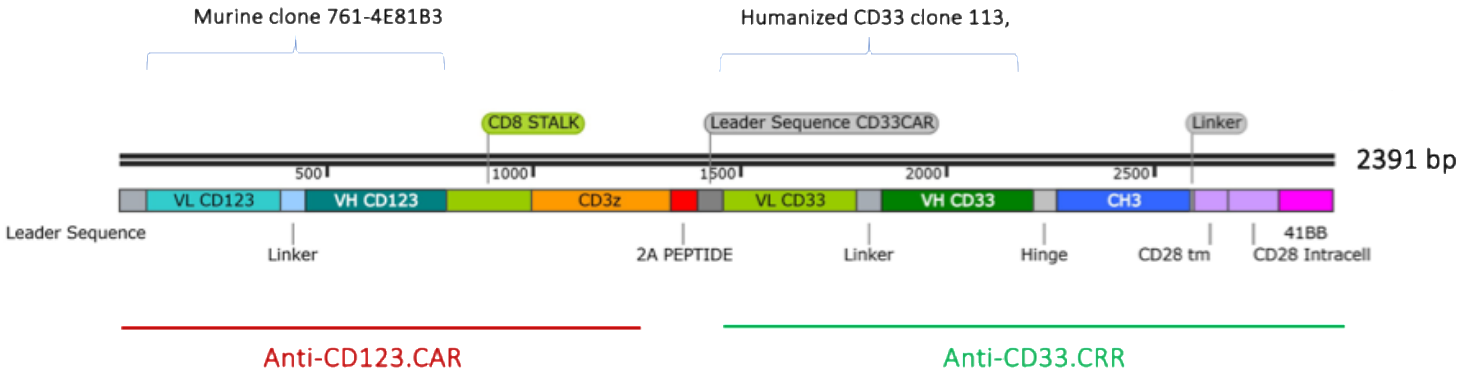
International Consensus Guidelines for the Conduct and Reporting of CAR T Cell Clinical Trials in AML

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]

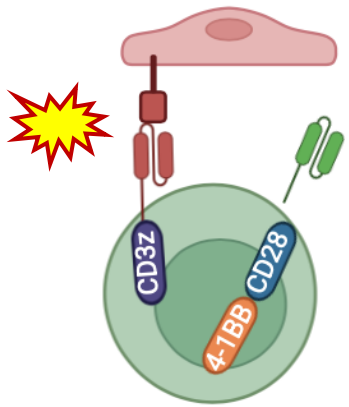


Naik S et al. *Blood Adv* 2025

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



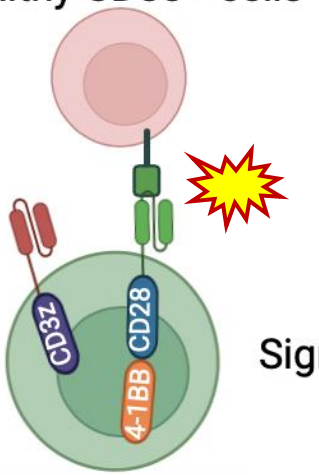
Healthy CD123+ cells



Signal 1

Limited Activation

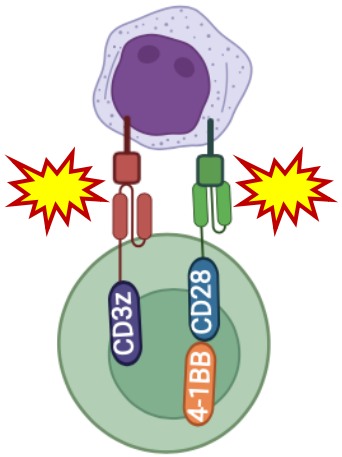
Healthy CD33+ cells



Signal 2

No Activation

CD123+ CD33+ AML cells



Signal 1
+
Signal 2

Full Activation

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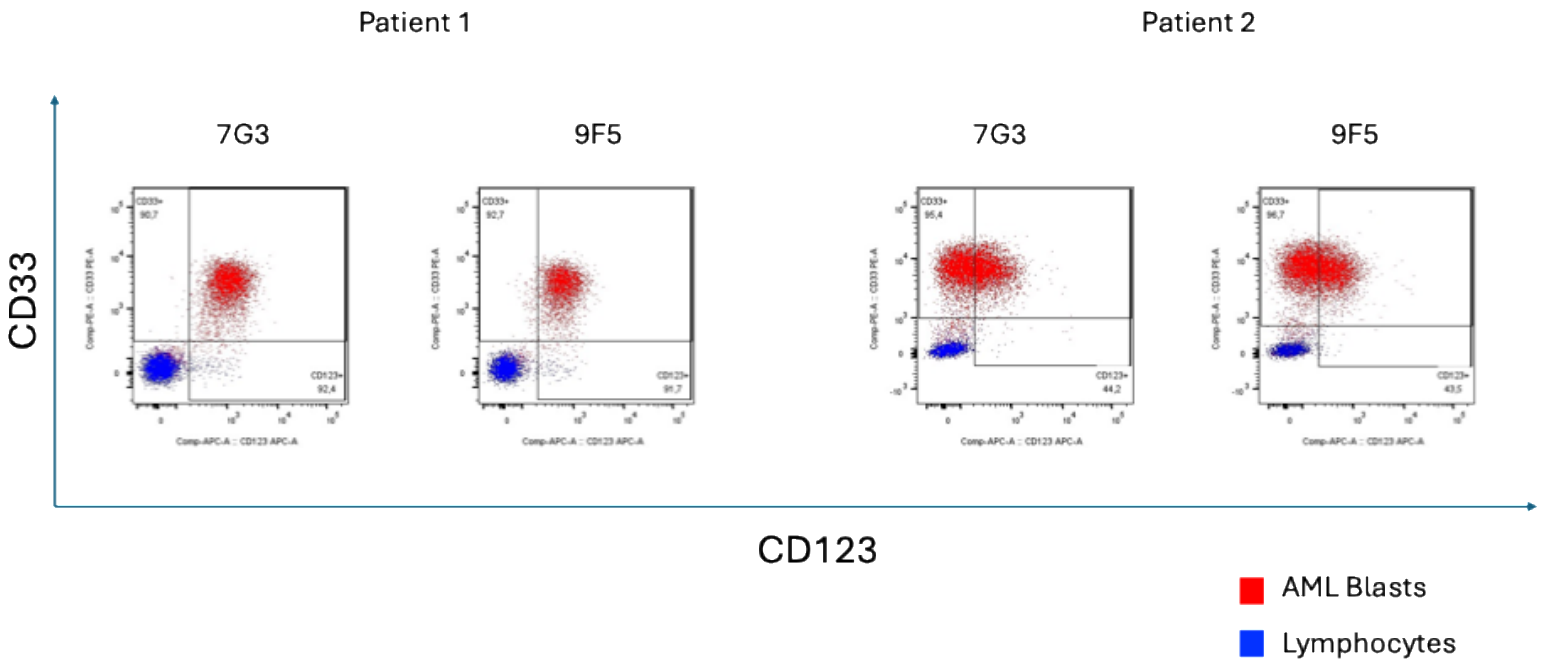
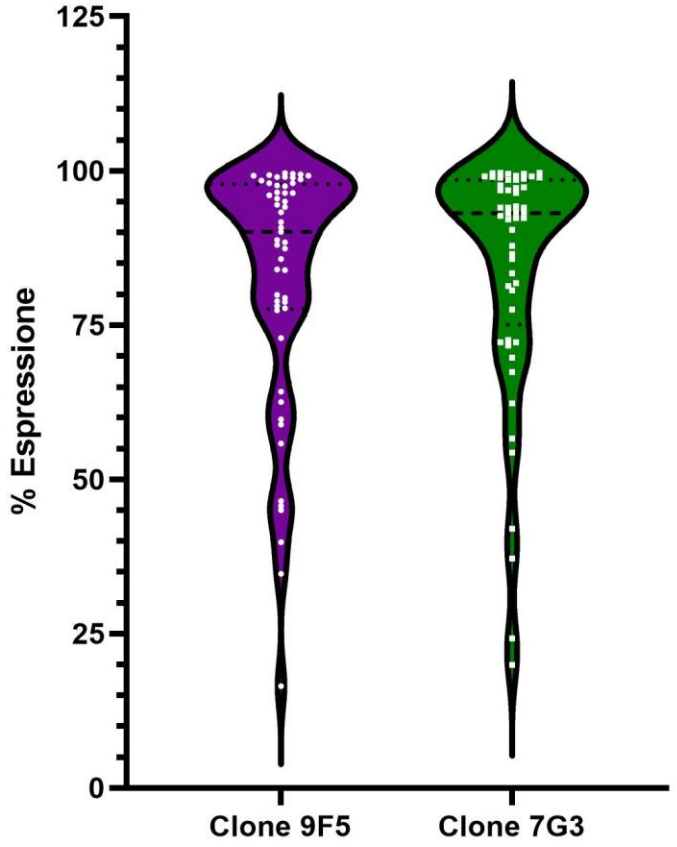
Perriello V, Rotiroti MC,....Tettamanti S, Blood Advances 2022

CD123 and CD33 expression in AML Bergamo Cohort



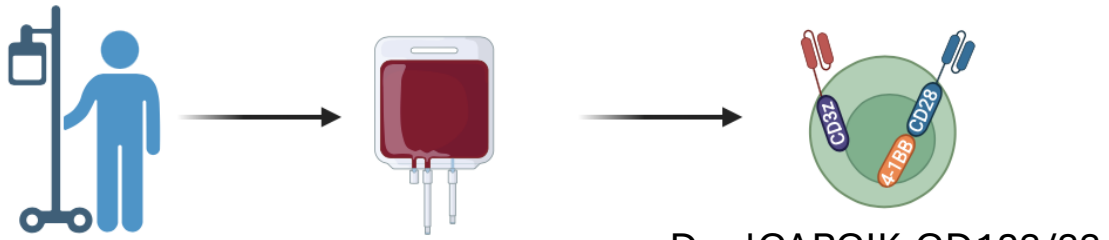
% CD123⁺ cells in 53 AML samples



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



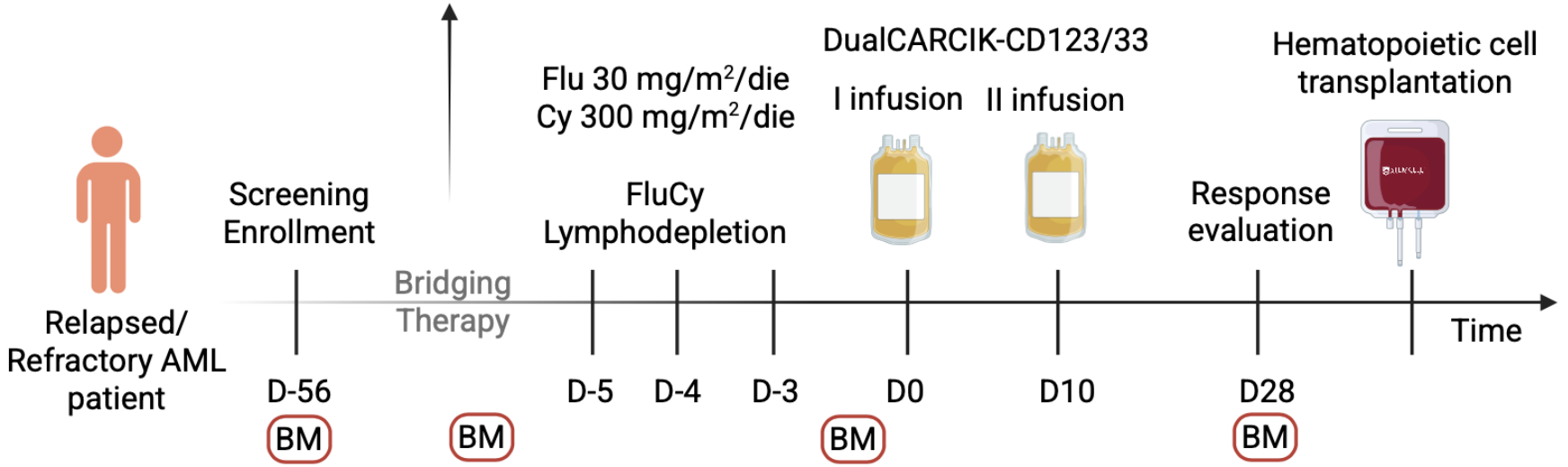
Healthy donor (haploidentical or matched)

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)

Azienda Ospedaliera Papa Giovanni XXIII Bergamo

Key Inclusion Criteria

1. Morphologically confirmed (bone marrow blasts > 5%) diagnosis of AML or quantifiable MRD by flow cytometry (>0.01%) or molecular MRD (any positivity), 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

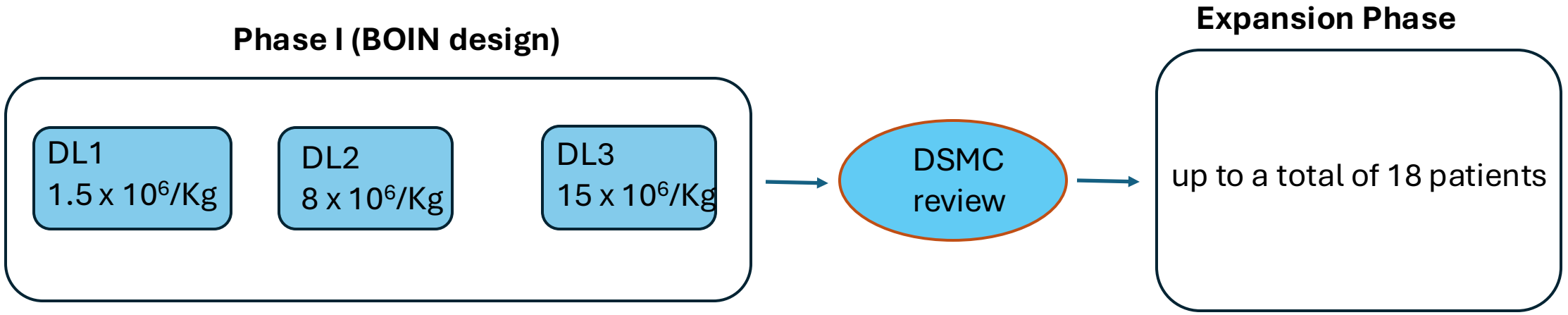
4. Eligibility to proceed to a HCT, different donor is allowed

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

- DLI is an effective post-transplant treatment, but still associated with GVHD and suboptimal rate of response
- 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
- 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

Acknowledgements

Fondazione IRCCS San Gerardo dei Tintori — Sistema Socio Sanitario Regione Lombardia

Sarah Tettamanti
Giorgio Ottaviano
Giovanna Lucchini
Andrea Biondi
Adriana Balduzzi

Giuseppe Dastoli
Giuseppe Gaipa
Lisa Mercadante
Giusi Melita

Ospedale di Bergamo — Sistema Socio Sanitario Regione Lombardia ASST Papa Giovanni XXIII

Alessandro Rambaldi
Federico Lussana
Anna Grassi
Giuseppe Gritti
Silvia Ferrari
Alessandra Algarotti
Giuliana Rizzuto
Maria Chiara Finazzi
Antonio Scannella
Silvia Mariani
.. E tutto il team!

The Molecular Lab »Paolo Belli»
Cristian Meli. Alice Arnoldi Giorgia Peluso Anna Salvi
Manuela Tosi Roberta Cavagna Clara Belotti Silvia Salmoiraghi

Università degli Studi di Milano Bicocca
Biostatistics and Bioimaging Centre
University of Milano - Bicocca
Stefania Galimberti

GMP-CELL FACTORIES

Laboratorio di Terapia Cellulare e Genica Stefano Verri

Daniela Belotti
Benedetta Cabiati
Stefania Cesana

Valentina Colombo
Michele Quaroni
Giada Matera

Laboratorio di Terapia Cellulare Gilberto Lanzani

Chiara Capelli
Elisa Gotti
Irene Cattaneo

Silvia Panna
Rut Valgardsdottir
Luca Cantamessa

Martino Introna
Josee Golay

University of Perugia
Prof. Brunangelo Falini
Dr. Vincenzo Perriello

Quelli che con Luca O.N.L.U.S. per lo studio e la cura della leucemia infantile
Fondazione Regionale per la Ricerca Biomedica — Regione Lombardia — Progetto Plagencell
AIRC 5x1000 — Ricerca
Ministero della Salute
Comitato Maria Letizia Verga per lo studio e la cura della leucemia del bambino ONLUS