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Oltre la trasduzione virale

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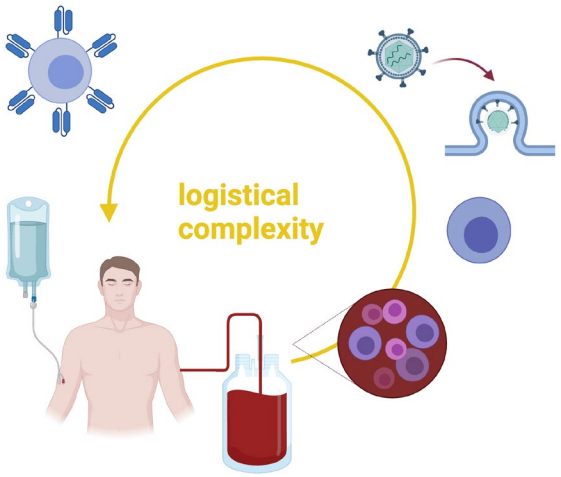
Disclosures of Federico Lussana

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Pfizer					X	X	
Abbvie					X	X	
Amgen					X	X	
Incyte					X		
Clinigen						X	
Astellas					X		
Bristol Myers Squibb					X	X	
Jazz Phamraceuticals					X		

Program overview

- Transposon-based vector systems
- Allogeneic CAR-T platform
- Clinical results with Sleeping Beauty transposon-engineered CARCIK-CD19

Viral vectors for gene therapy



Advantages:

- Efficient gene transfer
- Elevated expression of the CAR molecule
- Standardization of production
- Safety based on thousands of patients treated so far

Disadvantages:

- Viral vectors are limited in DNA cargo size capacity
- High cost of viral batch
- Limited number of specialized facilities (biosafety level 2)

Concerns about the accessibility considering the advancement of CAR-T cell therapy to earlier lines of therapy and their possible use in solid tumors and autoimmune diseases

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

Barbara McClintock's crucial discovery in 1944

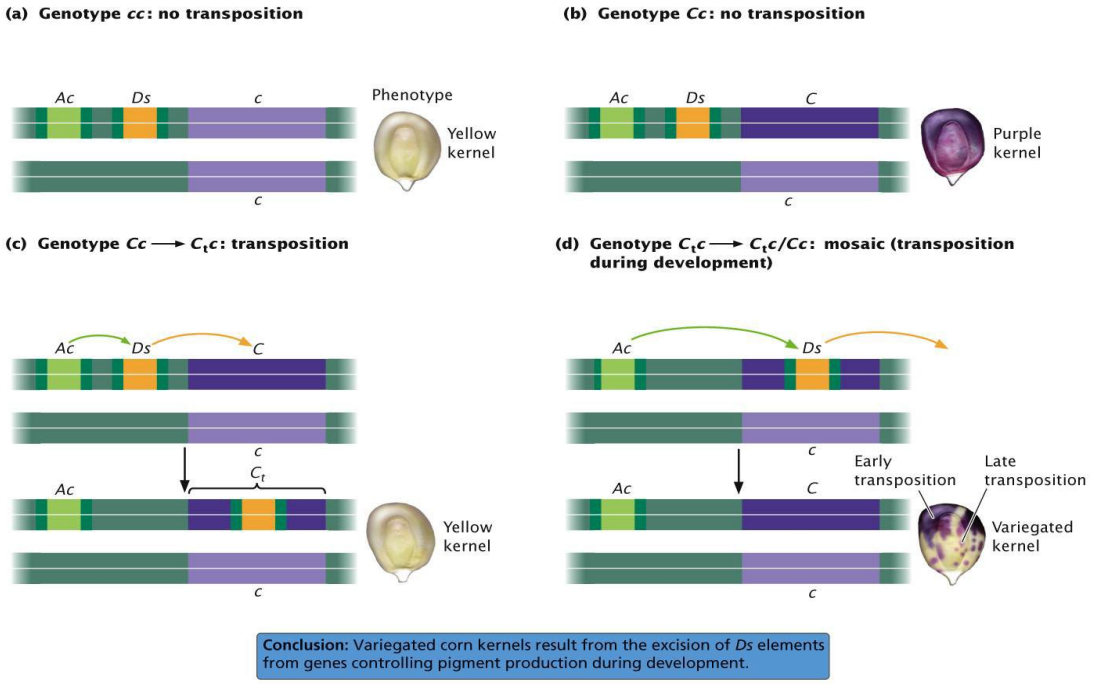
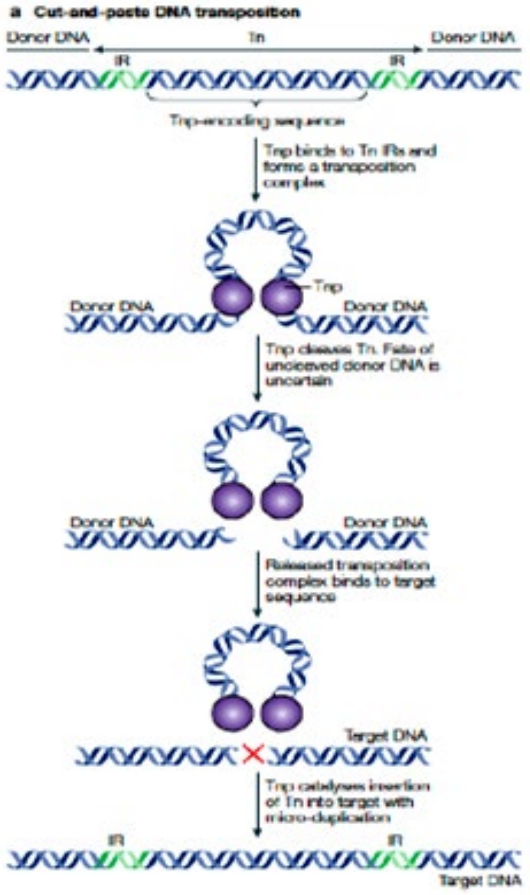


Fig_11-27 *Genetics, Second Edition* © 2005 W.H. Freeman and Company

She discovered that genes controlling the color of cariossidi are regulated **by other genetic elements that can change their position within the chromosome**. This led to the understanding that the genome is not static but subject to changes and alterations.

She was awarded the Nobel Prize for Medicine in 1983

Transposons: a “easy” alternative to viral vectors for gene therapy



TRANSPOSONS mobile DNA elements naturally occurring in the genome that can transpose to new positions **without the need of sequence omology**

Advantages:

- Large cargo size (11kb)
- Low immunogenicity

Disadvantages:

- Low rates of integration of transgenes
- Needs “help” to get into the nucleus

Optimization of disadvantages:

1) **Transposase** renders the system not dependent on low rates of integration of transgenes
2) **Electroporation** corrects the low rates of delivery to target-cell nuclei

“Transposon vector systems are less complex and can be handled under lower biosafety level regulations”

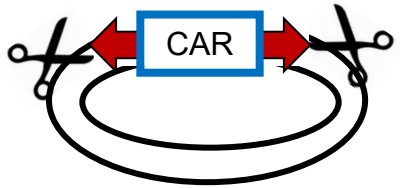
modified from Bordenstein S.R. (2005), *Nature Review Microbiology*, **3**, 688-699

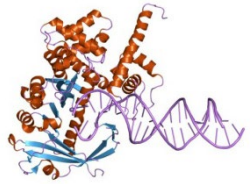
T CELL ENGINEERING WITH CAR BY SLEEPING BEAUTY (SB) SYSTEM: Clinical-grade Protocol of Gene Therapy for CIK Cells With CAR

SB

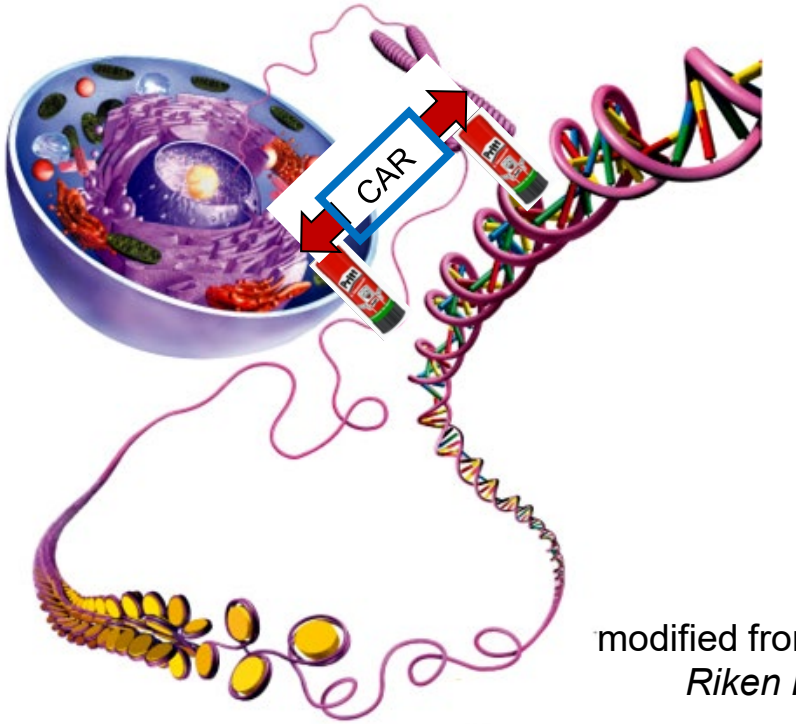
system

The Transposon plasmid contains CAR gene enclosed by IR/DR sequences





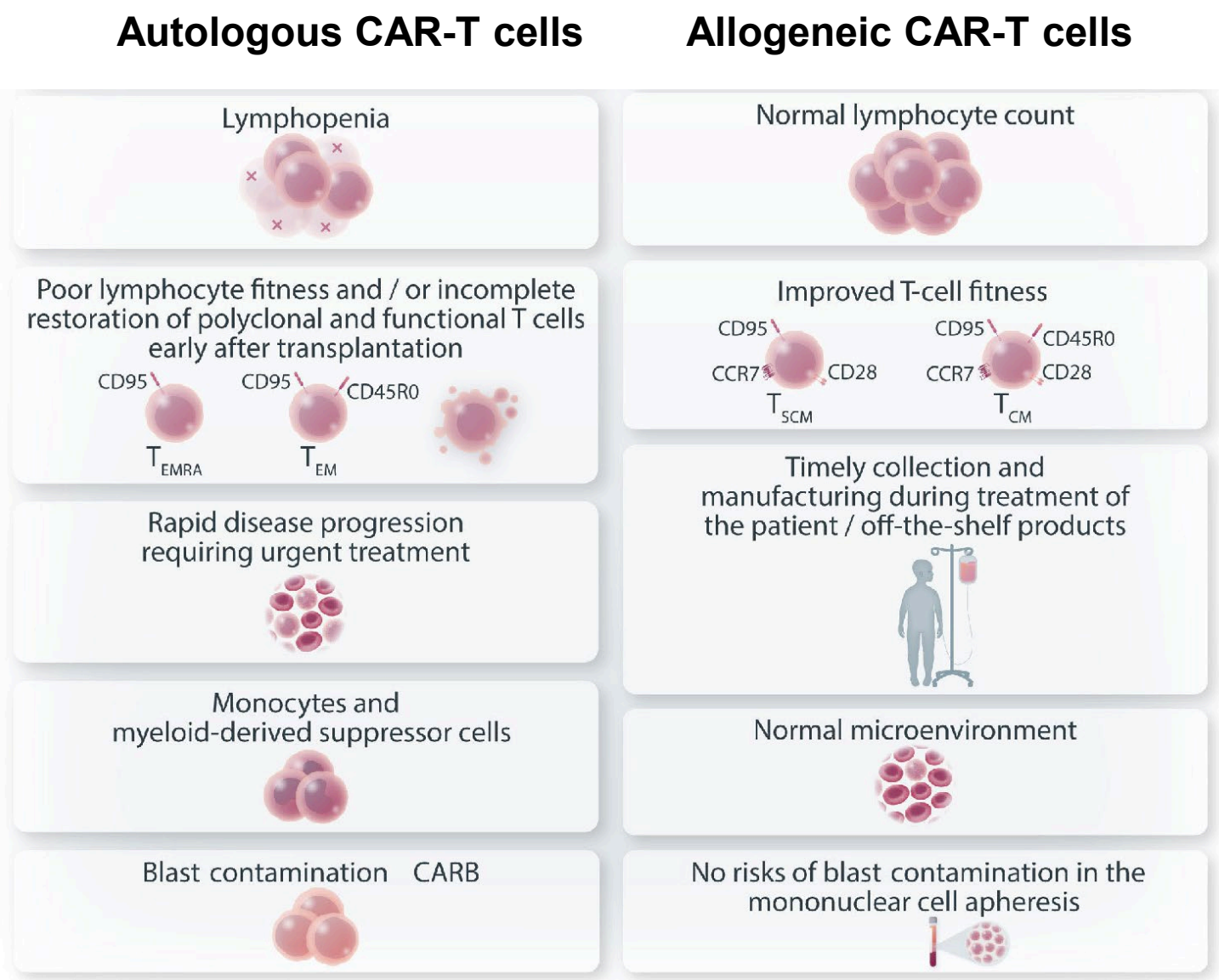
Another plasmid contains the Transposase that cuts IR/DR allowing insertion in genomic DNA



modified from Koseki H. (2008), *Riken Research*, 3, 7

Electroporation uses an electrical pulse to create temporary pores in cell membranes

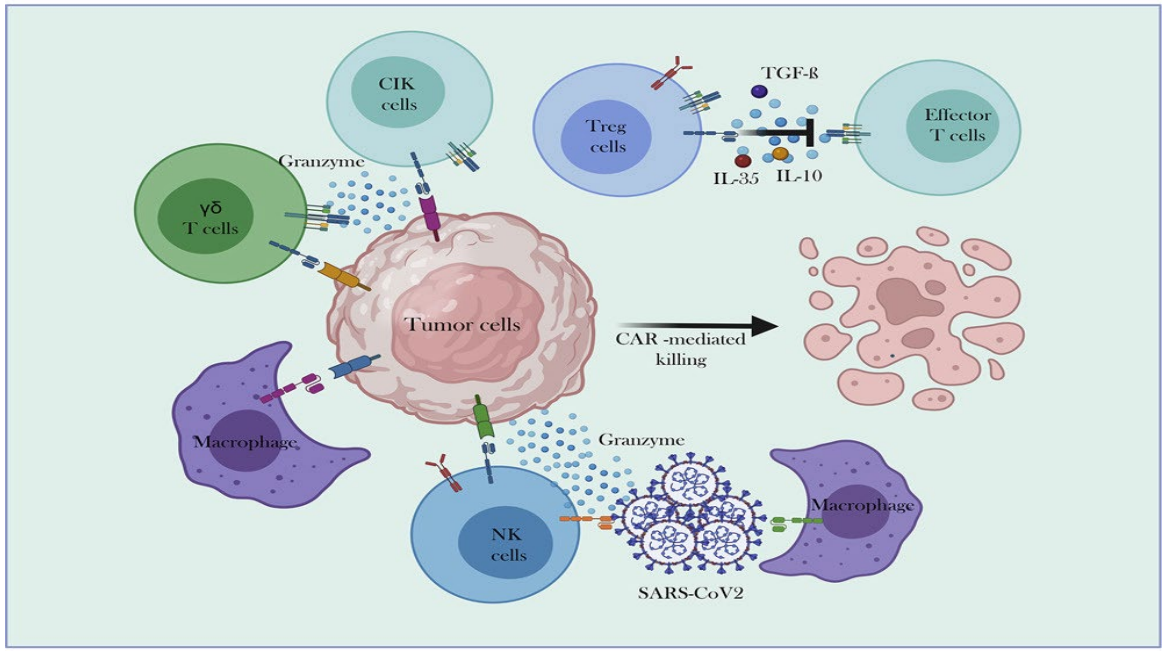
Potential advantage of the development of allogeneic CAR-T platforms



Locatelli F et al. Haematologica 2024 Jun 1; 109(6): 1689–1699.

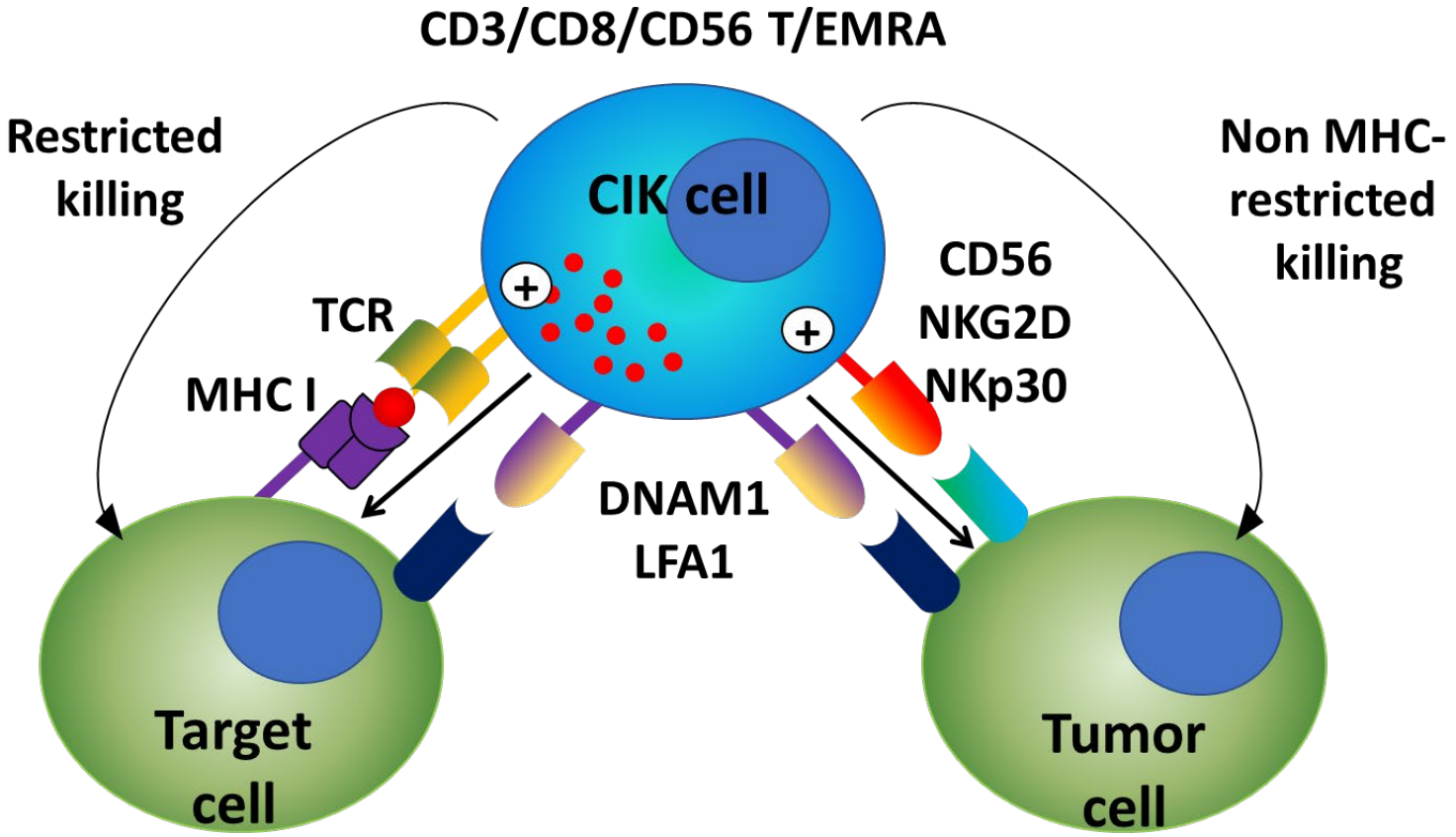
How to prevent the risk of GvHD

- Genetically edited T cells (e.g. targeting TCR or CD52)
- NK cells
- Invariant NK T cells (iNKT cells) or iPSC-derived NK
- CIK



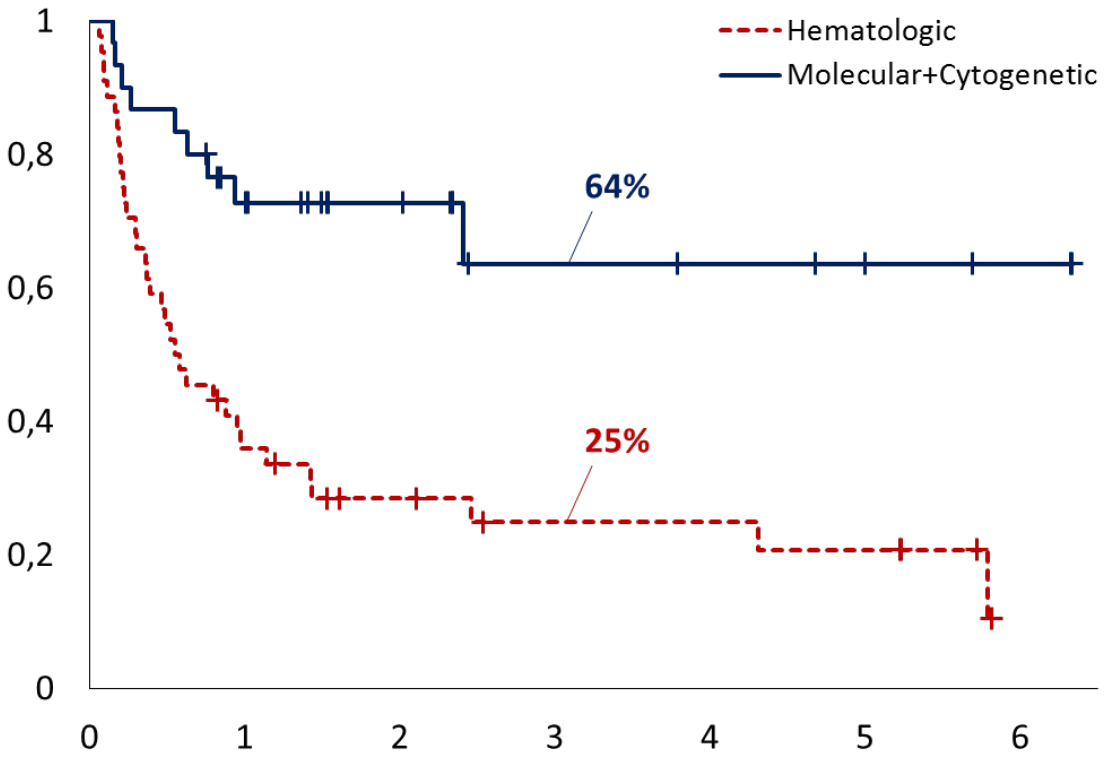
Adapted from Nawshad Hossian KM et al. Molecular Therapy DOI: 10.1016/j.ymthe.2022

Allogeneic CIK cells may represent an ideal platform to transduce chimeric antigen receptors with a reduced risk of inducing GvHD and cytokine release syndrome

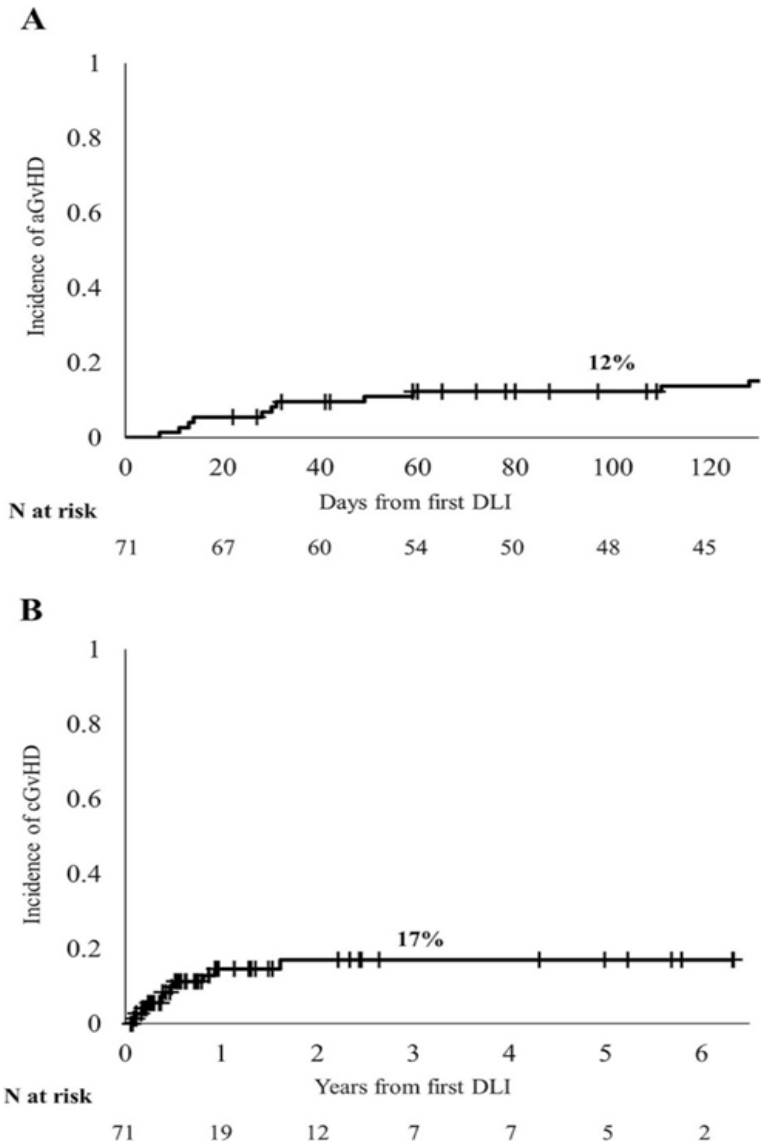


1 Introna M, et al. Bone Marrow Transplant. 2006; 2 Pievani et al, Blood, 2011; 3 Linn et al. Journal of Biomed and Biotech 2010; 4 Sangiolo et al. Journal of Cancer 2011; 5 Introna et al, Haematologica 2007; 6 Rambaldi A (2015) Leukemia 29(1):1-10; 7 Introna M (2017) Biol Blood Marrow Transplant. 23(12):2070-8;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

Sequential Infusion of DLI and CIK

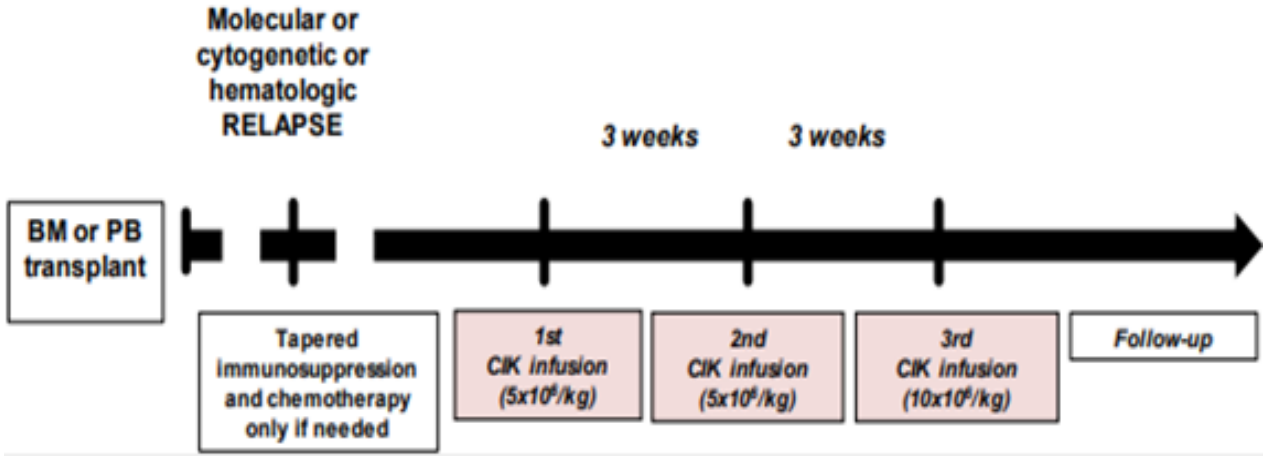


N at risk (events)													
Hematologic	44	(28)	15	(3)	9	(1)	6	(0)	6	(1)	5	(1)	0
Molec+Cytog	30	(8)	19	(0)	12	(1)	6	(0)	5	(0)	3	(0)	2



Introna M., Lussana F. et al.: Biology of Blood and Marrow Transplantation 2017

CIK from haploidentical or HLA-mismatched donor: single center, non-randomized, open label, phase I/II study

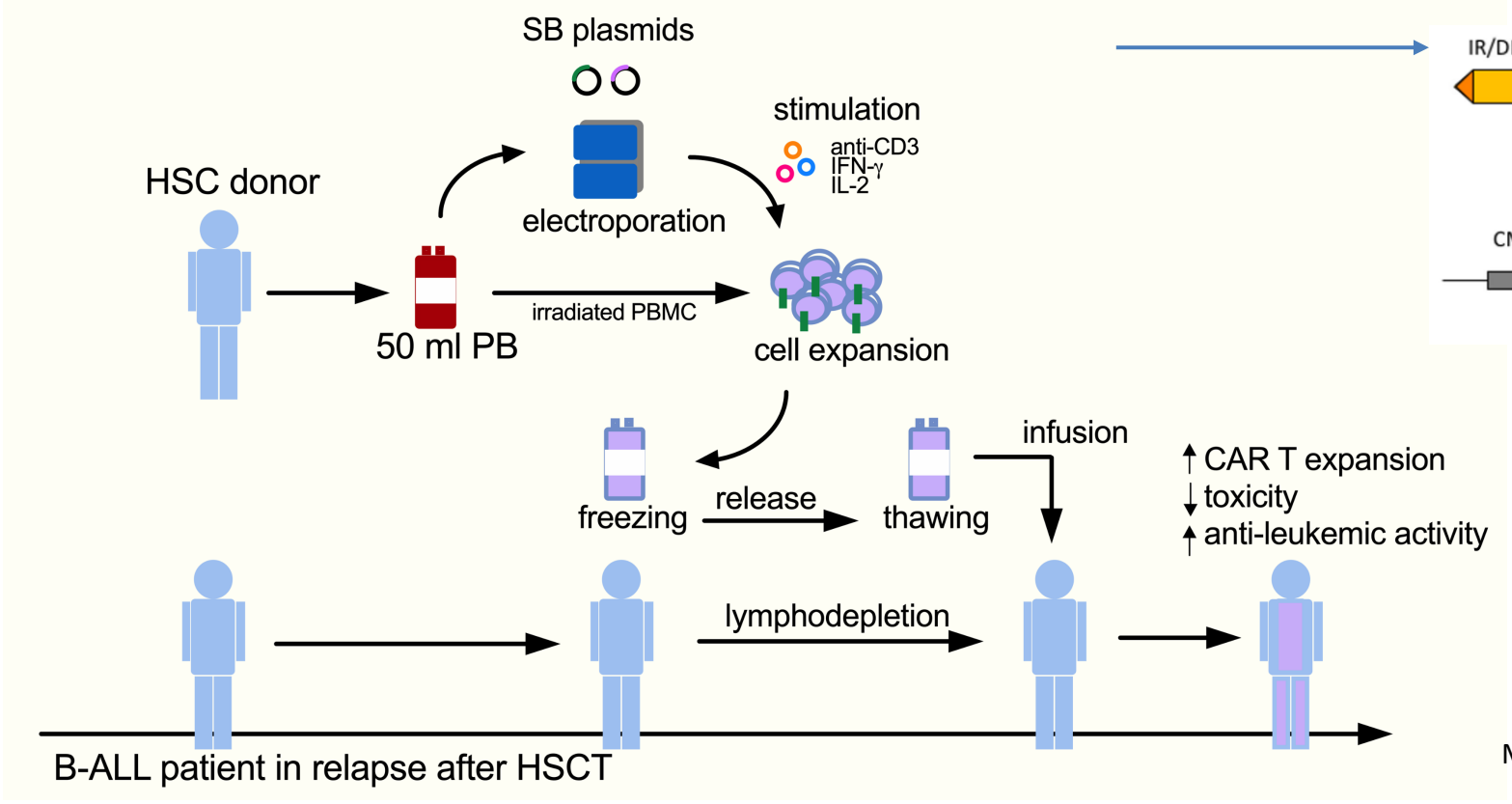


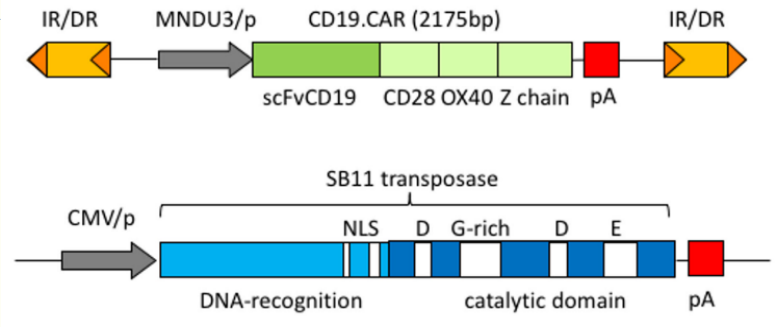
- **No dose-limiting toxicity** was observed
- **GVHD** after CIK infusion was **not observed**

Scannella A et.: Milano, SIE 2024
Lussana F. et al Manuscript in preparation

Clinical experience with SB-engineered CARCIK-CD19 in B-ALL post HSCT

Multicenter Dose Escalation Study FT01CARCIK (Phase I/II NCT03389035)→N=15
Compassionate use FT02CARCIK→N=6
Multicenter Phase 2 Study FT03CARCIK (Phase II NCT05252403)→N=15
Enrolling in ASST-Monza and ASST-Bergamo, IT
Manufactured in-house





Magnani CF, et al. *J Clin Invest*. 2020 Nov 2;130(11):6021-6033
Lussana F, et al. *Blood Cancer Journal,* 2025

Patient and disease characteristics

Characteristic	Phase 1/2 (FT01) (n = 15)	Compassionate (FT02) (n = 6)	Phase 2 (FT03) (n = 15)	Overall (n = 36)
Median age (range), year	35 (1-62)	44 (29-67)	45 (7-66)	39 (1-67)
Pediatric, n (%)	2 (13)	0 (0)	2 (13)	4 (11)
Philadelphia chromosome positive, n (%)	6 (40)	2 (33)	3 (20)	11 (31)
Median number (range) of Prior Lines of Therapy	4 (3-8)	3 (2-5)	3 (1-6)	3 (1-8)
Extramedullary disease, n (%)	2 (13.3)	3 (50)	2 (13.3)	7 (19)
Median BM Blasts at enrolment (range), %	50 (5-100)	17.5 (5-54)^	10 (4-86)	24 (4-100)
No. of previous allo-SCT, n (%)				
1	9 (60)	4 (67)	14 (93)	27 (75)
2	6 (40)	2 (33)	1 (7)	9 (25)
Type of Transplant Donor, n (%)				
Sibling donor	5 (33)	1 (17)	8 (53)	14 (39)
Matched-unrelated donor	5 (33)	3 (50)	5 (33)	13 (36)
Haploidentical donor	5 (33)	2 (33)	2 (13)	9 (25)
Months from Allo-HSCT to Relapse, median (range)	11 (1-25)	5.5 (3-54)	8 (2-168)	7 (1-168)
Median % (range) BM Blasts after lymphodepletion	7 (0-80)°	0 (0-0)°	0 (0-58)°	0 (0-80)

Safety: GvHD, CRS and ICANS

Events	Phase 1/2 (FT01) (n = 15)	Compassionate (FT02) (n = 6)	Phase 2 (FT03) (n = 15)	Overall (n = 36)
GVHD, n (%) Grade 1-4	0 (0)	0 (0)	0 (0)	0 (0)
CRS, n (%)				
No CRS	8 (53)	4 (67)	9 (69)	21 (58)
Grade 1	3 (20)	1 (17)	4 (27)	8 (22)
Grade 2	4 (27)	1 (17)	2 (13)	7 (19)
Grade ≥ 3	0 (0)	0 (0)	0 (0)	0 (0)
ICANS, n (%)				
No ICANs	14 (93)	6 (100)	15 (100)	35 (97)
Grade 1	0 (0)	0 (0)	0 (0)	0 (0)
Grade 2	1 (7)	0 (0)	0 (0)	1 (3)
Grade ≥ 3	0 (0)	0 (0)	0 (0)	0 (0)
Neurotoxicity, n (%)				
Grade 3	2 (13)	0 (0)	0 (0)	2 (6)

Investigation of product-derived lymphoma following infusion of *piggyBac*-modified CD19 chimeric antigen receptor T cells

Two T-NHL cases in patients treated with CAR T cells generated with PiggyBac transposon

No mechanism was identified that could precisely determine the cause of this transformation of the gene-modified T cells into neoplastic cells

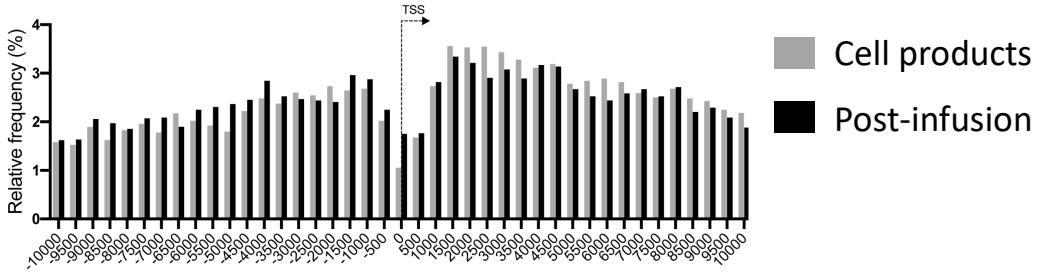
High transgene copy number (>20 per cell), which was integrated into the DNA of the malignant cells

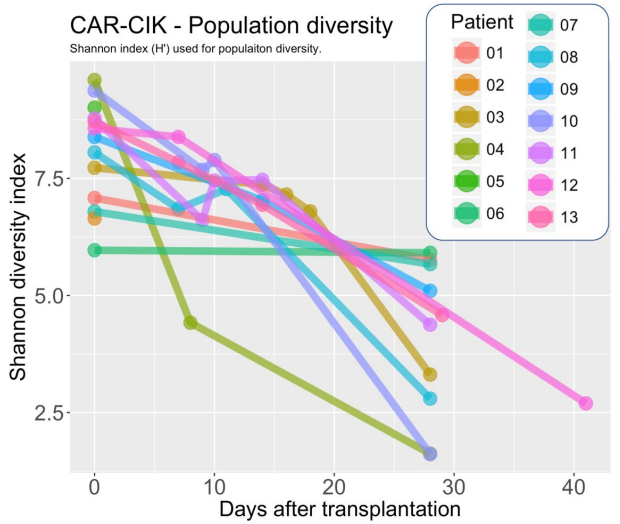
Mickletwaite et al. Blood 2021; Schambach A et al; Mol Ther2021 Sep 1;29(9):2631-2633

Safety of the use of SB transposon for human gene therapy

CARCIK-CD19 release criteria and integration site analysis:

- Transgene copy number release criteria <5 copies/cell
- No preference for transcriptional start sites and promoter/enhancer sequences
- No Common insertion site classified as cancer-related gene
- High polyclonal marking and population diversity



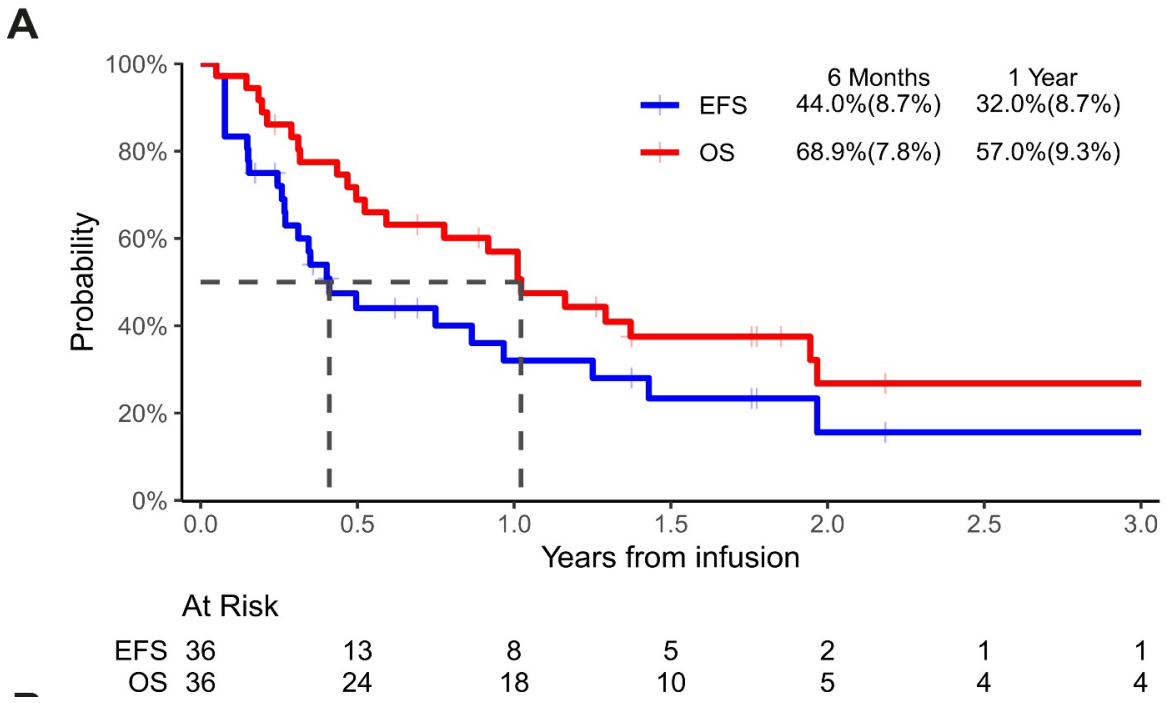


Magnani CF, et al. *J Clin Invest*. 2020 Nov 2;130(11):6021-6033
Lussana F, et al. *Blood Cancer Journal,* 2025

Survival after CARCIK-CD19

Efficacy

- 30/36 reached CR (83%)
- 86% were MRD-negative
- Median follow-up 2.2 years
- 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)



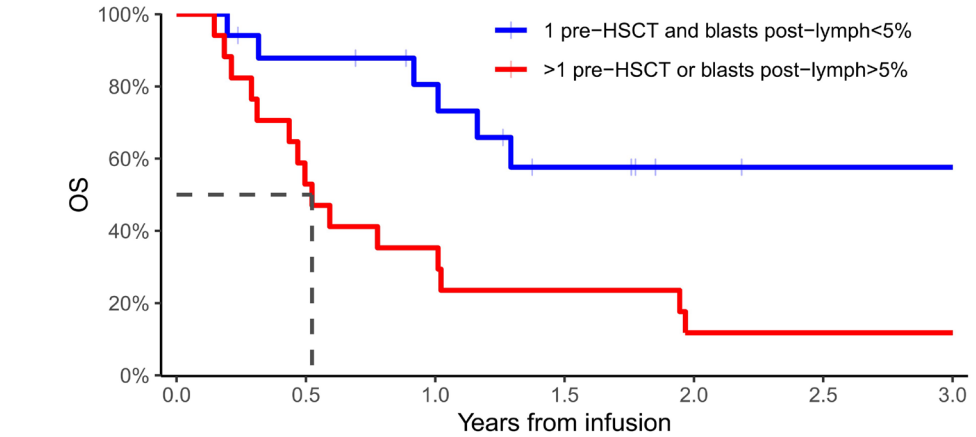
Lussana F, et al. *Blood Cancer Journal,* 2025

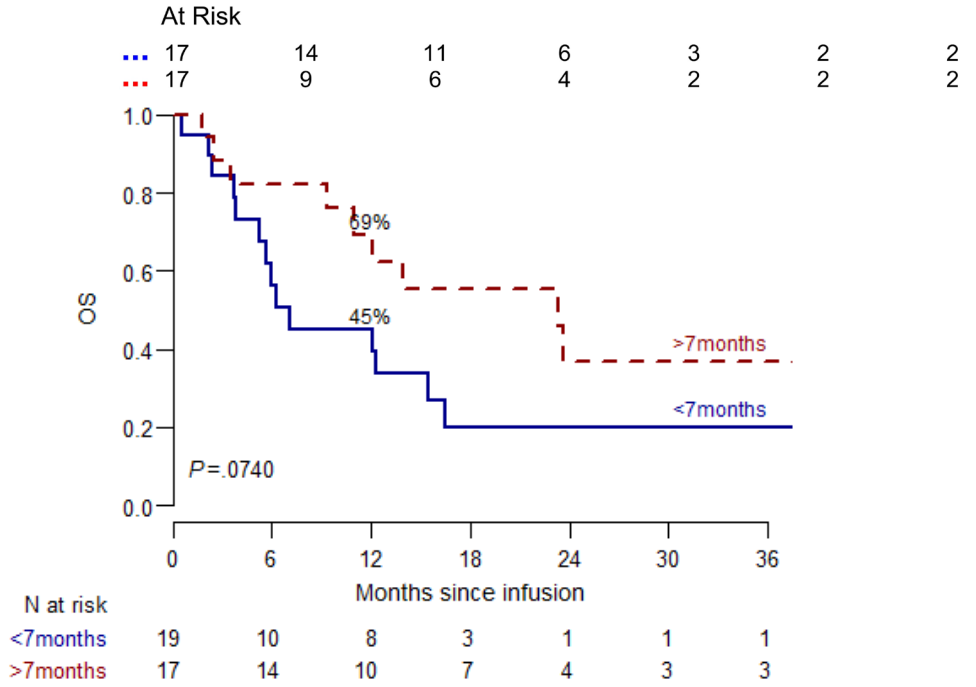
Outcomes by patient characteristics

Efficacy

- For patients with <5% blast post-lymphodepletion and 1 prior allotransplantation, the 12-month EFS and OS rates were 48% and 81%, respectively. In contrast, those with >1 prior alloHSCT or >5% blast had rates of 21% and 35%.

- For early relapses (<7 months post-alloHSCT), the 12-month EFS and OS were 21% and 45%, compared to 43% and 69% for relapses after 7 months





Lussana F, et al. *Blood Cancer Journal,* 2025

THE FT04 PROTOCOL: ALLOGENEIC CARCIK-CD19 IN NHL

Aut. AIFA 23/11/23
n° EudraCT /EU CT 2023-505511-20-00 (NCT 05252403)

Patients

- Pediatric or adult (age ≥1 years)
- R/R CD19+ B-cell NHL or CLL
- HIV-related B-NHL eligible
- ≥2 prior therapies
- Ineligible due to age or comorbidities (including HIV) to commercially available CART
- Adequate organ functions
- ECOG PS ≤2

Primary objectives

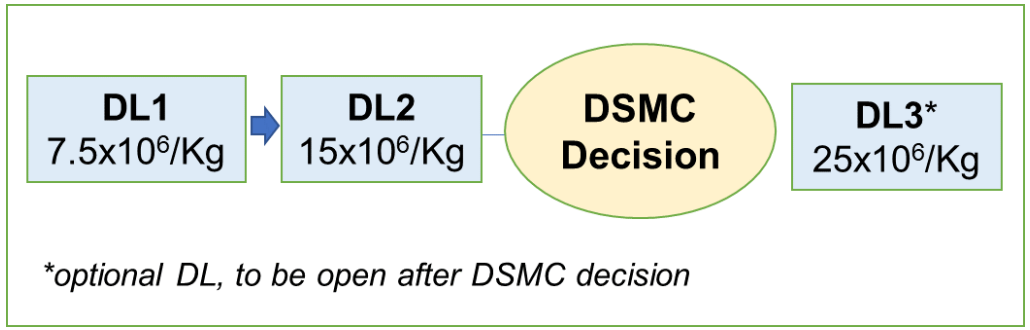
- Safety and RP2D (Phase I)
- Efficacy (ORR) (Phase II)

Dose escalation (Phase I) and expansion (Phase II)

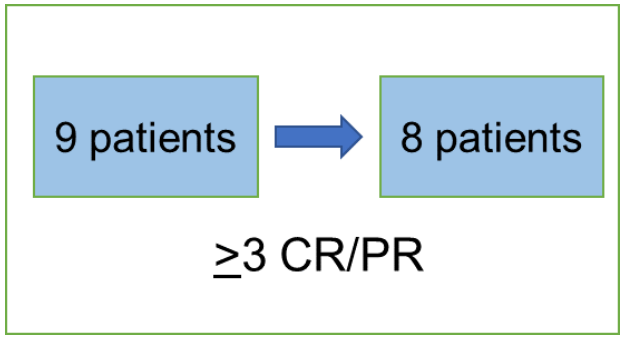
Donor: at least haploidentical (i.e. 4/8 HLA matched by allele typing)

[redacted]

Phase I
(3+3 design)
9-12 patients



Phase II (Simon Design)
Up to 17 patients



Conclusions

- Allogeneic CARCIK-CD19 cells can be reproducibly produced under GMP conditions using a transposon-based vector system
- Donor-derived CARCIK-CD19 allogeneic cells are safe, can induce a deep CR in more than 80% of patients with B-ALL relapsing after alloHSCT, and can persist in vivo up to 2 years
- The safety and the activity of CARCIK-CD19 and the reduced cost of production support its potential use in a preemptive setting after alloHSCT (i.e., MRD pos at the time of conditioning)
- These cells are currently being tested in a new clinical trial for patients with B-NHL for whom no commercial CAR-T cells are currently available

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