9° WORKSHOP IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Bologna, Aula "G. Prodi", 19-20 maggio 2025

"Nuove prospettive di terapia cellulare con effettori allogenici nelle neoplasie ematologiche (Allo-CART; NKCART; CIK) "

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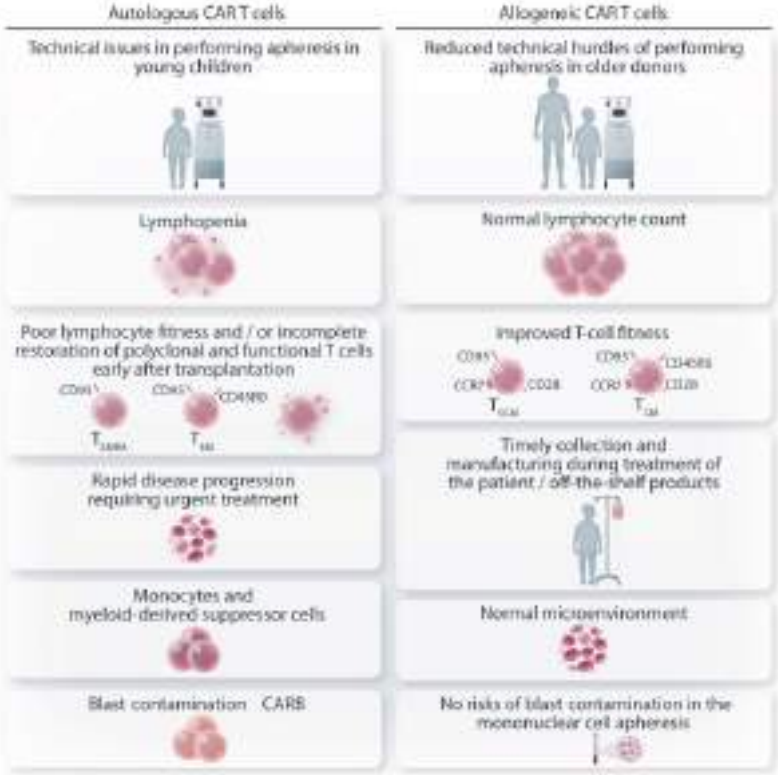
Fondazione IRCCS San Gerardo dei Tintori

Regione Lombardia

Disclosures di Andrea Biondi

No disclosures

Limitations of autologous and possible solutions offered by allogeneic CAR T cells

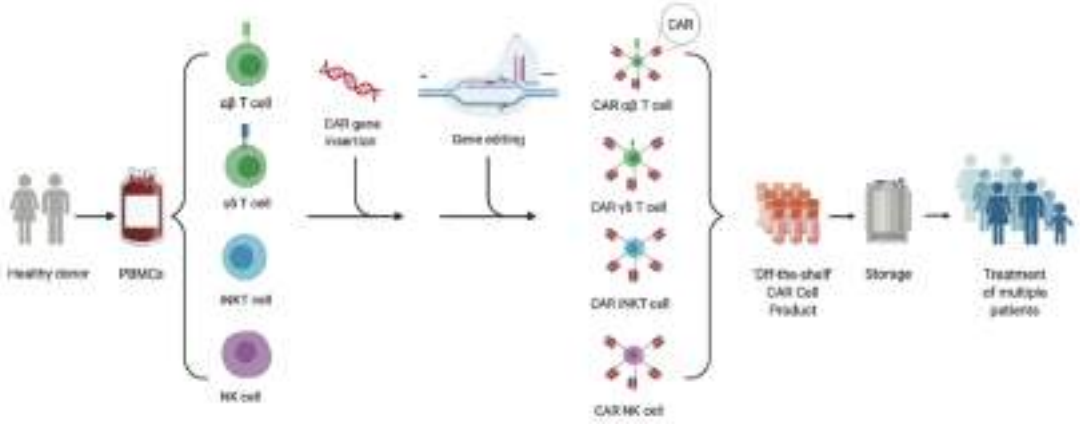


Locatelli F. *et al* , Haematologica 2024

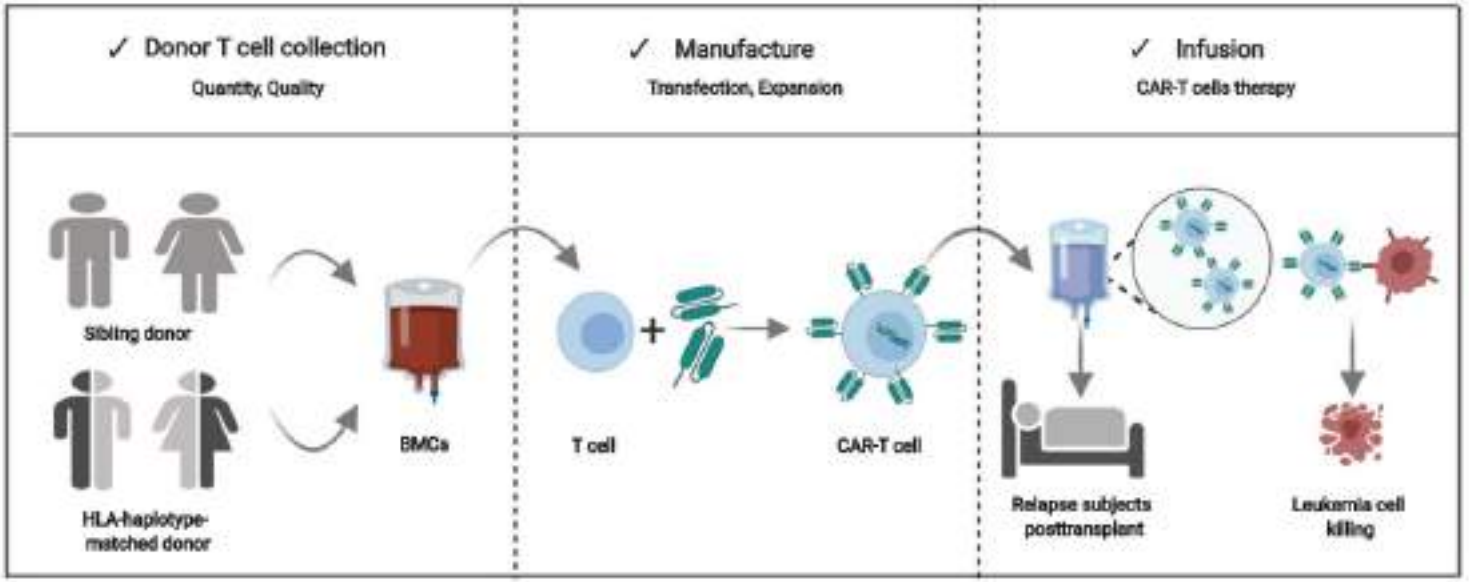
Allogenic sources of CAR T cell therapy

1. Donor-derived (CART and CARCIK)
2. Universal, gene-edited
3. Non-T cell based (iNKT, NK)
4. Induced pluripotent stem cell

→ Towards universal, **Off the shelf** CAR T cell therapy?



1. Donor-derived CAR T cells



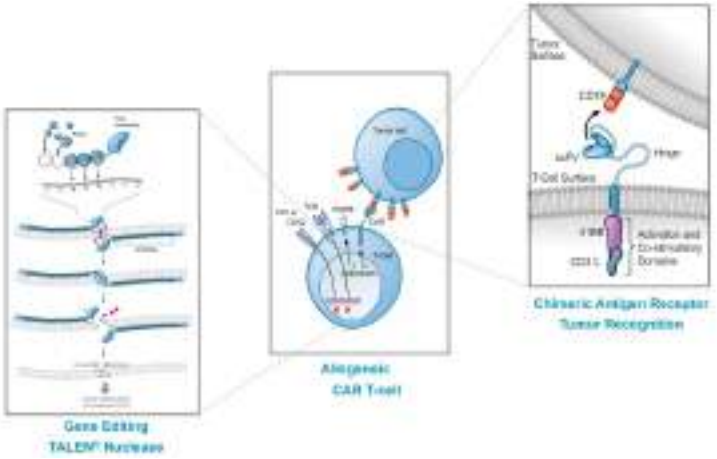
Zhang *et al* 2021, Leukemia

1. Donor-derived CD19 CAR T cells for lymphoproliferative diseases

Population	Disease	N of pts	CAR	Gene delivery	Prior LD	Cell dose	Donor	Prior DLI	GvHD	Clinical responses	Ref
Adults and children	CLL B-ALL	8 (6 adults and 2 children)	2nd gen, CD28	Retrovirus	No	5.8x10⁷ - 1.13x10⁸ (5 pts multiple infusions)	URD, MSD	Yes (2 pts only)	No	1 CR, 2 pts treated in CR and maintaining it 1 PR 1 SD 3 PD	23
Adults	CLL B-NHL	10	2nd gen, CD28	Retrovirus	No	0.4-7.8x10⁶/kg	URD, MSD	Yes	No	1 CR 1 PR 6 SD 2 PD	24
Adults	B-NHL B-ALL	2	2nd gen, 4-1bb	Lentivirus	Not reported	4.5x10⁶/kg 4.2x10⁶/kg	Not reported	Yes	G2-3 GvHD in both	1 CR in BM and PR in EM 1 PR (2)	26
Adults	B-ALL B-NHL CLL	20	2nd gen, CD28	Retrovirus	No	0.4-7.8x10⁶/kg	URD 10/10 or 9/10, MSD	Yes	G1-2 GvHD in 2 pts (pre-existing)	6 CR (4/6 MRD-neg) 2 PR 6 SD 4 PD	25
Adults	B-ALL (>5% blasts)	6	3rd gen (CD28/ CD27)	Lentivirus	Flu-Cy	0.38-4.1x10⁶/kg (4 pts: 2 infusions)	Haplo	Yes	50% GvHD (G2-3)	4 CR (all relapsed subsequently) 1 not evaluable 1 NR	27
Adults	B-NHL B-ALL	19	2nd gen, CD28	Sleeping Beauty	No	10⁶/m²-10⁸/m²	MSD, haplo	No	G1-4 GvHD in 8 pts	9 CCR 1 CR 2 AWD 2 DR 5 DOD	28
Adults and children	B-ALL	49	2nd gen, CD28 or 4-1bb	Lentivirus	Mainly Flu+Cy	0.4-12x10⁶/kg	MSD, haplo	Yes	G1-2 GvHD in 2 pts	2 died of toxicity 34 reach. CR	30
Children and young adults	B-ALL	13	2nd gen, 4-1bb	Retrovirus or lentivirus	Flu-Cy	1-3x10⁶/kg	MSD, URD, haplo	Yes	G3 GvHD in 1 pt	12 CR in BM and EM 1 CR in the BM and PR in EM	31

Locatelli F. *et al* , Haematologica 2024

2. Universal gene-edited CART – TALEN



TALEN (transcription activator-like effector nuclease)

- TCRα editing - avoid alloreactivity
- CD52 editing – alemtuzumab resistance

SCIENCE TRANSLATIONAL MEDICINE | REPORT

CANCER

Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells

Waseem Qasim,[1,2]* Hong Zhan,[1] Sujith Samarasinghe,[1] Stuart Adams,[1] Persis Amrolia,[1,2] Sian Stafford,[1] Katie Butler,[1] Christine Rivat,[1] Gary Wright,[2] Kathy Somana,[2] Sara Ghorashian,[1] Danielle Pinner,[1] Gul Ahsan,[1] Kimberly Gilmour,[1] Giovanna Lucchini,[2] Sarah Inglott,[2] William Wilson,[2] Robert Chiesa,[2] Karl S. Peggs,[1] Lucas Chan,[3] Farzin Farzaneh,[4] Adrian J. Thrasher,[1] Ajay Vora,[3] Martin Pule,[2] Paul Veys[1]

UCART19, a first-in-class allogeneic anti-CD19 chimeric antigen receptor T-cell therapy for adults with relapsed or refractory B-cell acute lymphoblastic leukaemia (CALM): a phase 1, dose-escalation trial

Reuben Benjamin
Department of Haematological Medicine, King's College Hospital NHS Foundation Trust, London, UK; Rayne Institute, School of Cancer and Pharmaceutical Sciences, Kings College London, London, UK
Nitin Jain
The University of Texas MD Anderson Cancer Center, Houston, TX, USA

2. Universal gene-edited - CRISPR-Cas9

Multiplexed CRISPR-Cas9 gene editing applications in combination with novel cell resources to enhance CAR T-cell efficacy and to produce a universal 'off-the-shelf' product

TRAC, B2M

- No GvHD
- No rejection
- Allogenic 'off-the-shelf' option

PD-1, CTLA-4, LAG-3, Fas

- Restrained differentiation
- Less exhaustion, greater persistance
- Reduced apoptosis

Cas9 variants to fine-tune transcriptional and epigenetic regulation

Enhanced Cytokine Profile

⊕ IL-7, IL-12, IL-15, IL-18

⊖ GM-CSF, IL-6, less inhibited by immunosupressive factors in TME

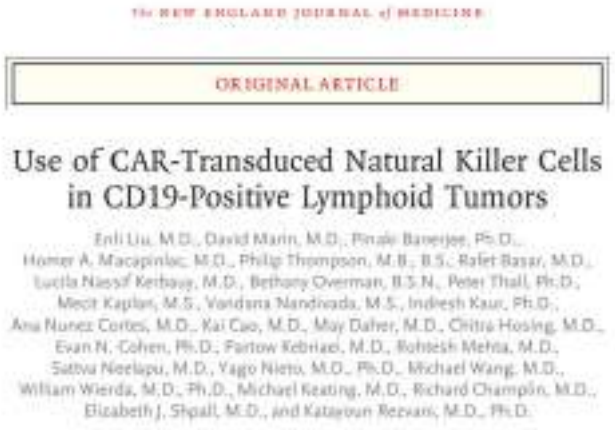
Dimitri *et al*. Molecular Cancer,2022

2. Clinical trials of engineering CAR-T cells based on CRISPR/Cas 9 in lymphoproliferative diseases

Study Title	Phase	CAR-T Cell	Cancer	Edited/ targeted Genes	Patients (n)	Location	Status	NCT Number
[illegible]	I	CD19 CAR-T	NHL	Unknown	[illegible]	United States	Recruiting	[illegible]
[illegible]	I	CD70 CAR-T	T cell malignancy, DLBCL	Unknown	[illegible]	United States	Recruiting	[illegible]
[illegible]	I	BCMA CAR-T	MM	Unknown	[illegible]	United States	Active, not recruiting	[illegible]
[illegible]	I	BCMA CAR-T	MM	Unknown	[illegible]	United States	Recruiting	[illegible]
[illegible]	I/II	CD19 CAR-T	B-cell Lymphoma, B-cell ALL	Unknown	[illegible]	United States	Recruiting	[illegible]
[illegible]	I	CD19 CAR-T	ALL, CLL, NHL	B2M, CIITA and TCR alpha chain knock out	–	–	Withdrawn	[illegible]
[illegible]	I	CD19 CAR-T	ALL, Lymphoma	HPK1 knock out	[illegible]	China	Recruiting	[illegible]
[illegible]	I/II	CD19 CD20 CAR-T	Leukemia, Lymphoma	Unknown	[illegible]	China	Unknown status	[illegible]
		CD22 CAR-T						
[illegible]	I/II	CD19 CAR-T	Leukemia, Lymphoma	TCR and B2M knock out	[illegible]	China	Unknown status	[illegible]
[illegible]	I	CD19 CAR-T	ALL	CD52 and TRAC knock out	[illegible]	United Kingdom	Active, not recruiting	[illegible]

Adapted from: Tao et al Front Immunology, 2024

3. Non-T cell based: Natural killer cells



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

Enli Liu, M.D., David Marin, M.D., Pinaki Banerjee, Ph.D., Homer A. Macapinlac, M.D., Philip Thompson, M.B., B.S., Rafet Basar, M.D., Lucila Nassif Kerbauy, M.D., Bethany Overman, B.S.N., Peter Thall, Ph.D., Mecit Kaplan, M.S., Vandana Nandivada, M.S., Indresh Kaur, Ph.D., Ana Nunez Cortes, M.D., Kai Cao, M.D., May Daher, M.D., Chitra Hosing, M.D., Evan N. Cohen, Ph.D., Partow Kebriaei, M.D., Rohtesh Mehta, M.D., Sattva Neelapu, M.D., Yago Nieto, M.D., Ph.D., Michael Wang, M.D., William Wierda, M.D., Ph.D., Michael Keating, M.D., Richard Champlin, M.D., Elizabeth J. Shpall, M.D., and Katayoun Rezvani, M.D., Ph.D.

- Among 11 patients with relapsed or refractory CD19-positive cancers, a majority had a response to treatment with CAR-NK cells without the development of major toxic effects.

Clinical Trial #	Target Antigen	Disease	NK Cell Source	Phase	Sponsor
NCT02742727	CD7	Lymphoma Leukemia	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co., Ltd
NCT02839954	MUC1	Solid tumors	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co., Ltd
NCT02892695	CD19	Lymphoma Leukemia	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co., Ltd
NCT02944162	CD33	Acute myeloid leukemia	NK-92 cell line	I/II	PersonGen BioTherapeutics (Suzhou) Co.
NCT03056339	CD19	Lymphoma Leukemia	UCB	I/II	MD Anderson Cancer Center
NCT03383978	HER2	Glioblastoma	NK-92 cell line	I	Johann Wolfgang Goethe University Hospital
NCT03415100	MICH2D ligands	Solid tumors	Autologous or allogeneic NK cells	I	The Third Affiliated Hospital of Guangzhou Medical University
NCT03579927	CD19	Lymphoma Leukemia	UCB	I/II	MD Anderson Cancer Center
NCT03656705	-	Non-small cell lung cancer	Modified NK-92 cell line	I	Xinxiang Medical University
NCT03841110	-	Solid tumors	iPSC-derived CAR-NK cells (FT500)	I	Fate Therapeutics

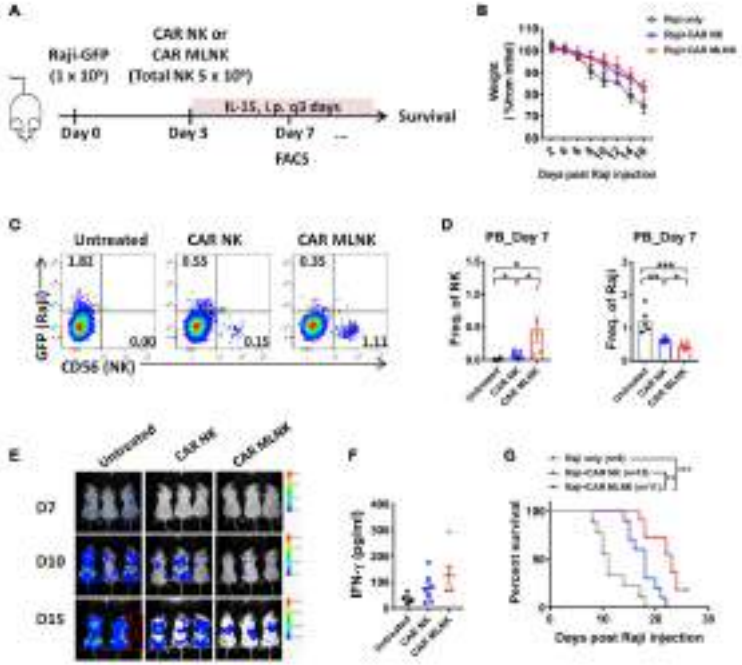
3. Non-T cell based: Natural killer cells

Memory like NK (MLNK)

(IL7 IL15 and IL18)

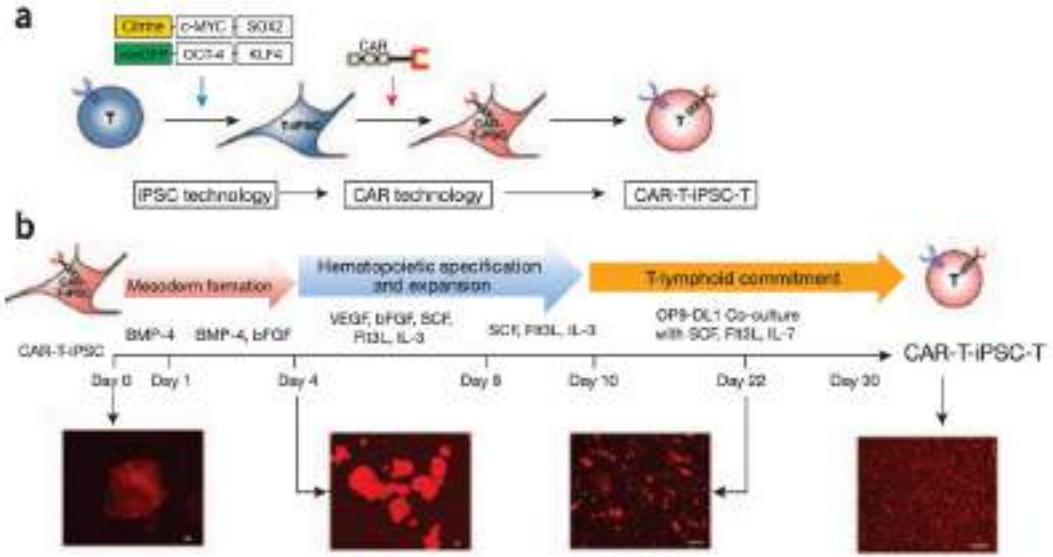
Sources:

- Peripheral blood,
- Cord blood,
- iPSCs,
- Immortalized cell lines.



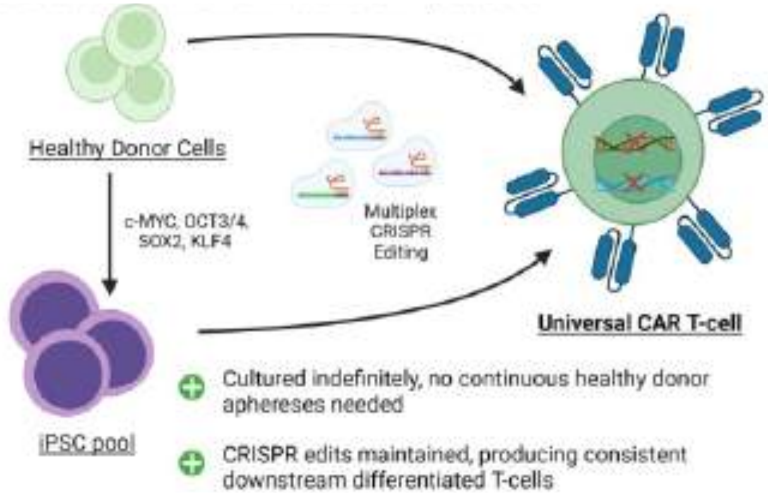
Hu et al, Frontiers Immunology, 2023

4. Induced pluripotent stem cells (iPSC) derived CART-CD19



Themeli *et al*, Frontiers Immunology, 2013

4. iPSC-derived CART-CD19



ASH 2022


704.CELLULAR IMMUNOTHERAPIES: EARLY PHASE AND INVESTIGATIONAL THERAPIES

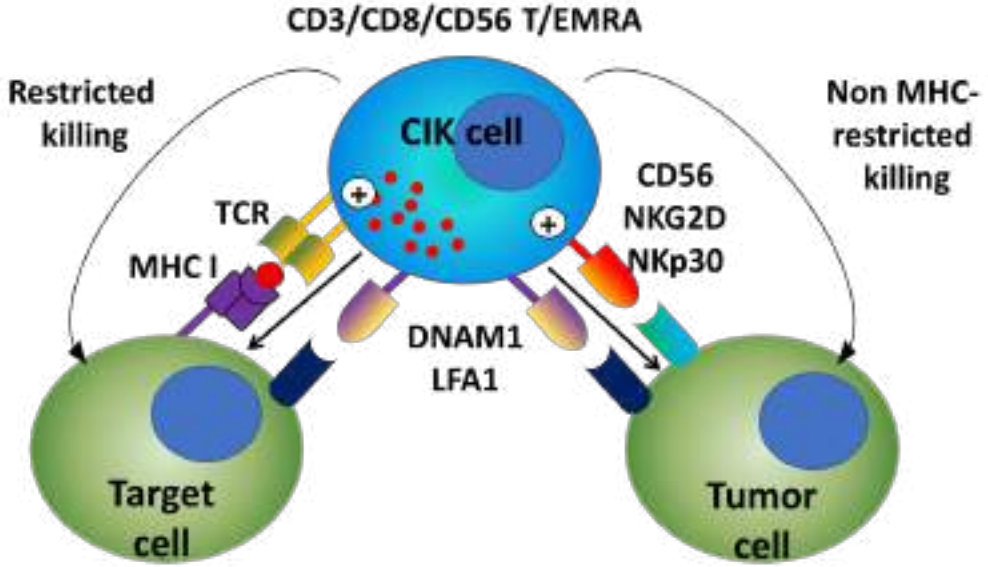
Interim Phase I Clinical Data of FT819-101, a Study of the First-Ever, Off-the-Shelf, iPSC-Derived TCR-Less CD19 CAR T-Cell Therapy for Patients with Relapsed/Refractory B-Cell Malignancies

As of June 25, 2022, **12 R/R BCL patients** were treated. No dose-limiting toxicities, ICANS, GvHD, or Grade ≥3 FT819-related adverse events were observed. **Cytokine release syndrome (CRS)** occurred in 3 patients (all Grade ≤2), indicating a **favorable safety profile** in early evaluation

•**Sponsor**: Fate Therapeutics

Status: Trial was ongoing, but Fate **terminated** all iPSC-derived cell therapy programs in **April 2024** due to financial and strategic setbacks.

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

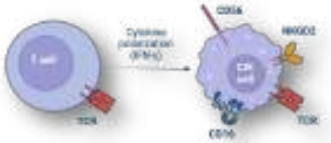


1 Introna M, et al. Bone Marrow Transplant. 2006; 2 Pievani et al, Blood, 2011; 3 Linn et al. Journal of Biomed and Biotech 2010; 4 Sangiolo et al. Journal of Cancer 2011; 5 Introna et al, Haematologica 2007; 6 Rambaldi A (2015) Leukemia 29(1):1-10; 7 Introna M (2017) Biol Blood Marrow Transplant. 23(12):2070-8;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

Non-viral Transposon based CARCIK cell platform

Cytokine-Induced killer (CIK) cells

- Basal antitumor activity
- Low GvHD
- Safe and well tolerated



Introna M et al., Haematologica 2007
Pievani A et al., Blood 2011
Introna M et al., BBMT 2017
Gatti et al., Cytotherapy 2022

Sleeping Beauty transposon system

- Random pattern of integration→ reduced genotoxicity
- Cost effective

Magnani CF et al., Oncotarget. 2016.
Magnani CF et al., Hum Gene Ther. 2018
Arcangeli S...Tettamanti S et al, Mol Ther 2017
Rotiroti MC...Tettamanti S et al., Mol Ther 2020.

Donor derived cells

- Healthy T cells

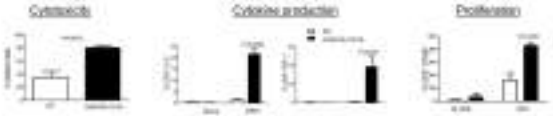
Magnani CF et al., JCI 2020.
Lussana et al., BCJ 2025
Pisani et al., JTM 2025

CARCIK-CD19 Study: Product of Internal Translational Research

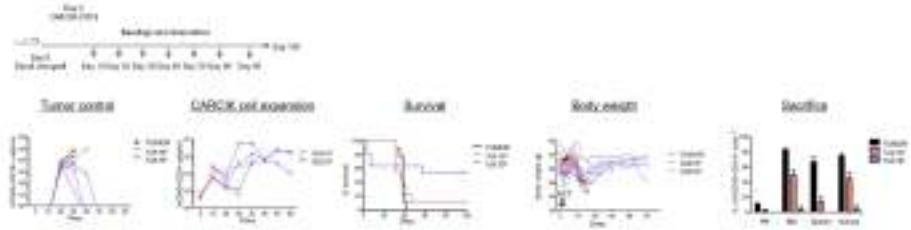
Research (2012-2015) → Preclinical Activities (2016) → Phase I/IIa CARCIK-CD19

CARCIK-CD19 generation: Expansion and Immunophenotype

***In vitro* functional activity of CARCIK-CD19 cells against CD19+ cell lines**

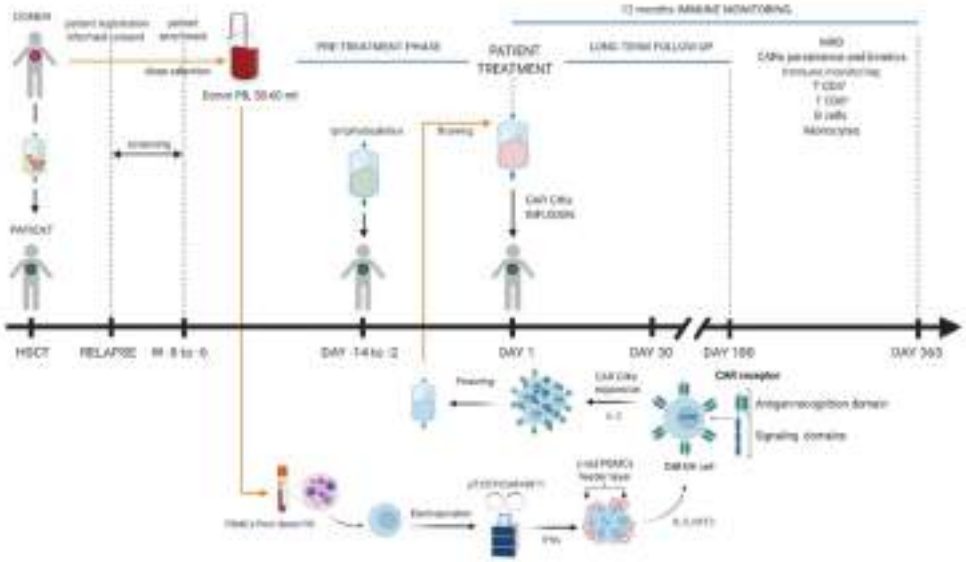


***In vivo* functional activity of CARCIK-CD19 cells against CD19+ cell lines: example of dose-finding in a survival model of DAUDI lymphoma xenograft**



CARCIK-CD19: Phase 1 trial

NCT03389035, Sponsor: Fondazione Tettamanti
PIs: A Biondi, A Rambaldi; Sites: ASST-Monza, ASST-Bergamo, IT

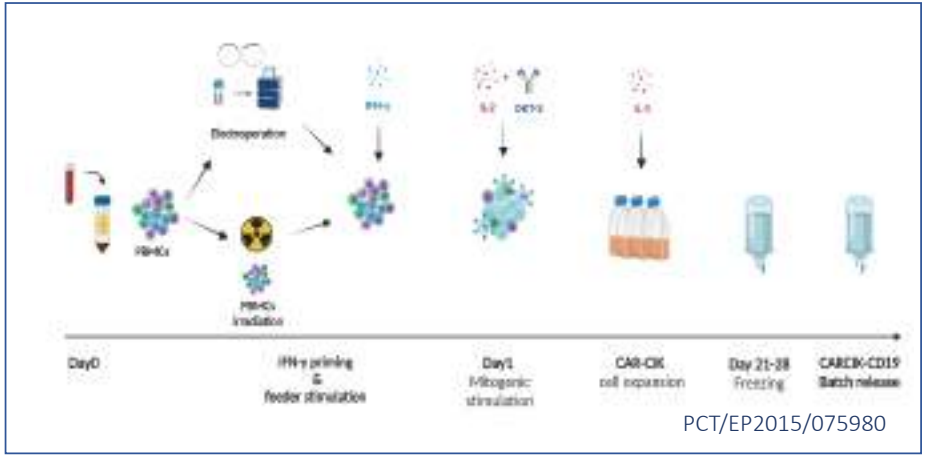


Primary objectives
- define the Maximum Tolerated Dose
- Safety and feasibility

Secondary objectives
- assessment of complete hematologic response
- CARCIK-CD19 cellular kinetics
- Anti-tumor activity (duration of response, PFS, EFS, OS)

Magnani CF et al, J Clin Invest 2020;130(11):6021-6033

Manufacturing Process & Batch Release Specifications



PCT/EP2015/075980

Parameter	method	specification	significance
% of CD3+ cells	Flow Cytometry	≥90%	purity/identity
% of CD3+/CD56+ cells (CIK)	Flow Cytometry	≥30%	purity/identity
% of CD3+/CAR+ cells	Flow Cytometry	≥20%	purity/identity
Cytotoxicity	Flow cytometry	≥25%	potency
Vector copy number	Q-PCR	<5 copies/cell	safety
Transposase	Q-RT-PCR	<20 copies/10000 GUS	safety
Cell viability	Trypan blue dye exclusion	≥80%	purity
Sterility	Eu Ph 2.6.1	sterile	safety
Mycoplasma	Eu Ph 2.6.7 – 2.6.21	absent	safety
Endotoxin	Eu Ph 2.6.14	<0.5 Eu/mL	safety

Tech transfer of CARCIK-CD19 manufacturing platform

Monza

meetings

materiali

procedure

metodi

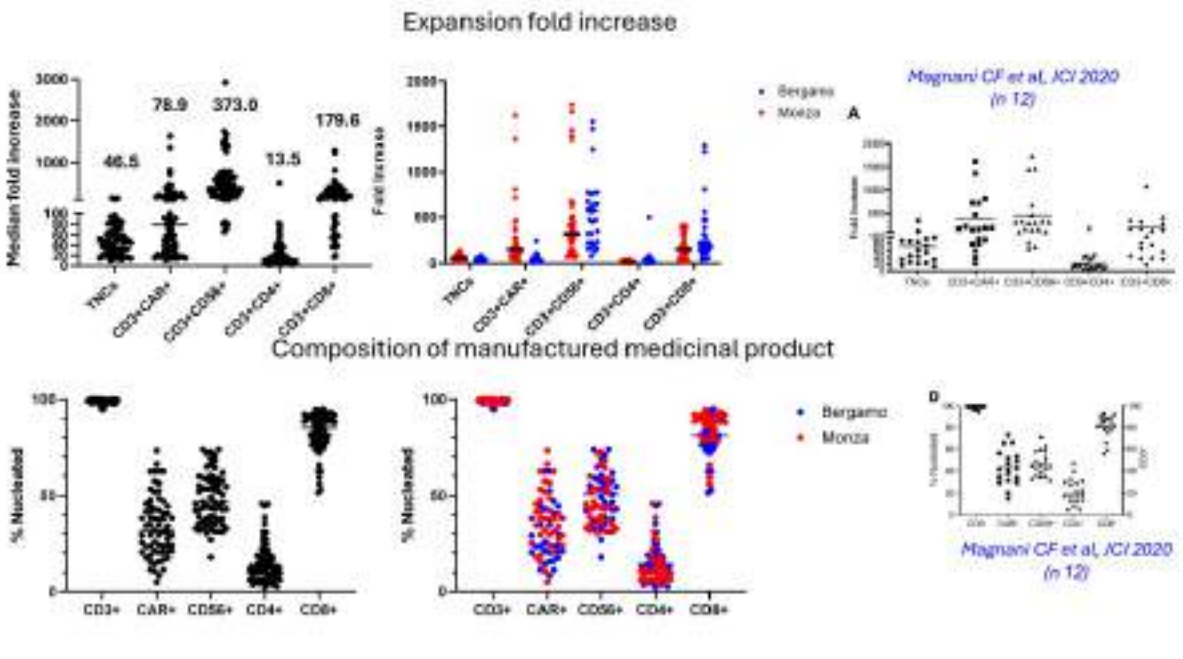
Specifiche
(Product Specification File)

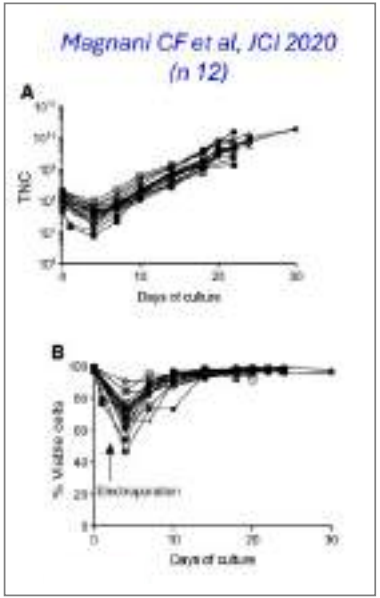
Verification runs

Bergamo

Manufacturing Process and Quality Control

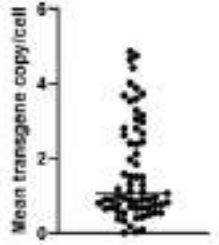
Expansion kinetics and cell viability in **64 CARCIK-CD19 batches** manufactured at **Verri laboratory (Monza, n 35) and Lanzani laboratory (Bergamo, n 29)** and **comparison with historical data** (Magnani CF et al, JCI 2020).

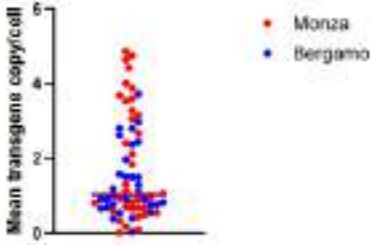




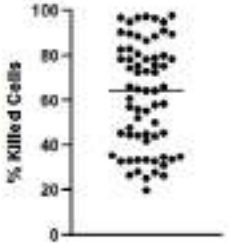
Quality Control: Vector Copy Number and Potency Assay

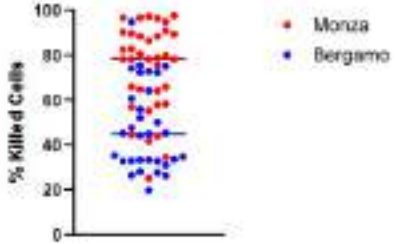
VCN

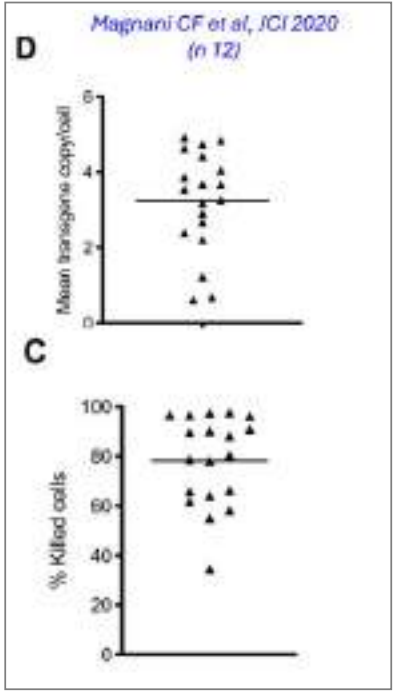




Cytotoxicity







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

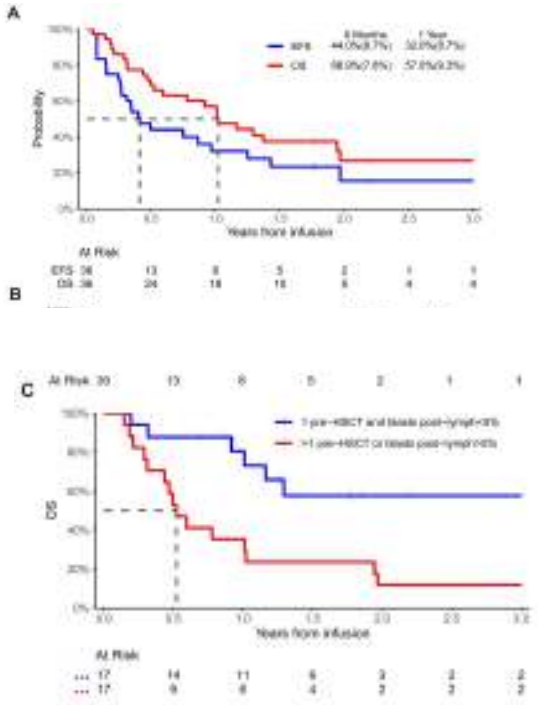
Characteristic	Phase 1/2 (FT01) (n = 15)	Compassionate (FT02) (n = 6)	Phase 2 (FT03) (n = 15)	Overall (n = 36)
Median age (range), year	35 (1-62)	44 (29-67)	45 (7-66)	39 (1-67)
Pediatric, n (%)	2 (13)	0 (0)	2 (13)	4 (11)
Female, n (%)	8 (53)	3 (50)	7 (47)	18 (50)
Philadelphia chromosome positive, n (%)	6 (40)	2 (33)	3 (20)	11 (31)
No. of Prior Lines of Therapy				
median (range)	4 (3-8)	3 (2-5)	3 (1-6)	3 (1-8)
No. of previous allo-SCT, n (%) 1 2	 9 (60) 6 (40)	 4 (67) 2 (33)	 14 (93) 1 (7)	 27 (75) 9 (25)
Inotuzumab ozogamicin before study enrolment, n (%)	2 (13)	0 (0)	0 (0)	2 (6)
Blinatumomab before study enrolment, n (%)	2 (13)	2 (33)	7 (47)	11 (31)
Inotuzumab ozogamicin + Blinatumomab before study enrolment, n (%)	2 (13)	0 (0)	3 (20)	5 (14)
Disease characteristics				
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)

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

Efficacy of anti CD19 CARCIK

Efficacy

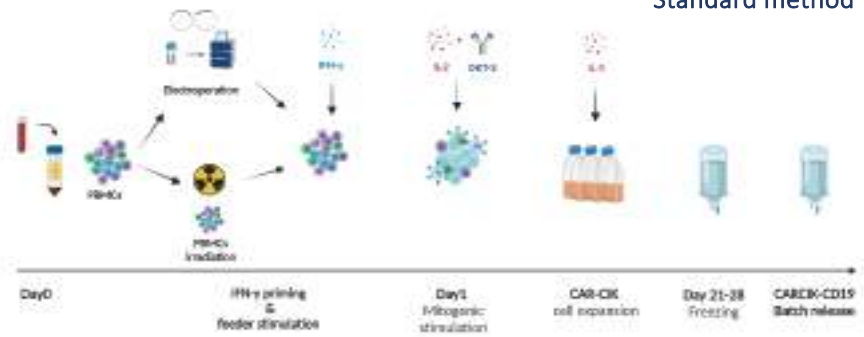
- Median follow-up 2.2 years
- 30/36 reached CR (83%)
- 86% were MRD-negative
- 12 patients (33%) did not experience a relapse:
  - 3 patients (25%) underwent consolidation with a second alloHSCT
  - 6 patients (17%) are still disease-free without additional therapies (1 with CAR-T circulating after 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

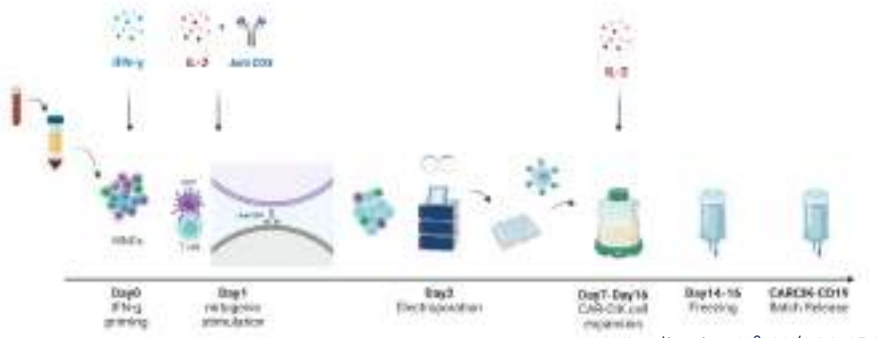
Simplification of the production process: feeder-free method and use of the G-REX bioreactor

Standard method



PCT/EP2015/075980

Feeder-free method

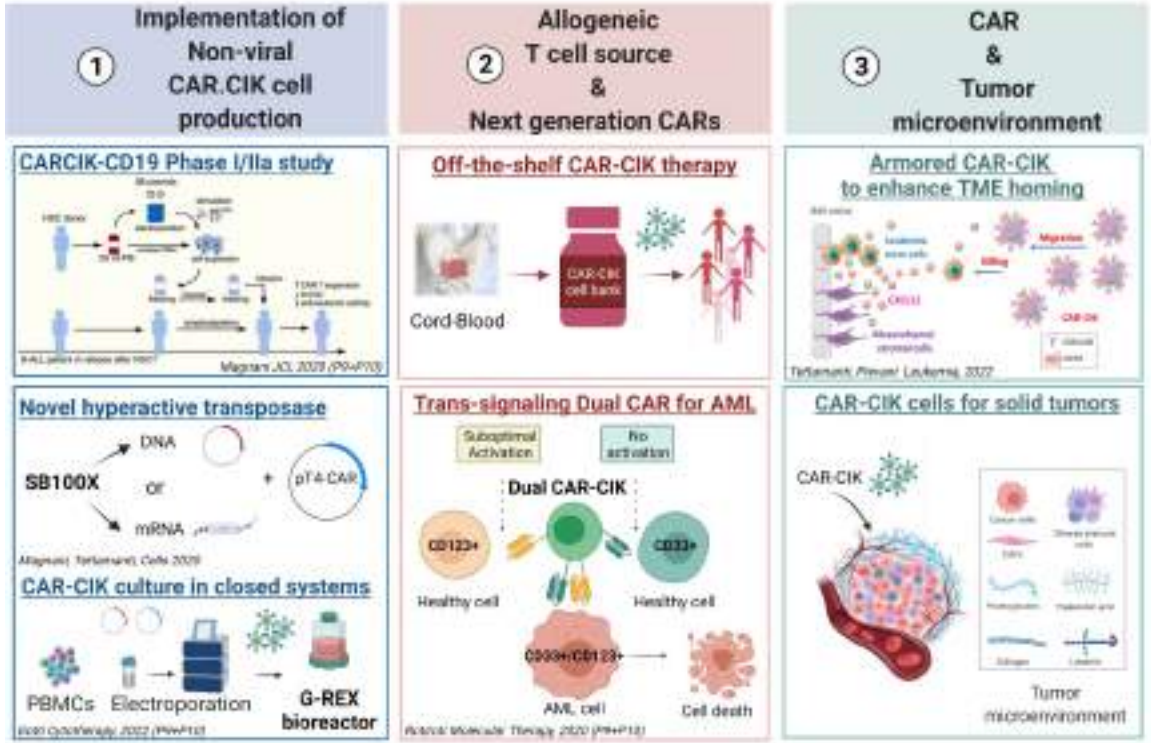


Application N° 63/386,454

Streamlining of CARCIK manufacturing:

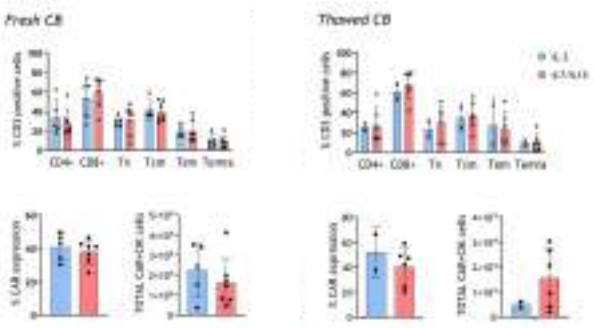
- No feeder
- G-Rex bioreactor
- Shorter cell culture time
- Next-gen SB plasmids: SB100X and pT4

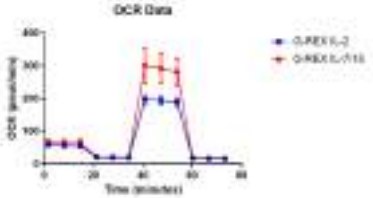
CARCIK R&D portfolio



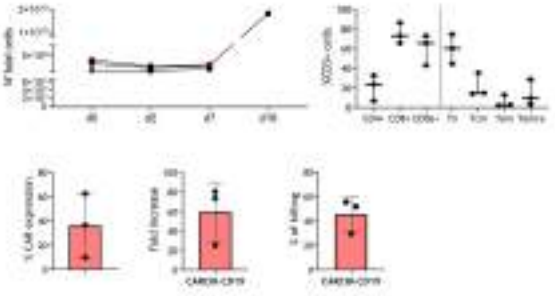
Cord-blood derived CARCIK in G-REX: toward off-the-shelf CARCIK therapy

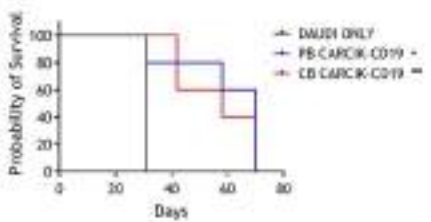
- Replacing IL-2 with IL7/IL-15 improve CARCIK **memory profile** and **fitness**

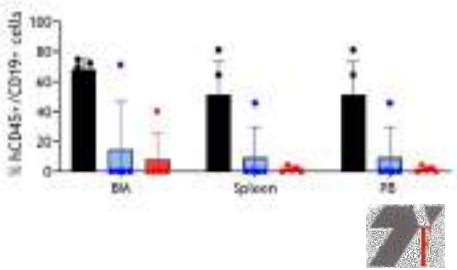




- GMP production CB-CARCIK product from thawed UCB unit







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)

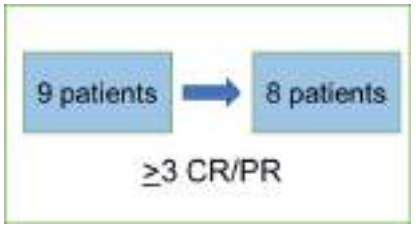
Dose escalation (Phase I) and expansion (Phase II)

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 CIK cells are active immune effector cells for the treatment of myeloid malignancies relapsing after allogeneic transplantation
- Allogeneic CIK cells do not mediate GvHD no matter the type of donor
- Genetically modified allogeneic CIK cells to express a CD19 car are effective for the treatment of childhood and adult BP-ALL patients relapsing after allogeneic transplantation
- These cells are currently tested in a new clinical trial with B-NHL for whom no commercial CAR-T cells are currently available
- New CAR-CIK cells targeting different antigens in lymphoid and myeloid malignancies as well as solid cancers are currently under investigation in our labs

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