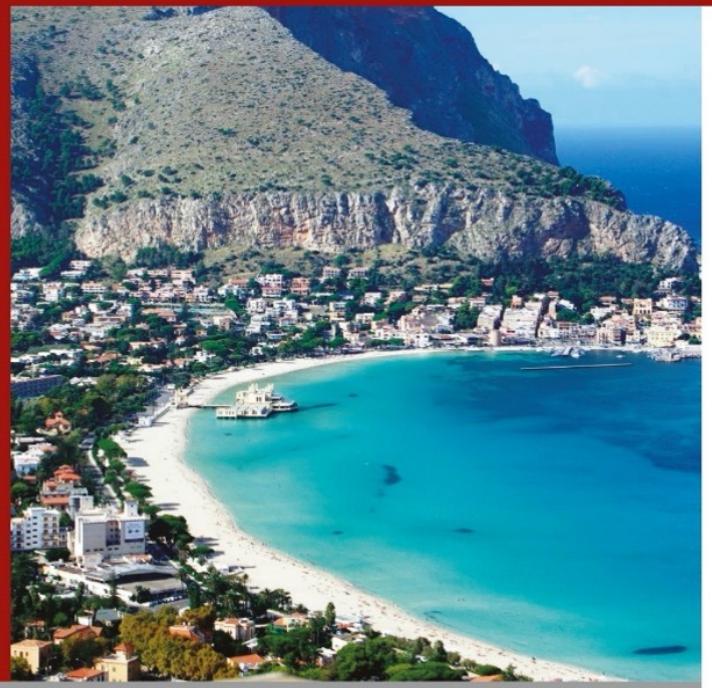
International Palermo Workshop on: Innovative Therapies for Lymphoid Malignancies



Palermo March 18, 2023
Hotel Federico II Central Palace

CAR-CIK

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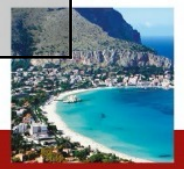
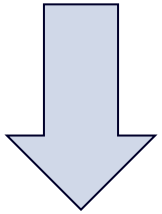
Disclosures of Name Surname

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



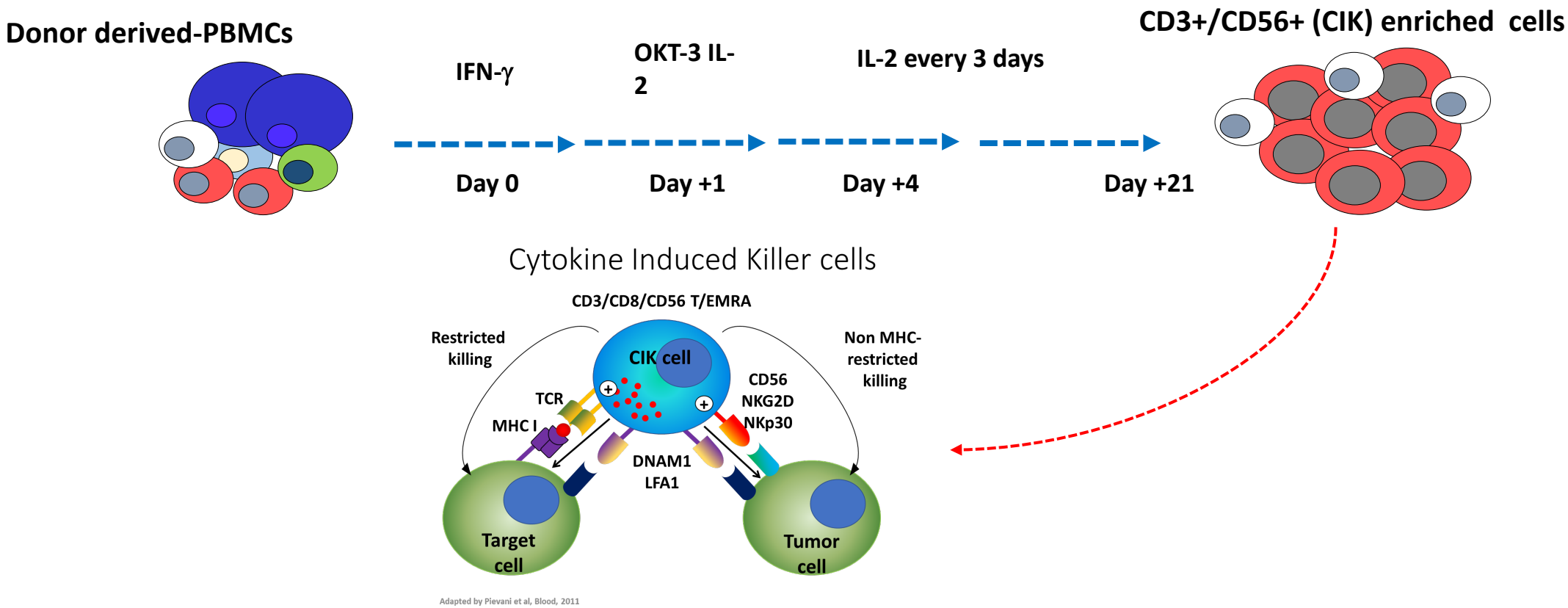
Background for a new CARs

- Despite the efficacy of CAR-T cell therapies, many adult patients fail to respond or relapse after initial response
- The logistics of autologous CAR T-cell therapy, including identifying an appropriate window to perform leukapheresis and the need to control disease while cells are being manufactured, confer a significant limitation in patients who have rapidly progressive disease
- In heavily pre-treated patients the quality and number of patient-derived CAR T cell may be sub-optimal
- Patients with high leukemic blast contamination might benefit from healthy allogeneic lymphoid cells
- Ready to go, off the shelf allogeneic CART cells represent an ambitious goal of the ongoing research



New platforms

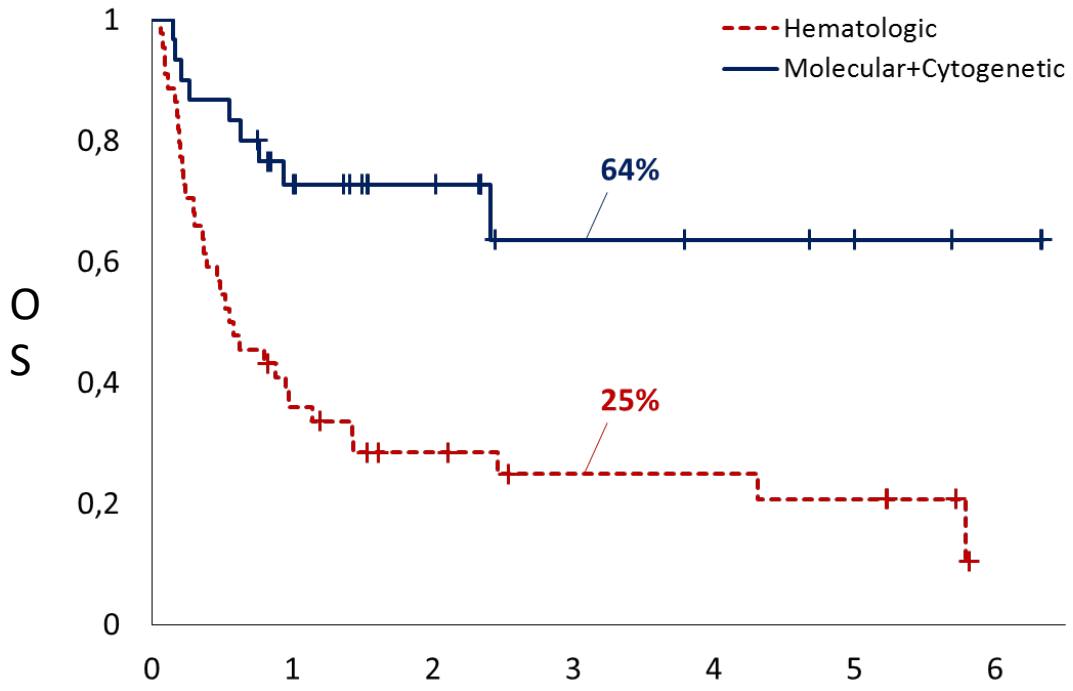
Donor-derived T-cell source differentiated in Cytokine Induced Killer Cells (CIK)



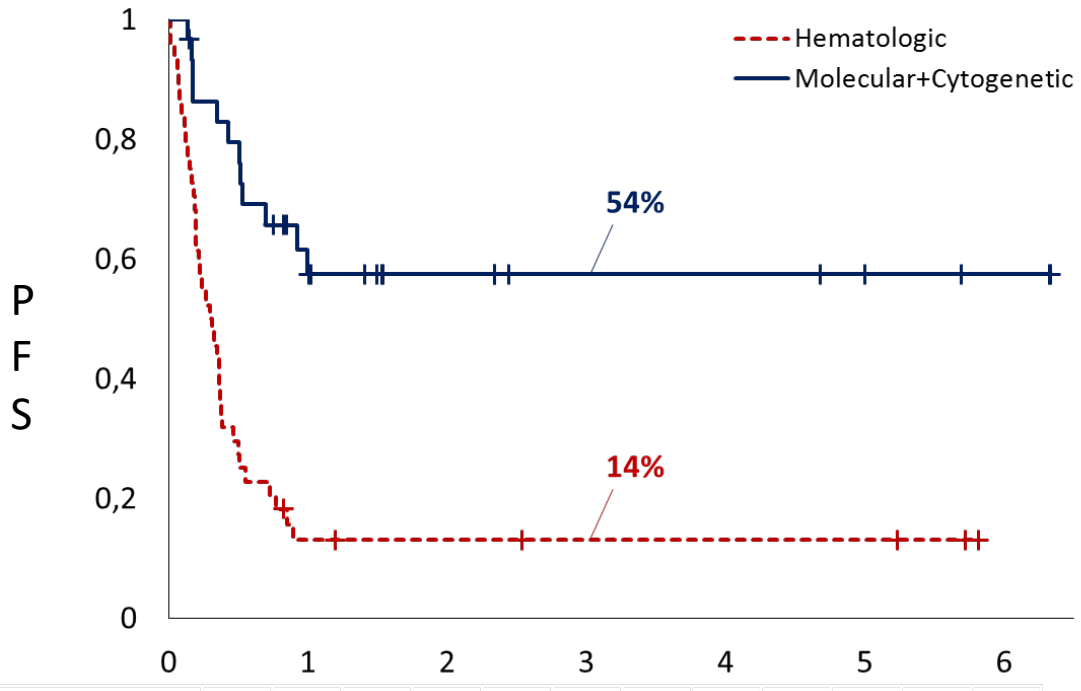
- Non MHC-restricted NK-like cytotoxicity, negligible alloreactivity and minimal GVHD
- Intrinsic capability of reaching leukemia-infiltrated tissues[1-3]
- Clinical experience with allogeneic CIK cells: feasible, safe and well tolerated[4-6]

1 *Pievani et al, Blood, 2011; 2 Linn et al. Journal of Biomed and Biotech 201; 3 Sangiolo et al. Journal of Cancer 2011; 4 Introna et al, Haematologica 2007; 5 Rambaldi A (2015) Leukemia 29(1):1-10; 6 Introna M (2017) Biol Blood Marrow Transplant. 23(12):2070-8*

Phase II Study of Sequential Infusion of Donor Lymphocyte Infusion and Cytokine-Induced Killer Cells for Patients Relapsed after Allogeneic Hematopoietic Stem Cell Transplantation



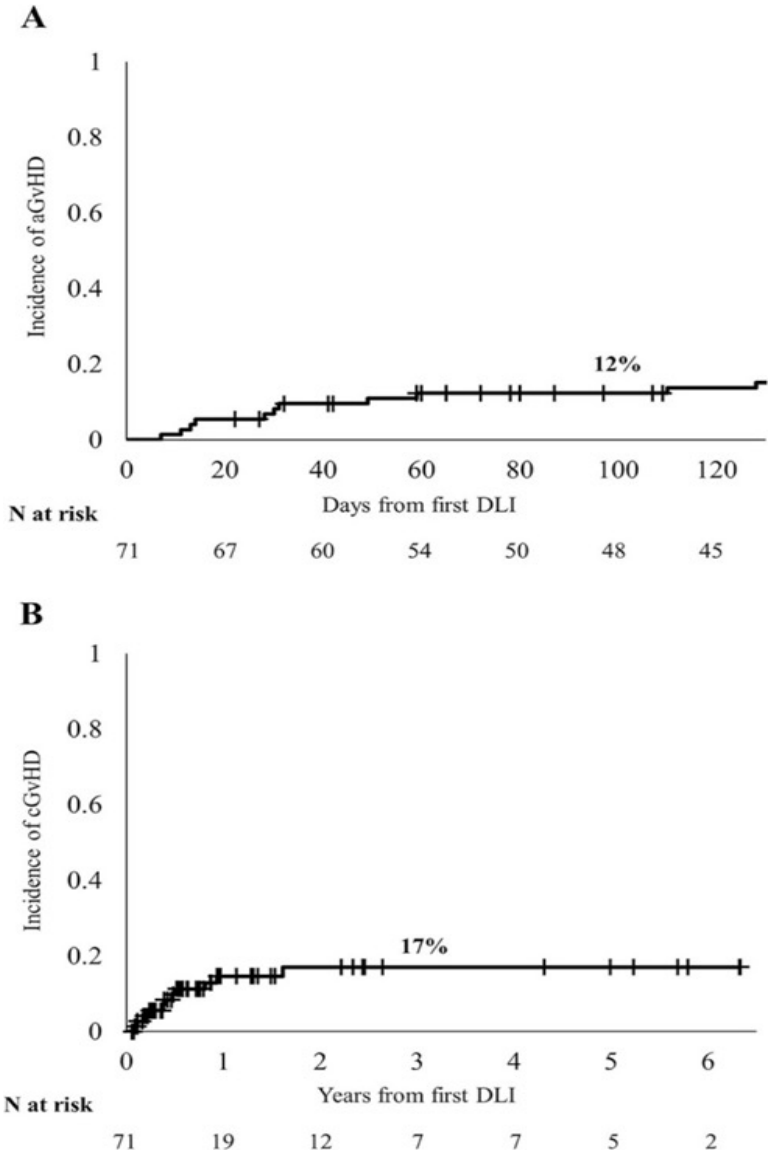
N at risk (events)													
Hematologic	44	(28)	15	(3)	9	(1)	6	(0)	6	(1)	5	(1)	0
Molec+Cytog	30	(8)	19	(0)	12	(1)	6	(0)	5	(0)	3	(0)	2



N at risk (events)													
Hematologic	44	(38)	5	(0)	4	(0)	3	(0)	3	(0)	3	(0)	0
Molec+Cytog	30	(12)	14	(0)	8	(0)	5	(0)	5	(0)	3	(0)	2

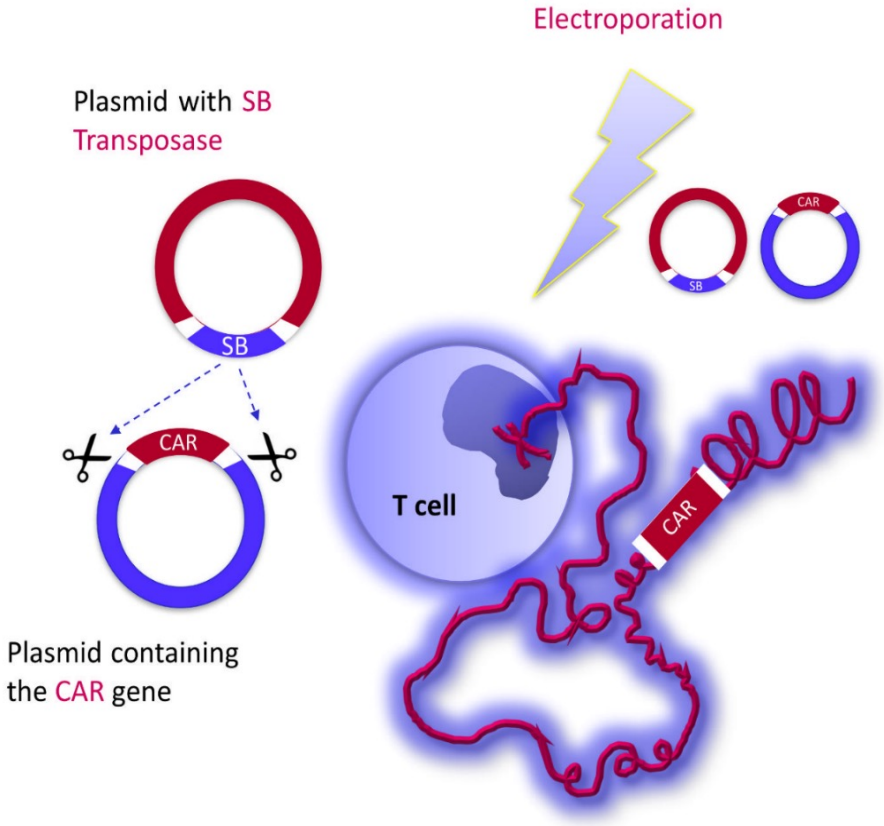
Introna M., Lussana F . Et al. : Biology of Blood and Marrow Transplantation 2017

Cumulative incidence of GVHD. (A) aGVHD; (B) cGVHD



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

Non-viral engineering of allogeneic CAR T cells



Cytokine induced killer cells (CIK)

Allogeneic memory T cells ($CD8^+CD56^+$)
Minimal GvHD occurrence
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

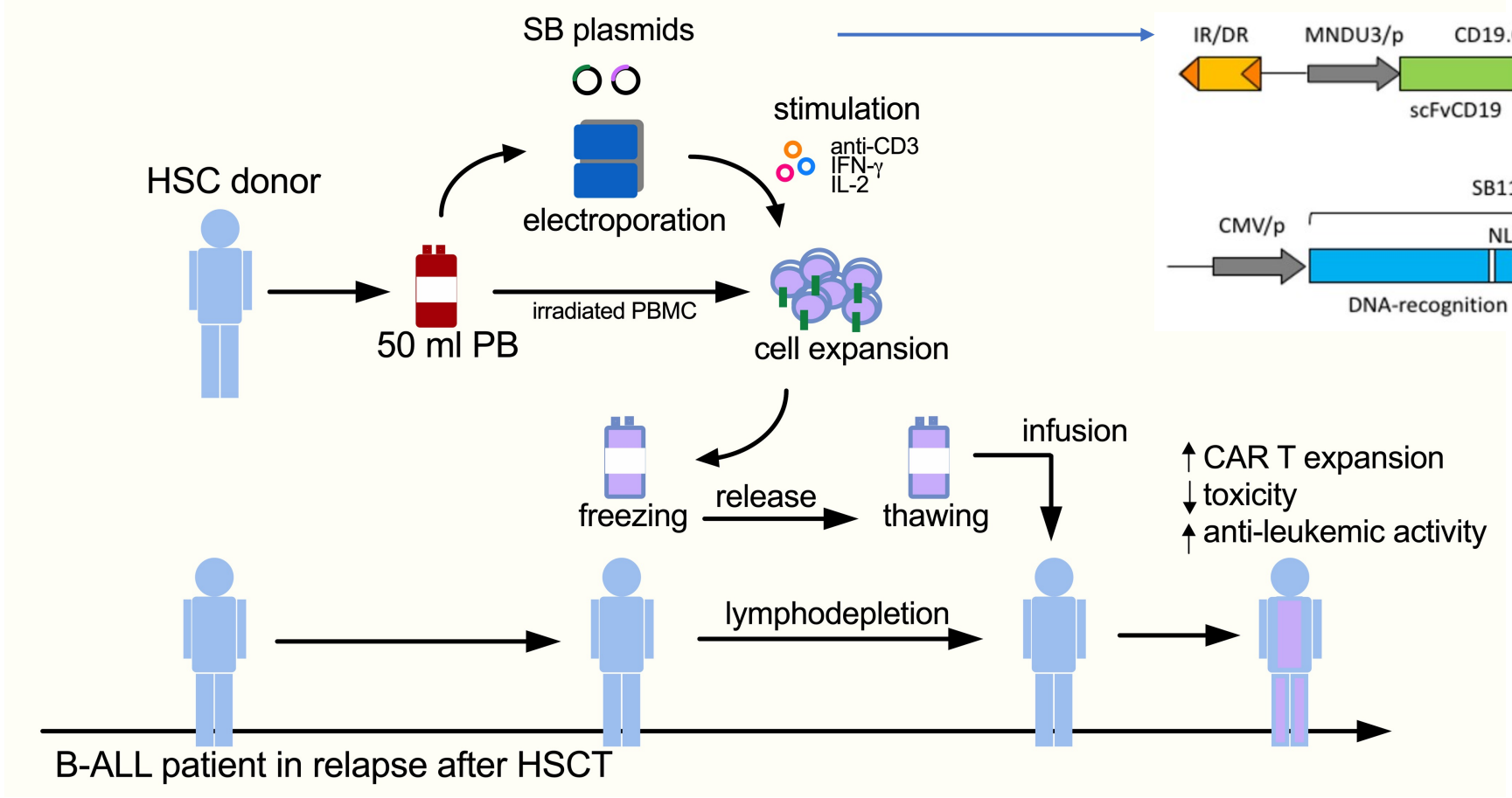
A non-viral vector derived from the Tc1/mariner family of DNA transposon validated in clinics

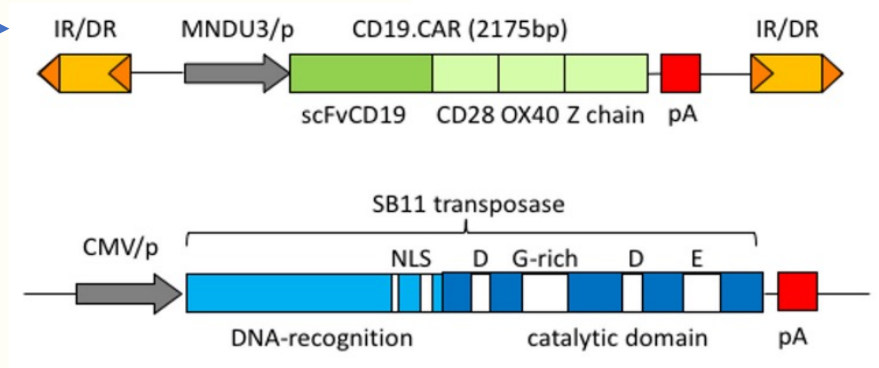
Ivics Z (1997) Cell 91(4):501-10; Kebriaei P (2016) J Clin Invest. 126(9):3363-76

Magnani CF, Oncotarget. 2016;7(32):51581-515
Turazzi N, Br J Haematol. 2017;182(6):939-943
Magnani CF, Hum Gene Ther. 2018; 29(5):602-613

Phase I/IIa trial with SB-engineered CARCIK-CD19 in B-ALL post HSCT

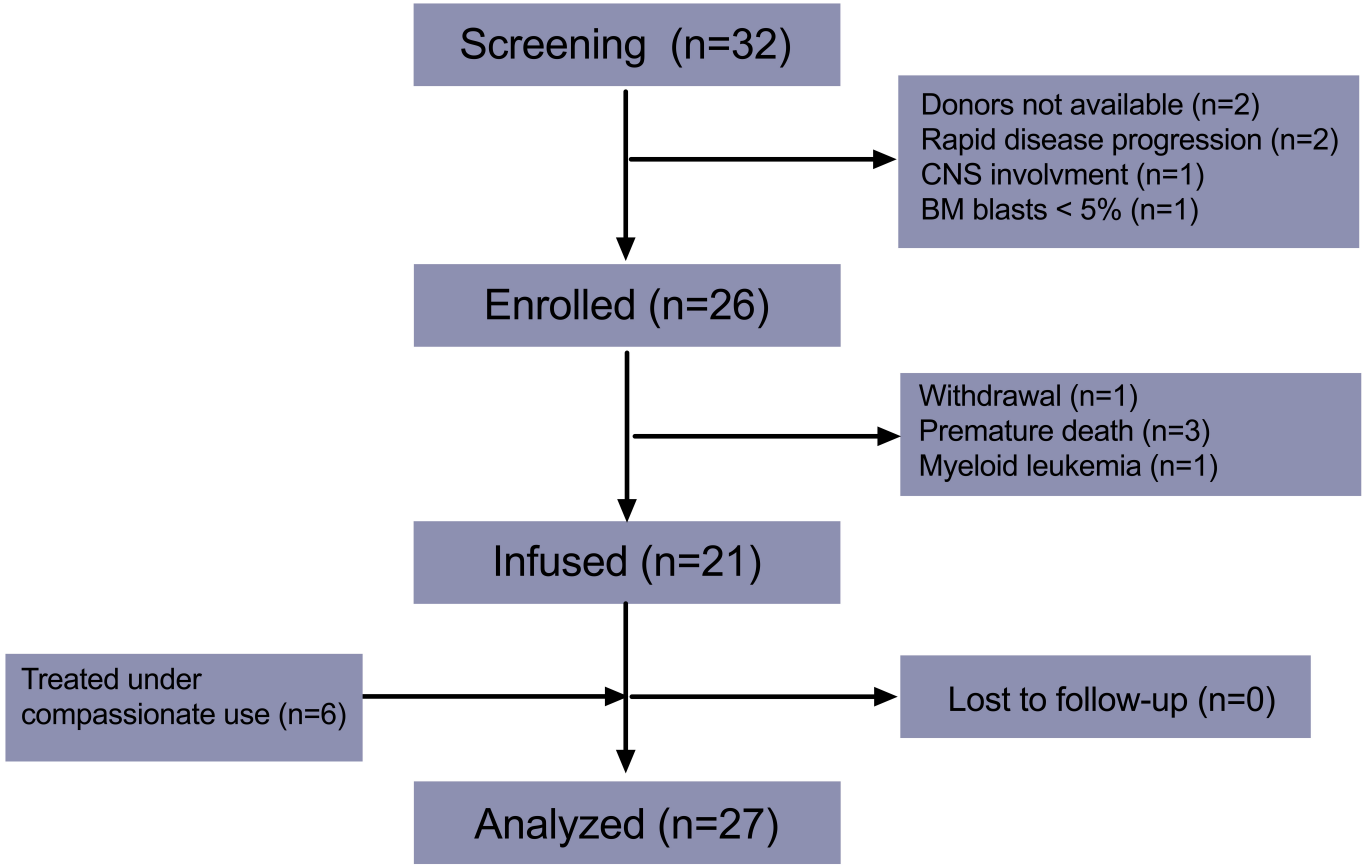
Multicenter Dose Escalation Study FT01CARCIK (NCT03389035) and compassionate use FT02CARCIK
Enrolling in ASST-Monza and ASST-Bergamo, IT
Manufactured in-house





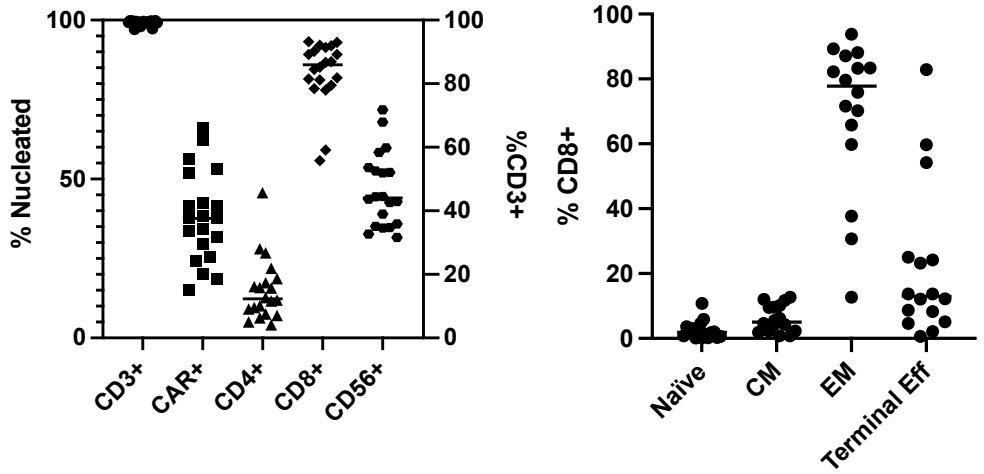
Magnani CF, J Clin Invest. 2020 Nov 2;130(11):6021-6033

CARCIK-CD19 in B-ALL post HSCT: Consort and feasibility



Product characteristics:

- Generated from 50 mL of peripheral blood from the HSCT donor
- Transduction by electroporation
- Manufacture failure (n=1)
- Mean CAR transduction 36.2% (range, 15.1-66.0)



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CARCIK-CD19 in B-ALL post HSCT: patient and disease characteristics

Characteristics	All patients (27)
Age, median (range)	38 (1-67)
Female, N (%)	14 (52)
Previous lines of treatment, median (range)	4 (2-8)
Previous lines of treatment • 2, N (%) • 3-5, N (%) • >5, N (%)	 3 (15) 14 (70) 3 (15)
Previous treatment with blinatumomab, N (%)	8 (30)
No. of previous allo-SCT, n (%) • 1 • 2	 18 (66,6) 9 (33,3)
Type of transplant, n (%): • Haplo • MUD • Sib	 10 (37%) 10 (37%) 7 (26%)
Bridge therapy • Inotuzumab, N (%) • Blinatumomab, N (%) • Low-intensity CT +/-TKI, N (%)	 3 (11) 5 (18) 19 (70)

Characteristics	All patients (27)
aGvHD post last Tx: • Grade I and II • Grade III	 8 (29%) 2 (7%)
cGvHD post last TX: • Grade I • Grade II	 3 (11) 1 (4)
Extramedullary disease, N (%)	4 (2-8)
BM Blasts at enrolment, median (range)	40 (0-100)
Blood values pre-lymphodepletion • LDH U/L, median (range) • Pletelet count 10^3/mmc, median (range)	 361 (148-1487) 76 (5-237)

CARCIK-CD19 in B-ALL post HSCT: selected adverse event

Events	Patients
CRS, n (%)	
• Grade 1	4 (15%)
• Grade 2	5 (19%)
• Grade 3	0 (0%)
ICANS, n (%)	
Grade 3	2 (7%)
GvHD, n (%)	
Grade I-IV	0 (0%)
Infection, n (%)	
• Grade 1-2	2 (7%)
• Grade ≥ 3	7 (26%)
Prolonged cytopenia, n (%)	
Severe neutropenia, day 28	7 (32%)
Severe thrombocytopenia, day 28	17 (68%)

- no dose limiting toxicity was observed
- CRS and ICANS were observed in patients treated with the highest doses and were manageable
- Although 10 out of 27 had experienced GVHD after the previous HSCT, secondary GVHD was never observed
- 17 out of 27 patients remained with persistent cytopenia at day 28

CRS criteria (Lee et al. Blood. 2014); ICANS, immune-effector cell-associated neurotoxicity syndrome; severe neutropenia <500/mmc; severe thrombocytopenia <50000/mmc

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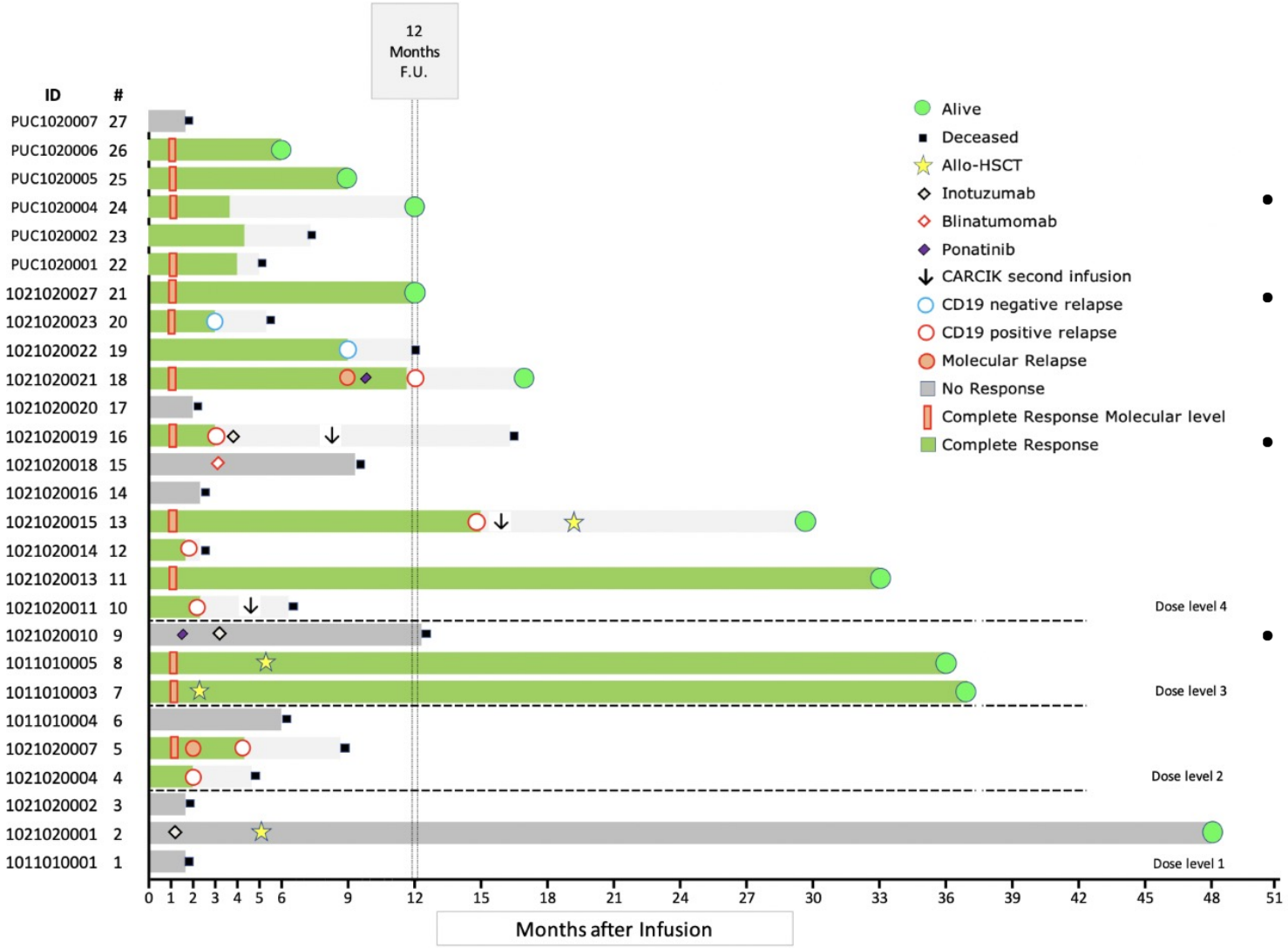
Response data



- CR: 18/27 patients (66.7%, 95%CI=46-84%)
- CR: 16/21 patients (76.2%, 95%CI=53-92%) treated with the 2 highest doses
- Fourteen (77.8%) of the overall responders and 13 of the responders at the highest doses (81.3%) achieved MRD negativity
- The type of donor did not influence the achievement of CR 28 days post-infusion

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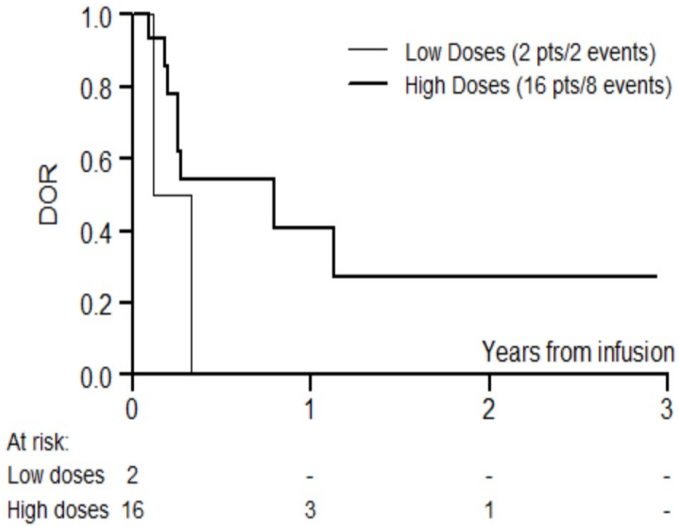
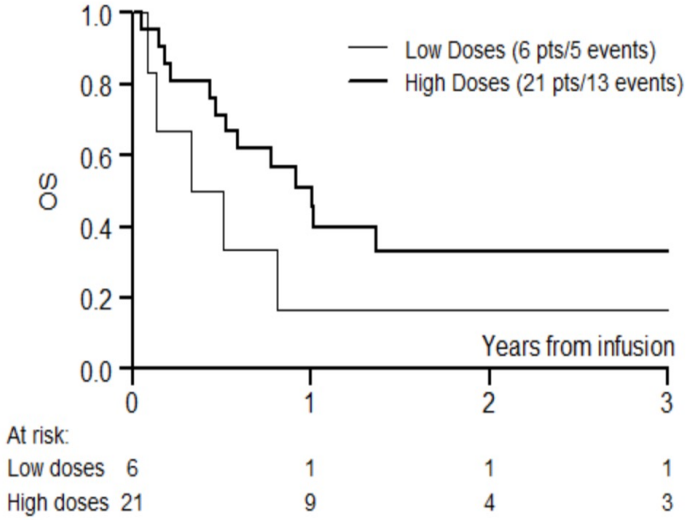
Main outcomes

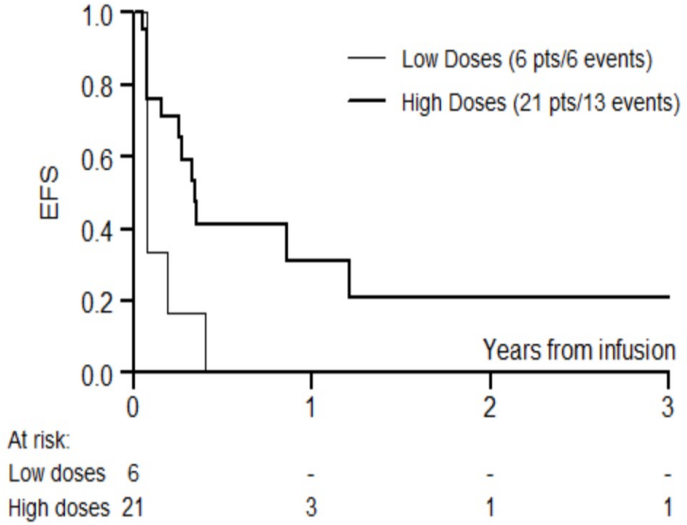
Duration of remission



Overall survival



Event free survival



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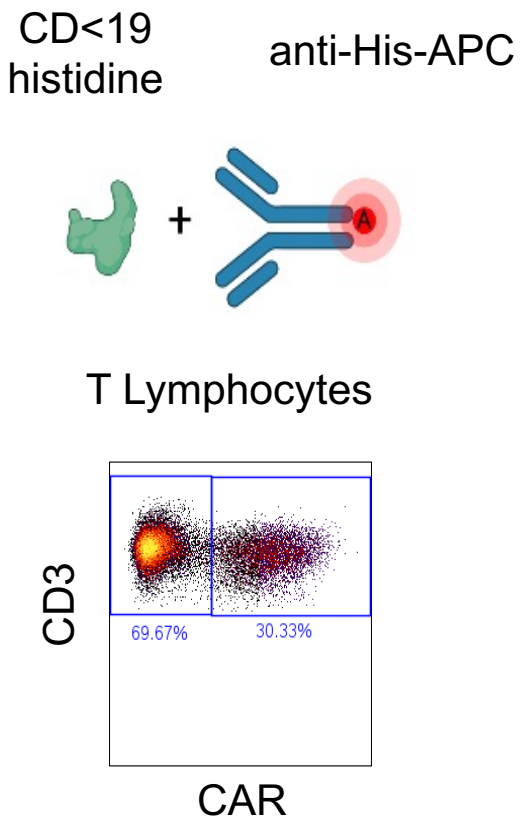
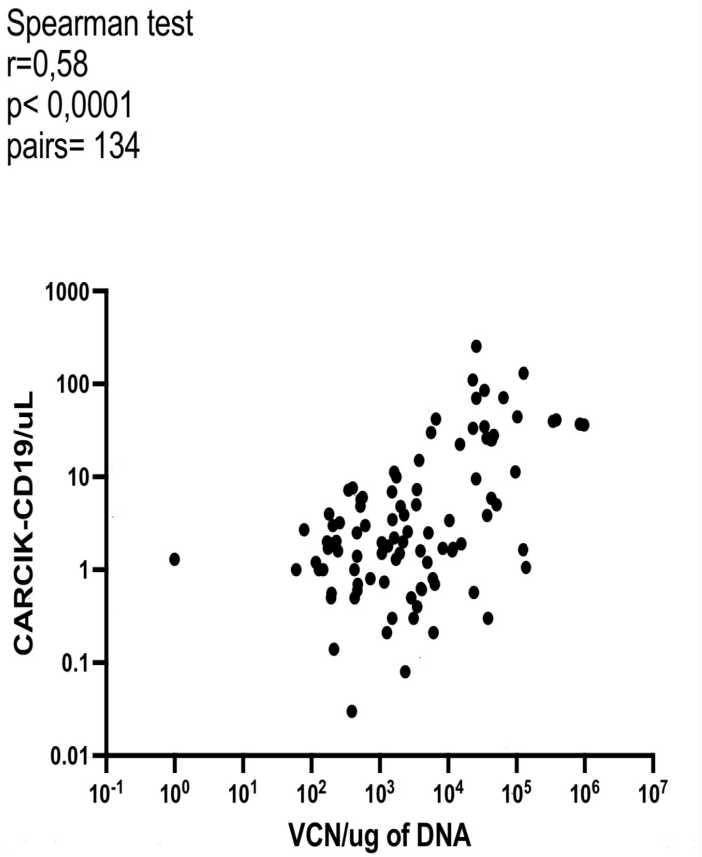
CARCIK flowcytometry detection methods





Advantages:

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

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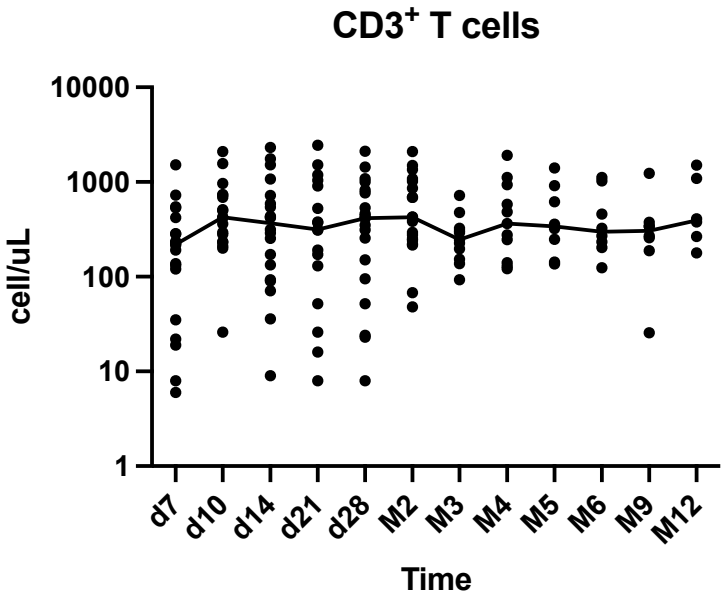
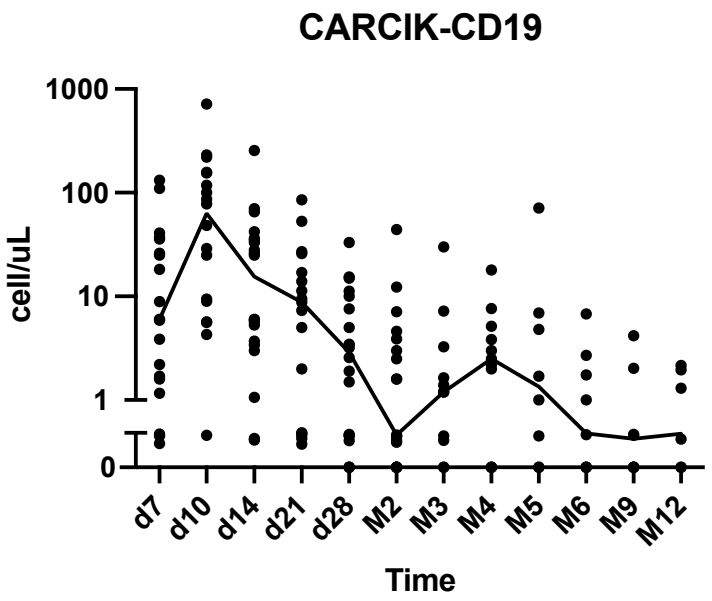
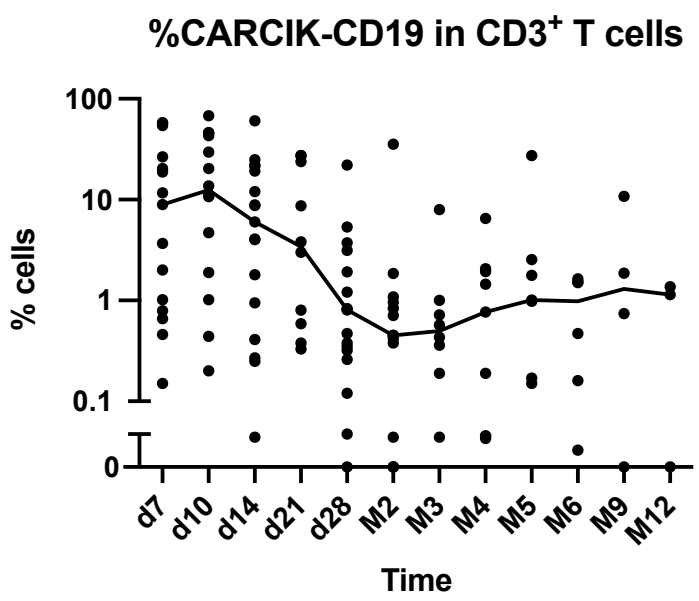
CD3+ T cells and CARCIK-CD19 reconstitution







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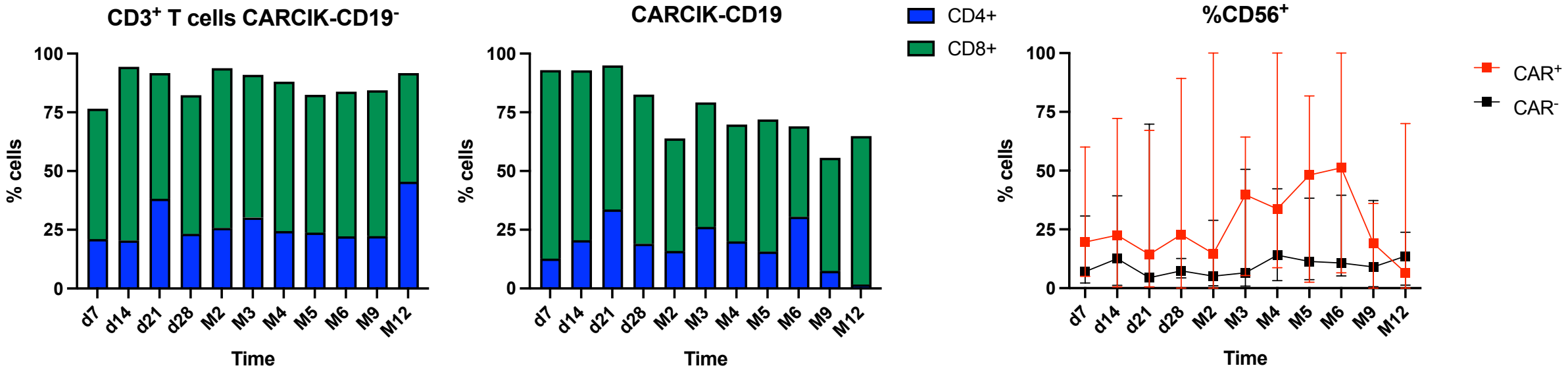
T cell subsets reconstitution in CD3^{+} T cells and CARCIK-CD19



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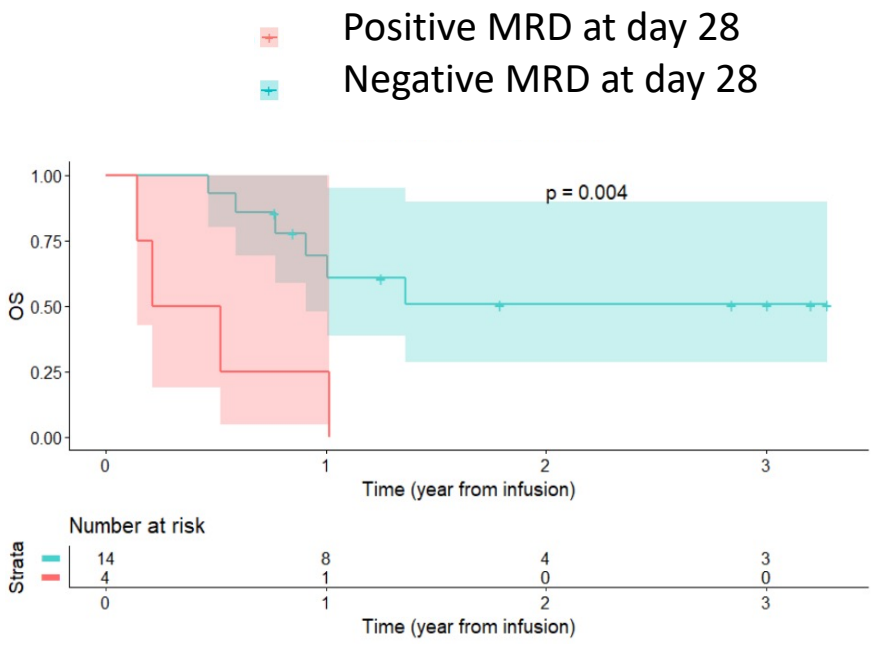
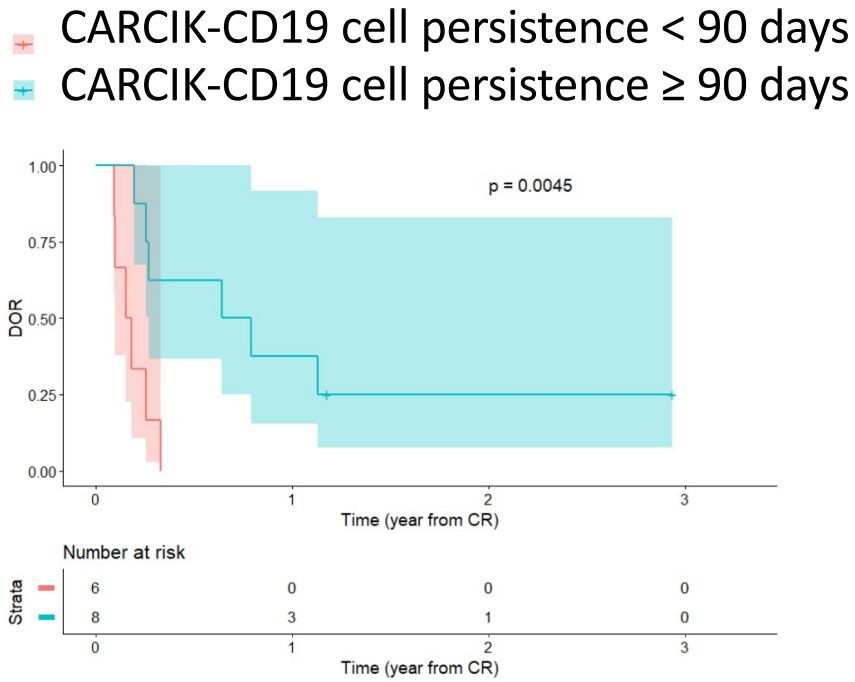
Minimal residual disease and CARCIK-CD19 persistence and clinical outcome





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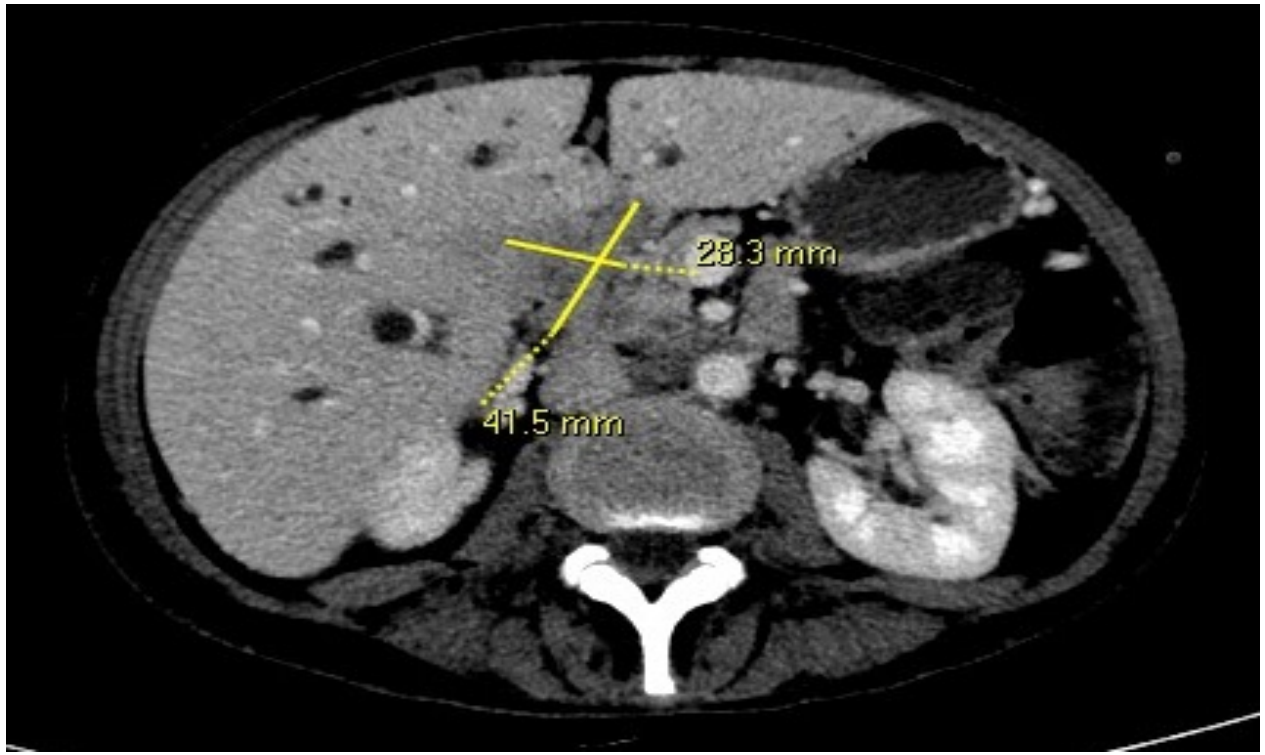
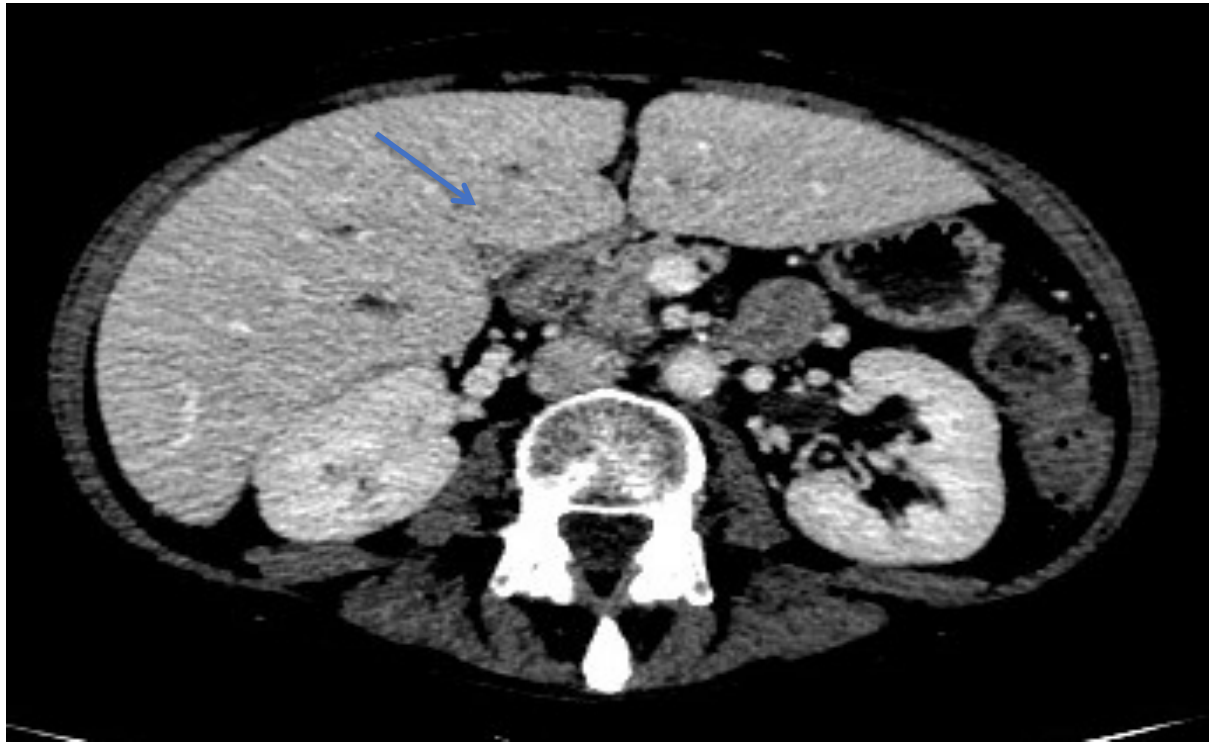
CARCIK-CD19 mediated anti leukemic activity on extrahematologic disease

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

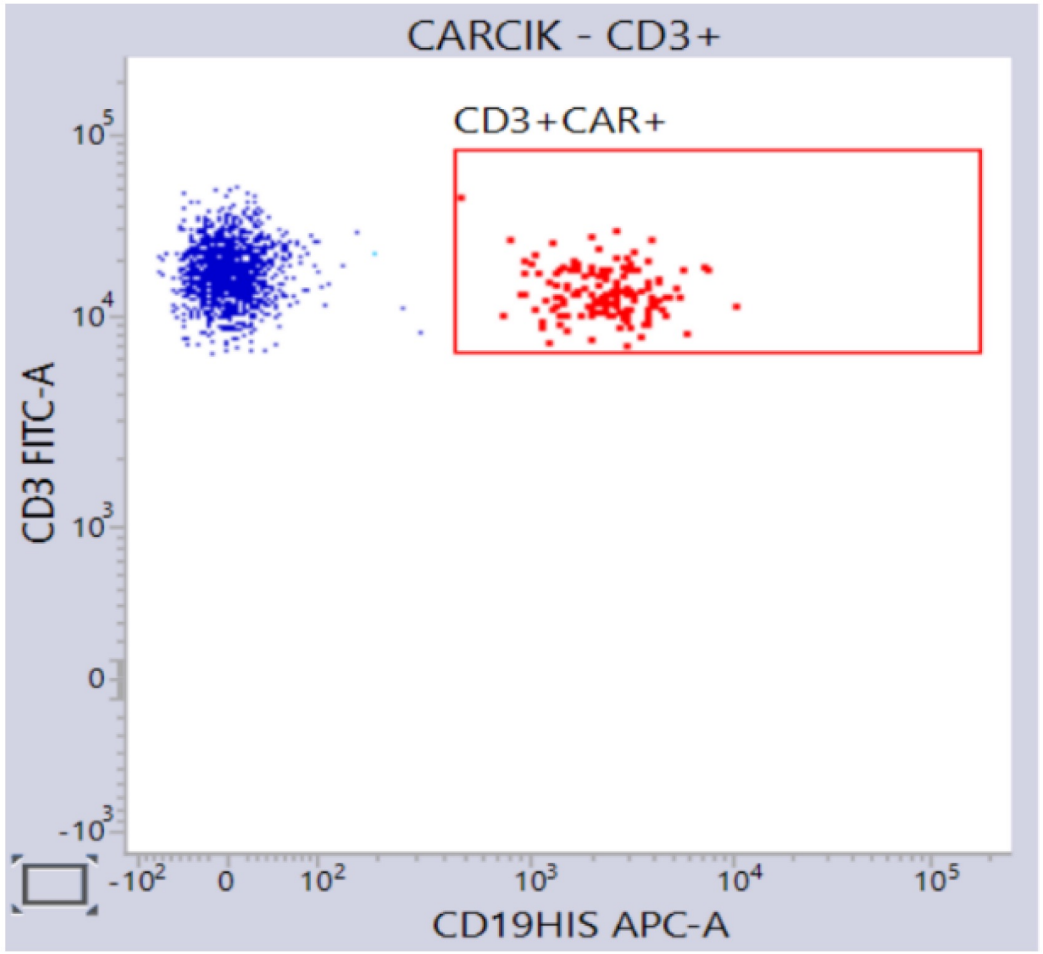




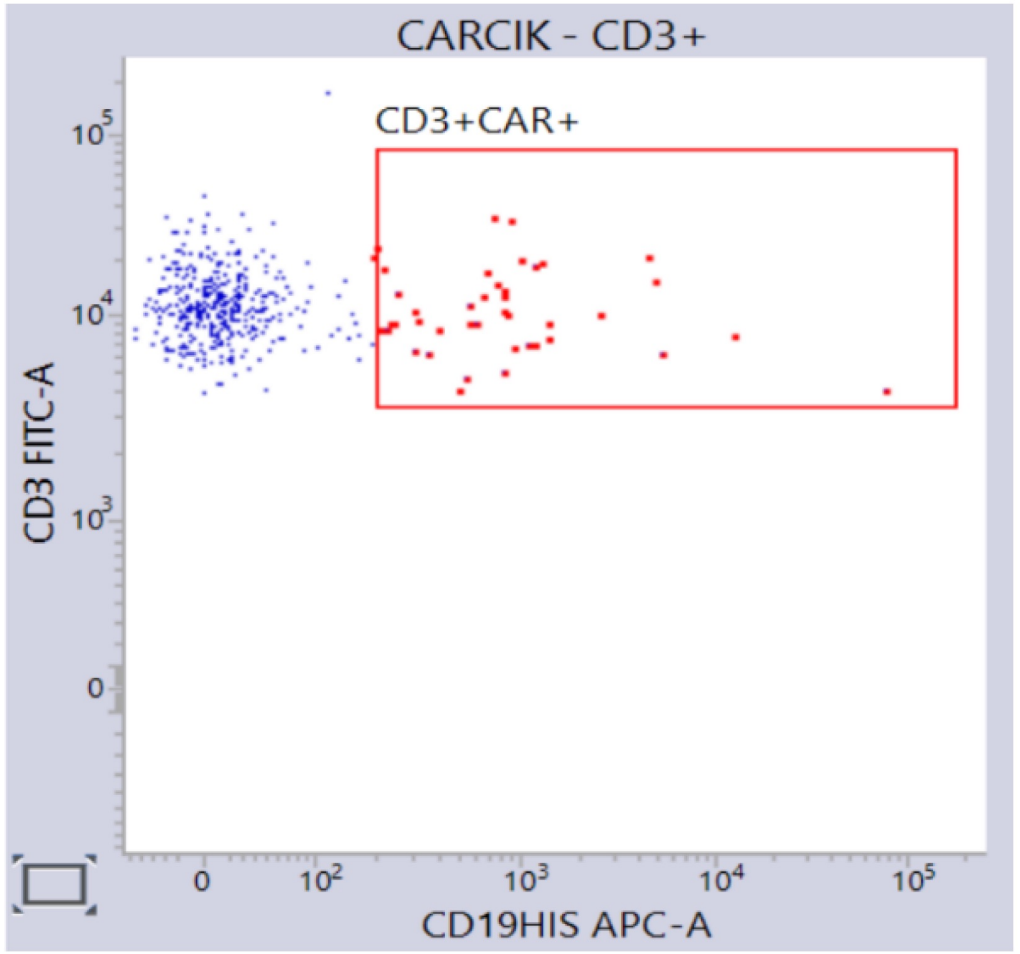
07 June 2019: Relapse post Allo-HSCT presenting liver adenopathy
27 June 2019:
- AST/ALT: 157/287 UI,
- γGT: 1183 UI
- Bilirubin: 18.8 mg/dl

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

CARCIK-CD19 mediated anti leukemic activity on extrahematologic disease



(**A**) (PB)

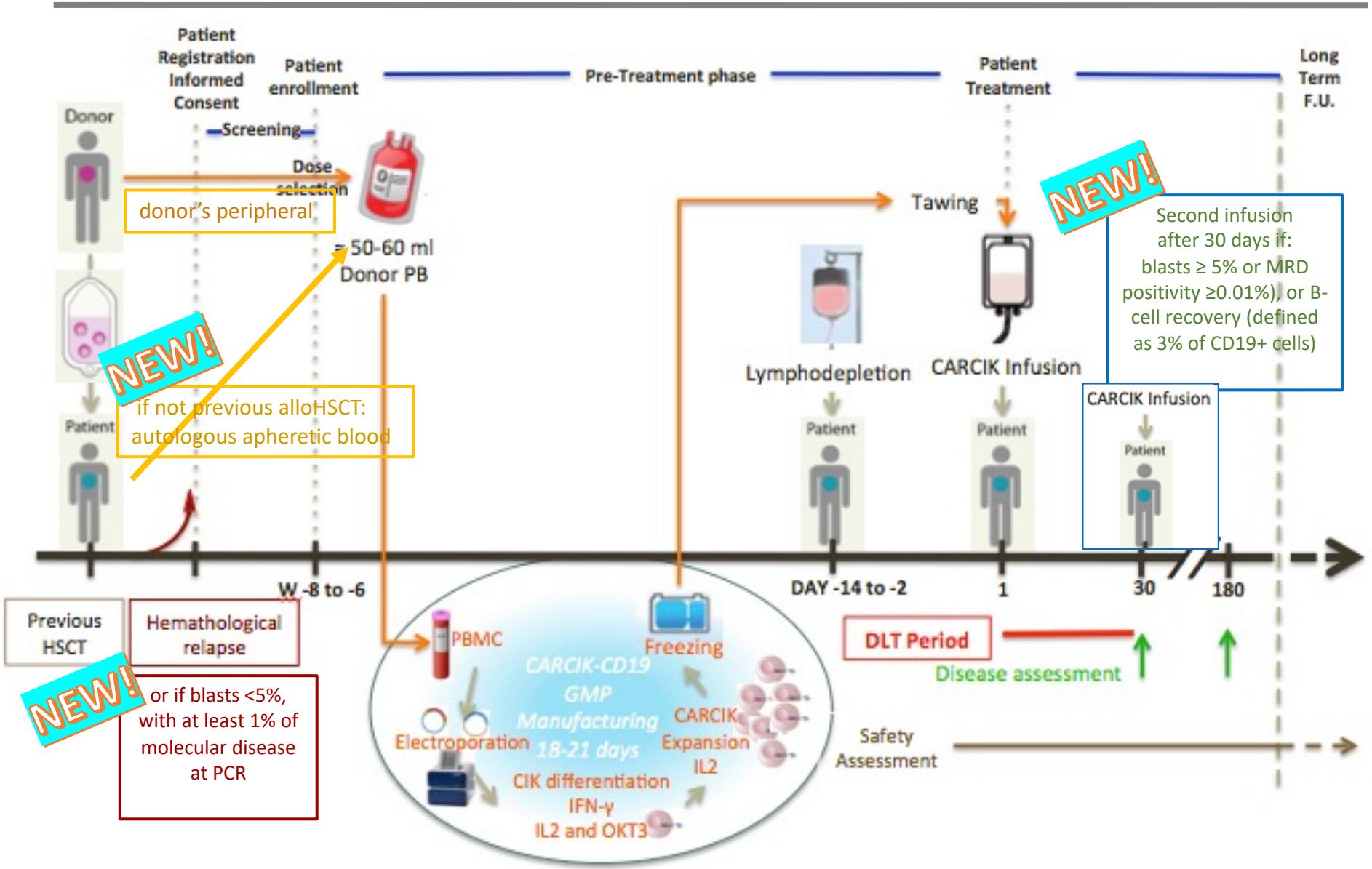


(**B**) (CSF)

Conclusions

- In this phase I/II dose-finding study, SB-engineered CAR T cells demonstrated:
  - A **successfully GMP production** for all patients **from 50 ml PB** of the previous transplant donor
  - An excellent **safety profile** associated with a **robust expansion** in most patients
  - Anti-leukemia activity in **heavily pretreated patients** with B-ALL relapsed after allo-SCT:
    - CR: 76% in patients receiving the highest dose levels
    - OS: 71% of patients receiving the highest dose levels at 6 months
    - Anti leukemic activity on extrahematologic sites
- The achievement of a negative MRD status at day 28 was crucial for patient's survival, suggesting the need of early additional treatment in patients failing this end point

FT03CARCIK Phase 2: Flow-chart



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