LEUKEMIA2020-2021

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Coordinator: A.M. Carella
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ASSOCIAZIONE ITALIANA CONTRO LEUCEMIE LINFOMI E MIELOMA

CAR-T in pediatric and adult patients ALL

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fondazione GIMEMA onlus per la promozione e lo sviluppo della ricerca scientifica sulle malattie ematologiche. FRANCO MANDELLI

GITMO

SIES Società Italiana Ematologia Sperimentale

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Disclosures

- Speaker Bureau: Novartis, Amgen
- Advisory Board: CoImmune, BMS, Incyte
- Research grants: CoImmune

Tisangelecleucel: CAR-T living cells; impressive efficacy in patients with very poor prognosis

Single infusion

Unexpected and fast to get results in ALL patients without therapeutic alternatives

"living" cell therapy vs chemotherapy

CAR T cell-based gene therapies have been able to provide unprecedented remission rates and have demonstrated success where other therapies have failed.

• Grupp SL et *al. NEJM* (2013); 368(16):1509-1518;
• Maude SL et *al. NEJM* (2018); 378(5):439-448;
•Park JH et *al. NEJM* (2018); 378(5):449-459;
•Gardner R et *al. Blood* (2017); 129(25):3322-3331

Efficacy/Outcome observed in Eliana, B2001X, real world UK & USA

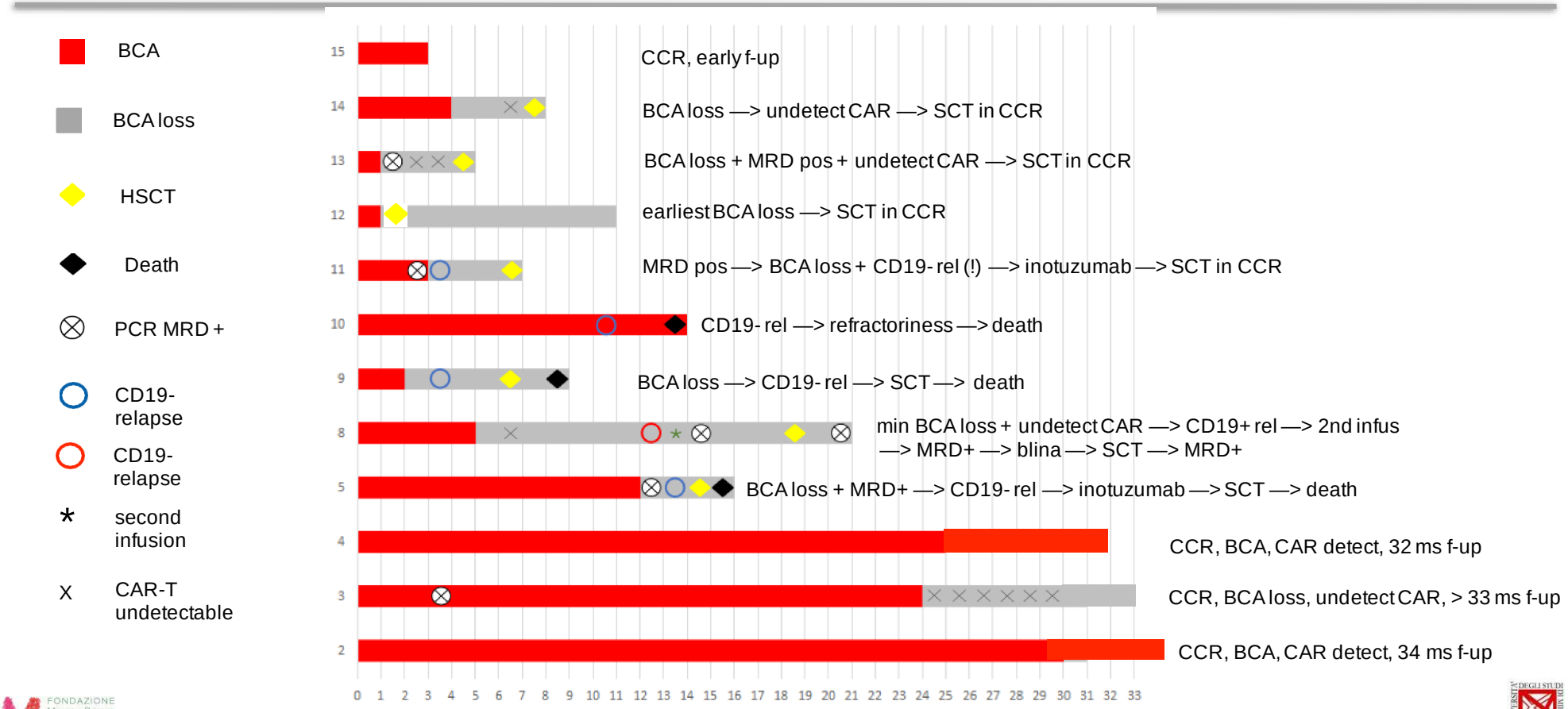
	denominator	Clinical trials		Real world life	
		updated Eliana	B2001X	UK	USA
	enrolled/infused	97 / 79	73 / 67	60 / 49	200 / 185
	follow-up	24.2 ms *(4.5-25.1)*	9.6 ms *(0.2-16.5)*		11.2 ms *(0.2-28.8)*
OR	infused	82% *(CI 72-90)*	85% *(CI 74-92)*	95%	79%
	ITT			85%	85%
OS	prior blina	-	**1-yr 53%** *(CI 19-78)*		
OS	infused	**2-yr 66%** *(CI 54-76)*	**1-yr 83%** *(CI 69-92)*		1-yr 72%
	high/low disease				**1-yr 58%** / 85%
EFS	respondent	2-yr 62% *(CI 47-75)*			
	infused				1-yr 51%
	high/low disease				**1-yr 34%** / 69%
Further treatment	in CCR (BCA loss)	8 SCT			
Composite endpoint*	infused			**34%**	

- composite endpoint: CCR in BCA w/o further treatment

BCA: B-cell aplasia; CCR: continuous complete remission; EFS: event-free survival; ItT: intrathecal therapy; OS: overall survival; yr: year

- Updated Eliana: Grupp SA, et al. ASH 2018. Oral 895
- Baruchel A, et al. EHA 2020. Oral S118
- Real life UK: Ghorashian S, et al. Blood. 2020;136(Suppl. 1):1016
- Real life: USA: Schultz LM, et al. Blood. 2020;136(Suppl. 1):14–15

Kymriah - outcome: BCA and disease monitoring by time



BCA: B-cell aplasia; CCR: continuous complete remission; EFS: event-free survival;; ms: months; rel: relapse; SCT: stem cell transplant; undetected: undetectable

CAR-T cells: Main critical issues

- Comparative data of efficacy in similar setting of pts?
- Apheresis and success of genetic manipulation;
- Time from apheresis to infusion;
- Levels of disease at the time of infusion;
- Toxicity
- Bridge to HSCT or its substitute?
- More sustainable approaches ?
- How to prevent “escape” mechanism(s) ?
- Perspectives (“off the shelf”, dual target?...)

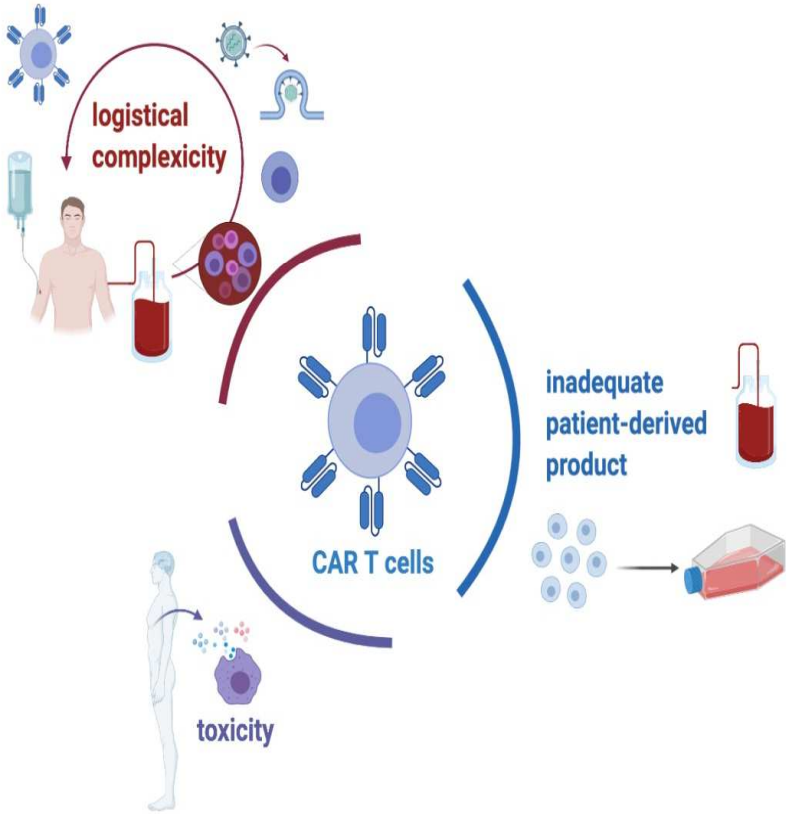
A non-viral Sleeping Beauty (SB) allogeneic platform

Allo-T cells differe
induced kill

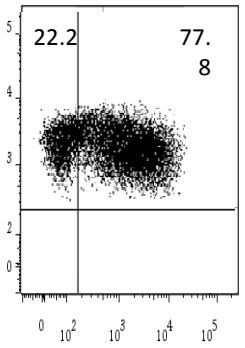
Cytotoxic T cells enr
Minimal GvHD af
Able to reach leu
tiss
Rambaldi A (2015) Leuke
(2017) Biol Blood Marrow

Sleeping Beauty

a non-viral vector
Tc1/mariner family
validated in cl
Ivics Z (1997) Cell 91(4):501
Invest. 126



Anti-BAFFR CAR



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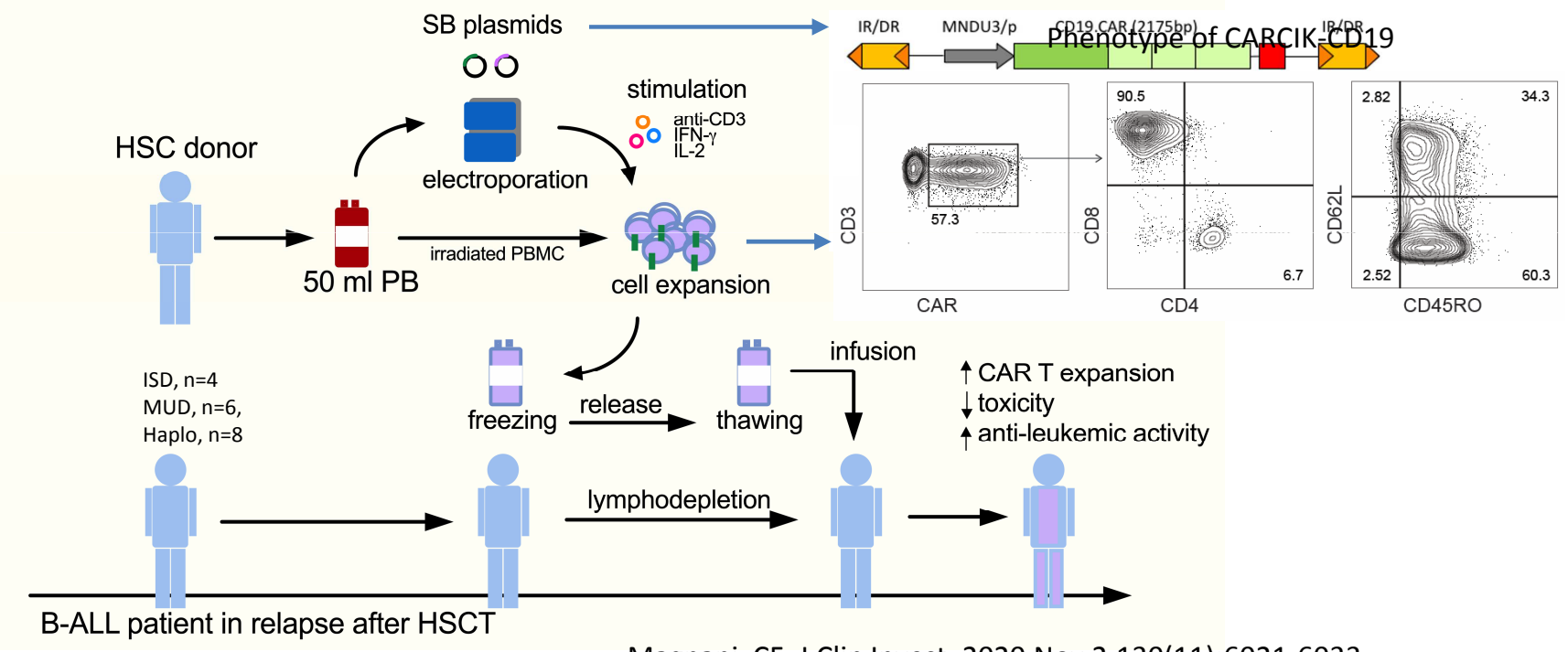
Molecular Therapy

35:141-152

CARCIK-CD19: allogeneic CAR T generated with SB in B-ALL post HSCT

Phase 1/2, Multicentric Dose Escalation Study, NCT03389035, Sponsor: Fondazione Tettamanti
PIs: A Biondi, A Rambaldi; Enrolling in ASST-Monza and ASST-Bergamo, IT

- 21 adult and pediatric **B-ALL** patients
- From 1 x 10^6 up to 15x10^6 /kg **dose escalation**



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

HSC= hematopoietic stem cell; ISD= HLA identical sibling; MUD= matched unrelated donor; Haplo= haploidentical donor

CARCIK-CD19: Patient characteristics

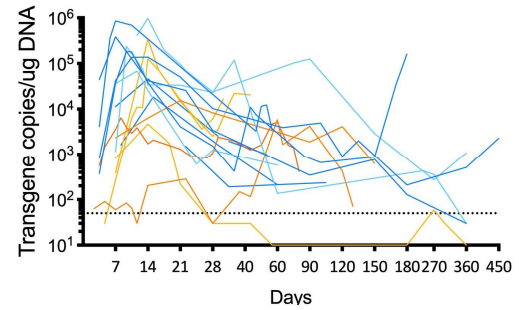
The first 13 patients were reported in Magnani CF, J Clin Invest. 2020 Nov 2;130(11):6021-6033

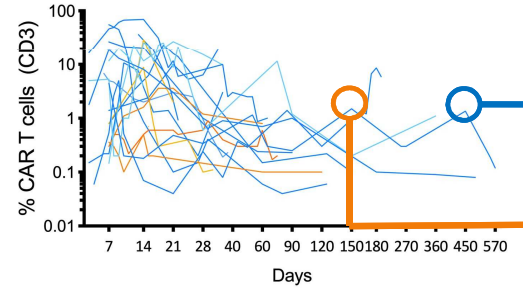
Characteristics		Patients (n = 20)
Median age (range), year		36.5 (1-62)
Female, (%)		10 (50%)
No. of Prior Lines of Therapy	median (range)	2 (1-8)
	No. of previous HSCT, n (%)	1, 14 (70.0%); 2, 6 (30.0%)
Prior HSCT		
Type of Transplant Donor, n (%)	Haplo	8 (40.0%)
	MUD	6 (30.0%)
	ISD	6 (30.0%)
	Disease status at HSCT, n (%) HCR PCR-, HCR PCR+, Hemato PR	4 (22.2%), 13 (72.2%), 1 (5.5%); N/A 2
	Months from Allo-HSCT to Relapse, median (range)	9.5 (1-30)
	aGVHD post last HSCT, n (%)	G-I 6 (33.3%); G-I 1 (5.5%); G-III 1 (5.5%); N/A 2
	cGVHD post last HSCT, n (%)	G-I 3 (16.6%); G-II 1 (5.5%)
Disease characteristics	Non isolated extramedullary disease, n (%)	6 (30.0%)
	BM Blasts at enrolment, median (range)	60% (5%-98%)
	Karnofsky/Lansky performance status before cellular therapy, n (%)	90-100, 10 (50.0%) 60-80, 10 (50.0%)
Blood values pre-lymphodepletion	LDH pre-LT CT (U/L), median (range)	378.5 (148-1487)
	PLT count pre-LT CT (mmc), median (range)	16100.5 (16-237000)
	WBC pre-LT CT (mmc), median (range)	5540.0 (120-5200)
	neutrophils pre-LT CT (mmc)	3390.0 (60-4150)
BM Blasts post lymphodepletion	Median (range)	13% (0-96)
	PCR +, n (%)	16 (80.0%)

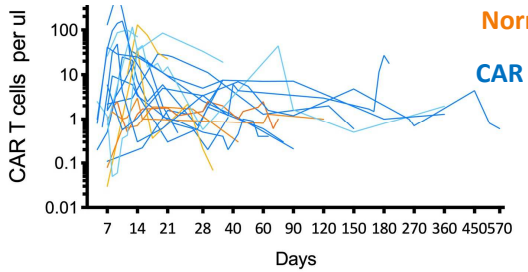
HSCT= hematopoietic stem cell transplantation; ISD= HLA identical sibling; MUD= matched unrelated donor; Haplo= haploidentical donor; HCR= hematological complete remission; G-I= grade I; G-II= grade II; G-III= grade III; CT= count

20 patients have been infused (data cut off 15.01.2021). Last patient was infused few wks ago.

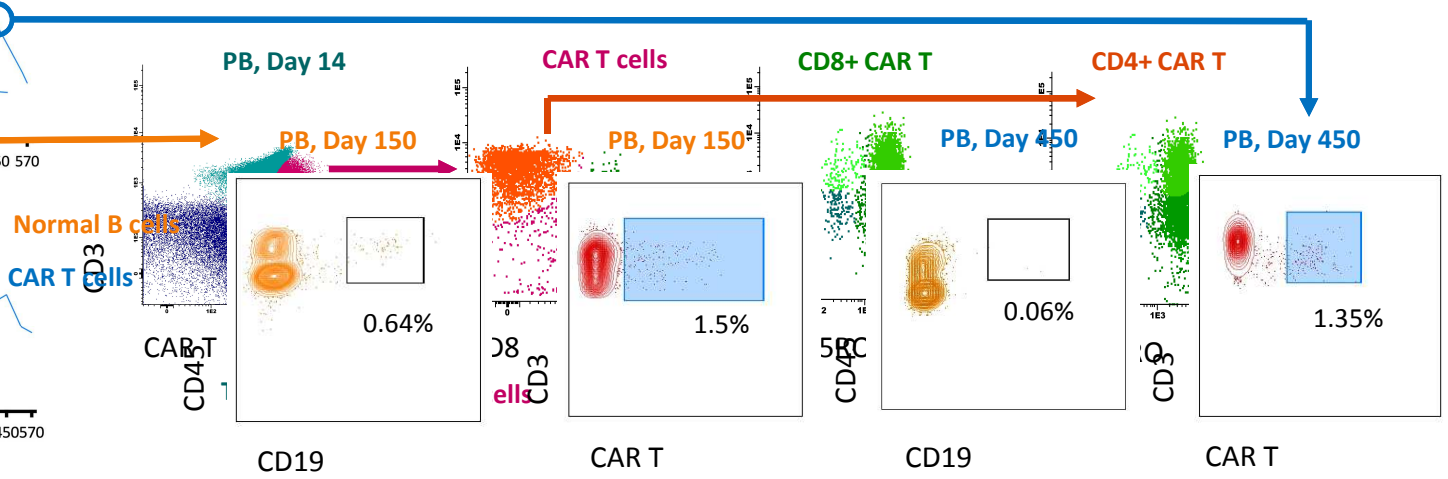
Post infusion expansion, phenotype and persistence of CAR T cells







Pharmacokinetic analysis	Geometric Mean (n)
AUC, copies/∝g	
AUC0-28d	403,607 (15)
AUC-last	1,040,117 (15)
Cmax, copies/∝g	
Cmax0-28d	61,369 (15)
Cmax-last	75,098 (15)
Tmax, d	
tmax0-28d	12.1 (15)
Tmax-last	12.9 (15)



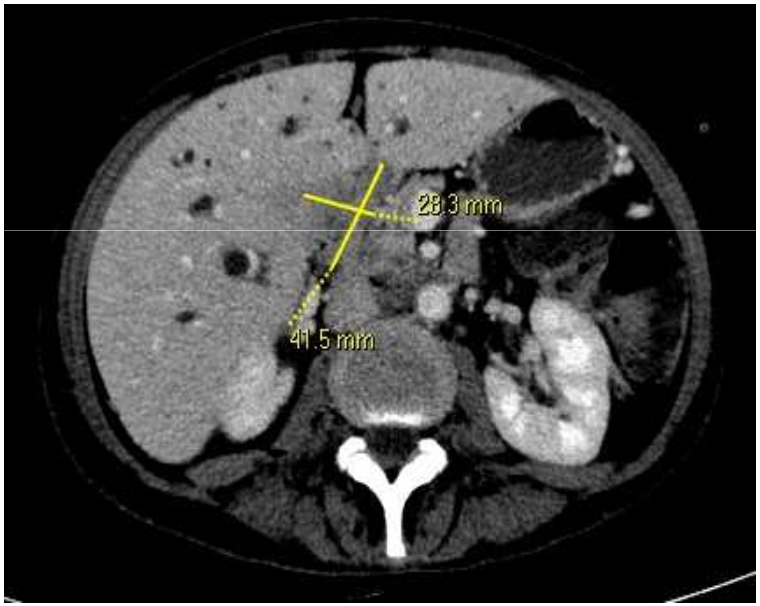
Selected adverse events

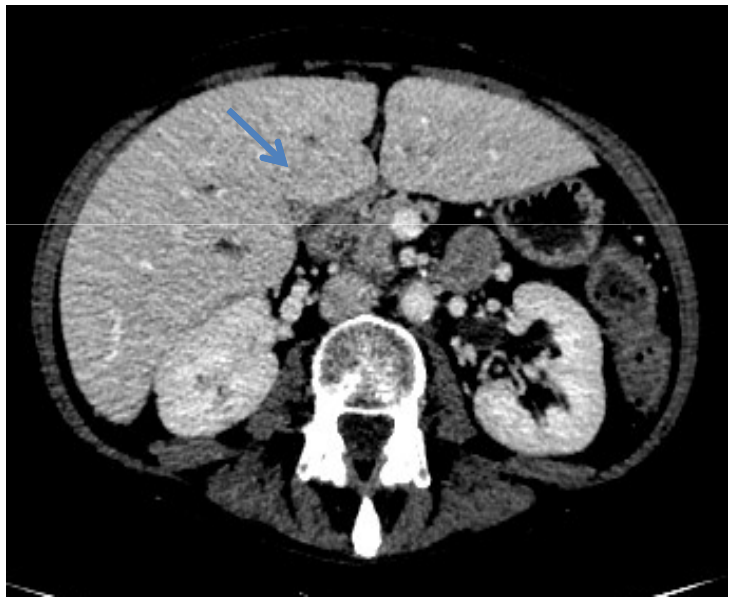
Events	Patients (n = 20)
CRS, n (%)	
Grade I	3 (15.0%)
Grade II	2 (10.0%)
Grade ≥ III	0 (0%)
ICANS, n (%)	
Grade III	1 (5%)
GVHD, n (%)	0 (0%)
DLT, n (%)	0 (0%)
Seizure, n (%)	2 (10.0%)
Severe pancytopenia, n (%)	2 (10.0%)
Infection, n (%)	6 (30.0%)

CRS, cytokine release syndrome, assessed with criteria in Lee et al. Blood. 2014;124(2):188–195; ICANS, immune-effector cell neurotoxicity syndrome, graded by Common Terminology Criteria for Adverse events (CTCAE) v4.03; GVHD, graft-versus-host disease; DLT, dose limiting toxicity

CAR-T mediated contraction of extramedullary disease

Patient #12: CT scan before and after CARCIK-CD19



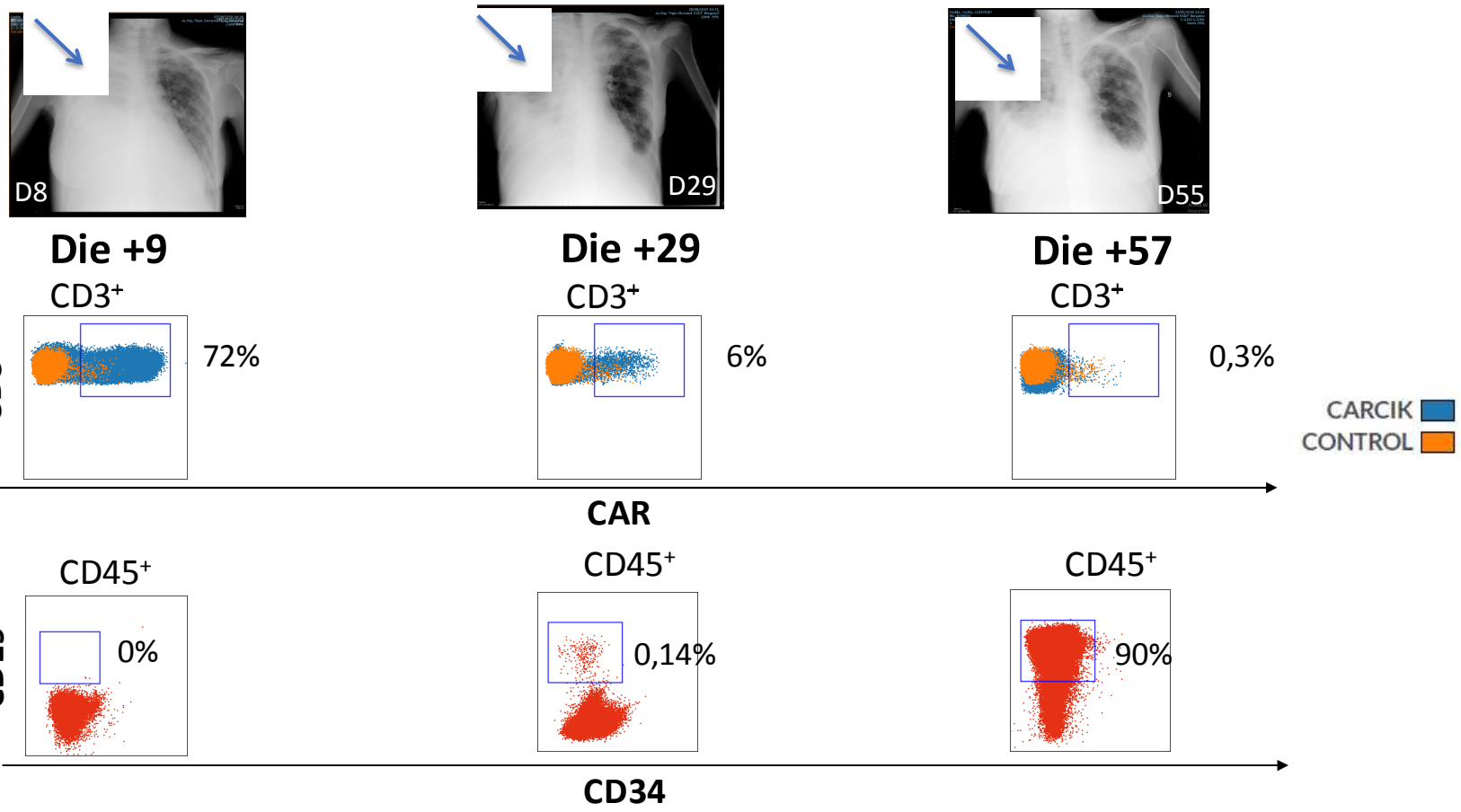


07 June 2019: Relapse post Allo-HSCT presenting liver infiltration
27 June 2019:
- AST/ALT: 157/287 UI
- GammaGT: 1183 UI
- Bil: 18.8 mg/dl

12 September 2019, day **+44** after CARCIK-CD19 infusion:
- AST/ALT: 12/58 UI
- Gamma GT: 82 UI
- Bil 0,8 mg/dl

CAR-T mediated contraction of extramedullary disease

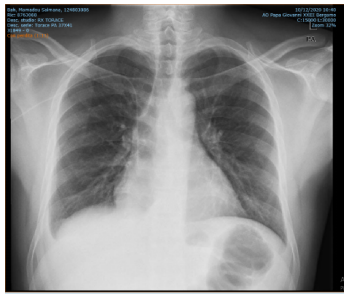
Patient #14: Massive pleural effusion after CARCIK-CD19 at Chest X-Ray



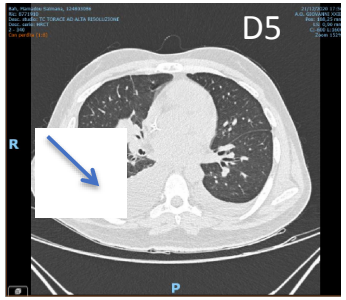
CAR-T mediated contraction of extramedullary disease: Pleural Effusion

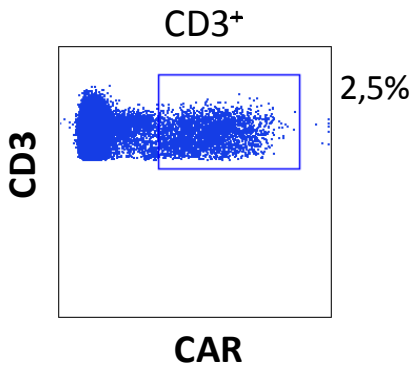
Patient #22: CARCIK-CD19 and disease detection in pleural effusion Day +7

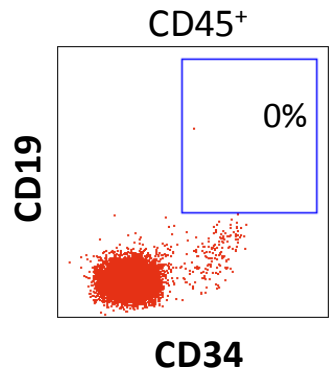
- 38 yrs, Ph+ ALL
- Highly pre-treated (8 lines)
- Pre-CARCIK BM blast 37%



Negative X-Ray



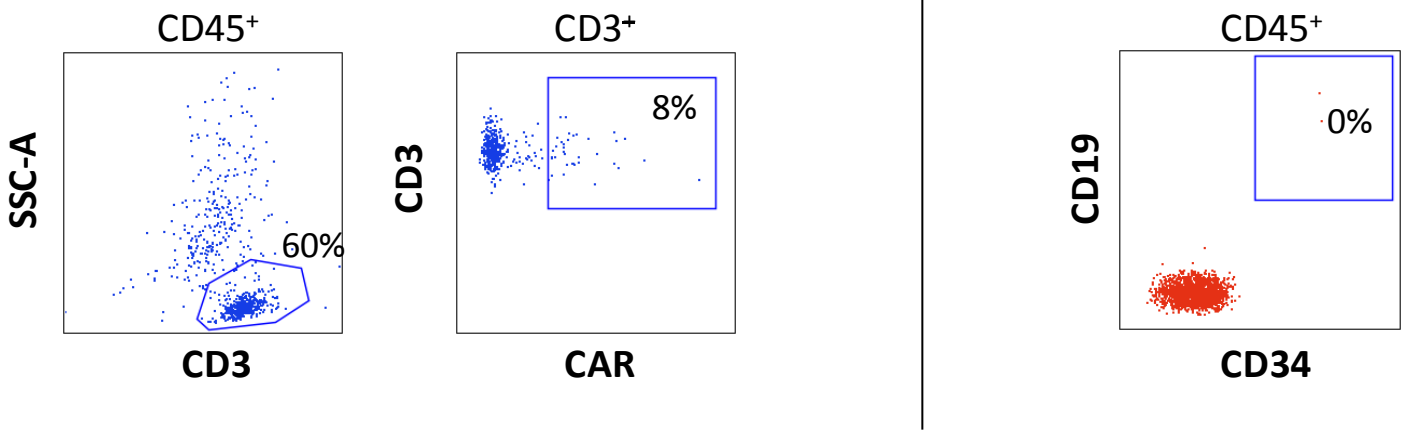




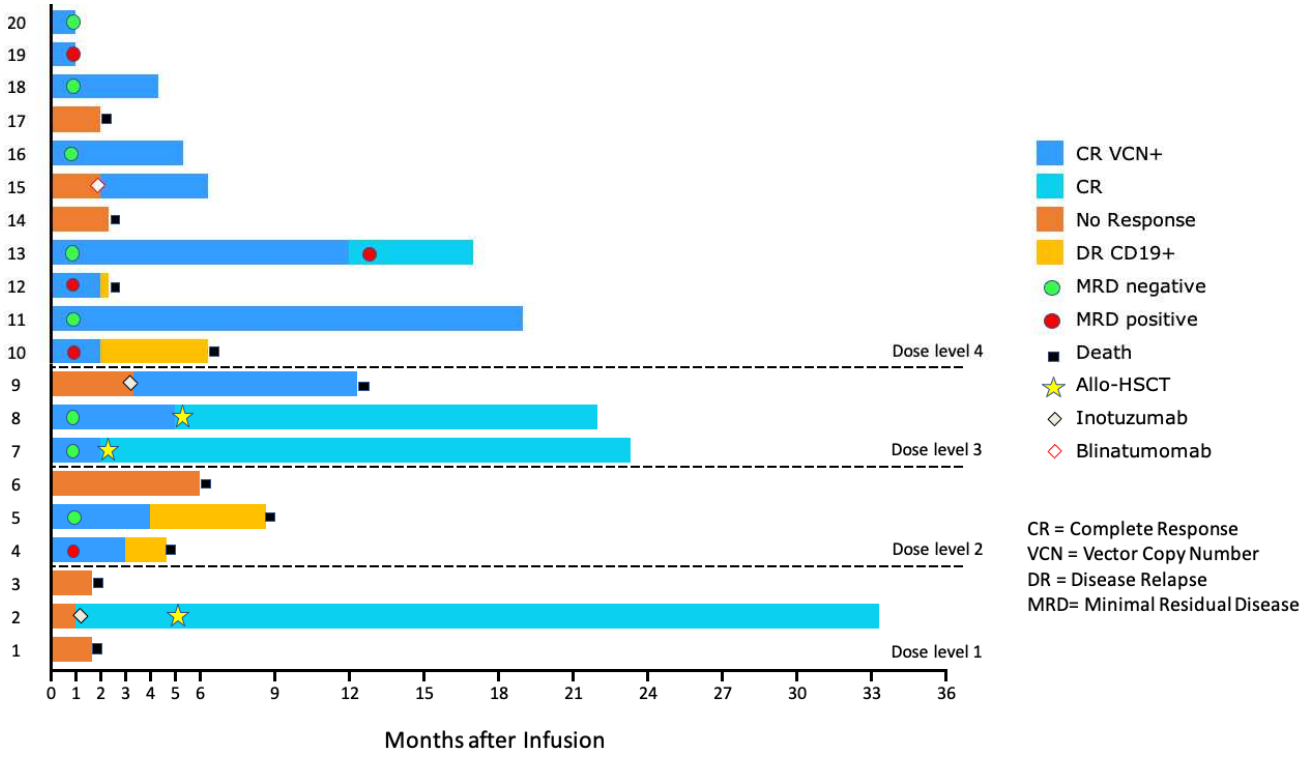
CAR-T mediated contraction of extramedullary disease: CSF

Patient #19: CARCIK-CD19 and disease detection in CSF at day +33

- 60 yrs, Ph+ ALL
- Pre-CARCIK BM blast 60%
- Highly pre-treated (5 lines)
- Pre-CARCIK CSF not performed due to low platelets count
- Pre-CARCIK Cerebral RMI negative for malignancy



Response

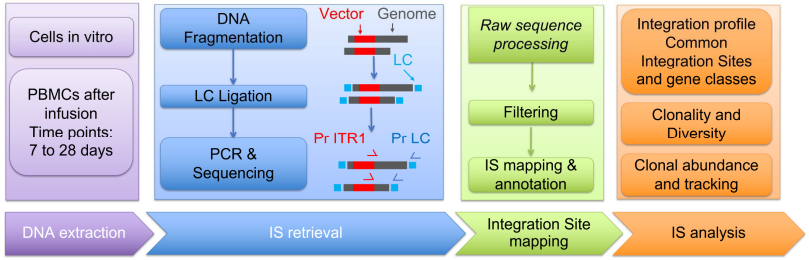


CR = Complete Response
VCN = Vector Copy Number
DR = Disease Relapse
MRD= Minimal Residual Disease

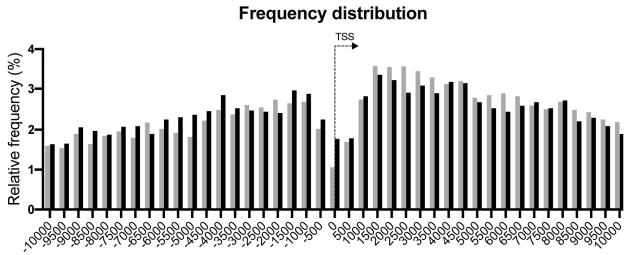
Response	Patients (n = 20)
CR/CRi	
CR/CRi	60% (12 of 20)
MRD- in the CR cohort	67% (8 of 12)
CR/CRi, High doses	
Dose levels 3 and 4	71% (10 of 14)

Consistent with the concomitant in vivo detection of CAR T cells, B cell aplasia (BCA) was observed in all treated patients. Thirteen of 20 patients had persistent BCA at the last follow-up.

Integration site analysis

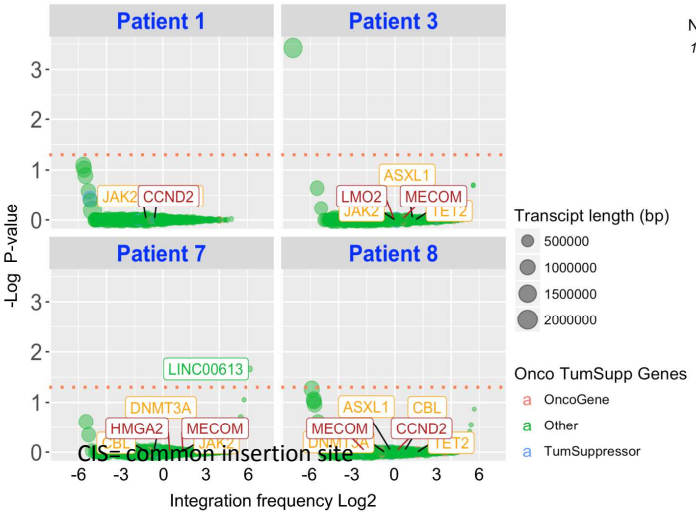


Cellular products Post-infusion samples

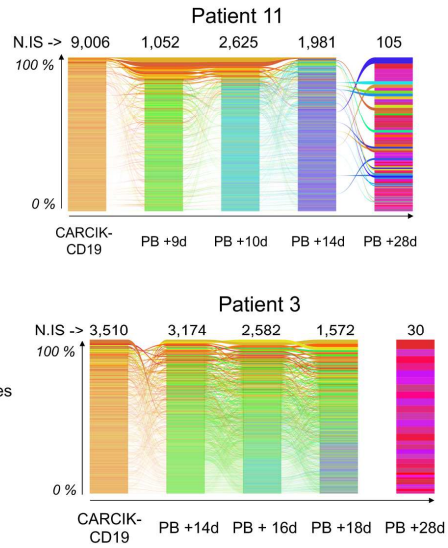


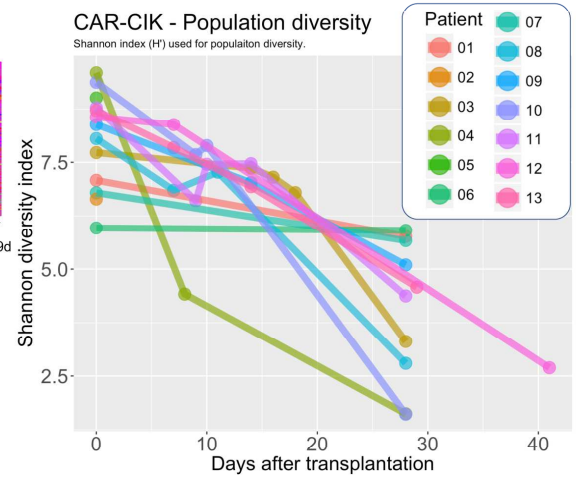
No CIS classified as cancer related gene

High polyclonal marking and population diversity



CIS= common insertion site

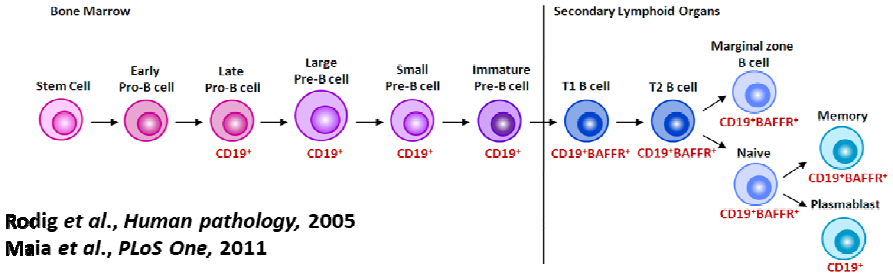




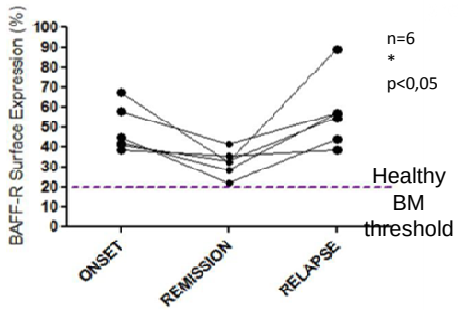
BAFF Receptor: an Alternative Target for Leukemia escape after CD19 targeting

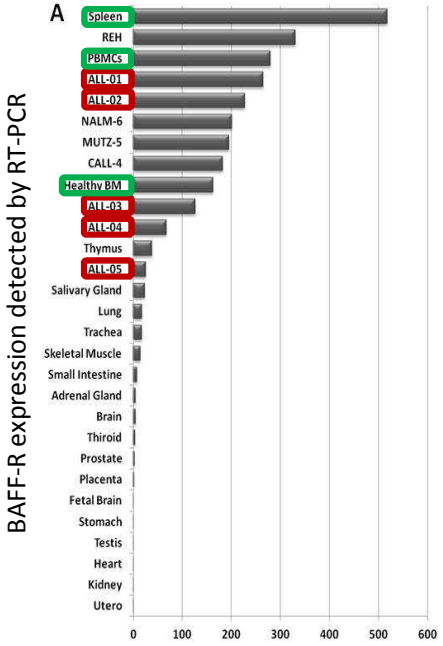
B cell-activating factor (BAFF) belongs to the TNF family and plays a key role in B cell survival and differentiation;
BAFF/BAFF-R pathway supports B-ALL cell survival and contribute to resistance of leukemic clone to therapy in BM microenvironment;

Ng LG et al J Immunol. 2004 Jul 15;173(2):807-17
Parameswaran R et al Cancer Res. 2010 Jun 1;70(11):4346-56
Rodig SJ et al um Pathol. 2005 Oct;36(10):1113-9



Rodig *et al.*, *Human pathology*, 2005
Maia *et al.*, *PLoS One*, 2011



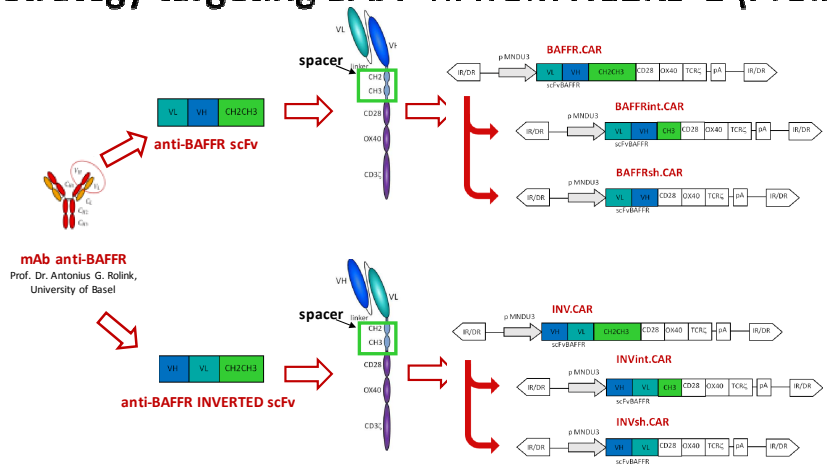


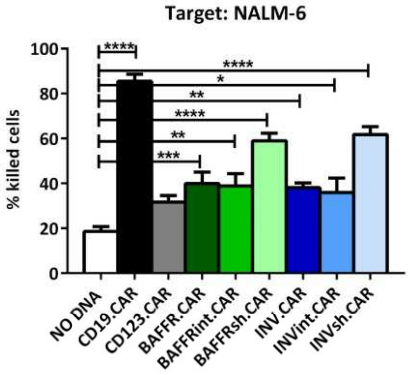
Fazio G. et *al.* (2017); *British Journal of Haem.*

BAFF-R is expressed on B-ALL diagnostic samples and is preserved during drug treatment and at relapse, further supporting his role on blast survival.

B-ALL patients can be targeted by BAFFR and CD19 combination

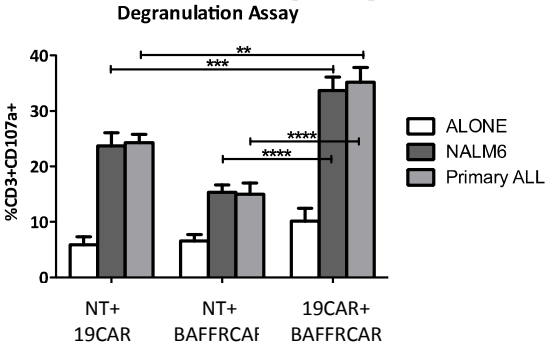
CAR cell strategy targeting BAFF-R: from HuBR9-1 (Prof. Rolink, Basel)

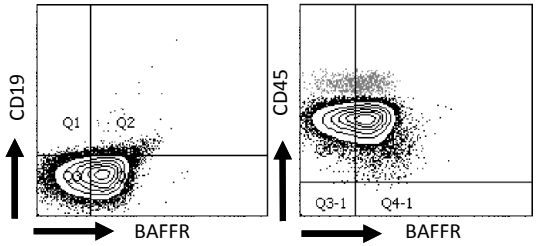


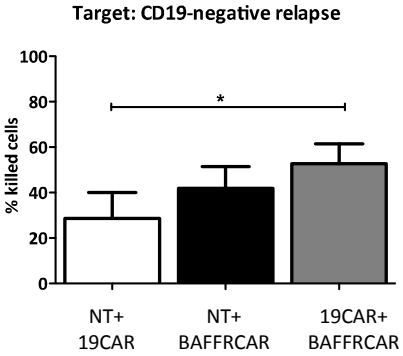


SPACER ELIMINATION AUGMENTS TUMOR RECOGNITION

Combination of SB CARCIK targeting BAFFR and CD19





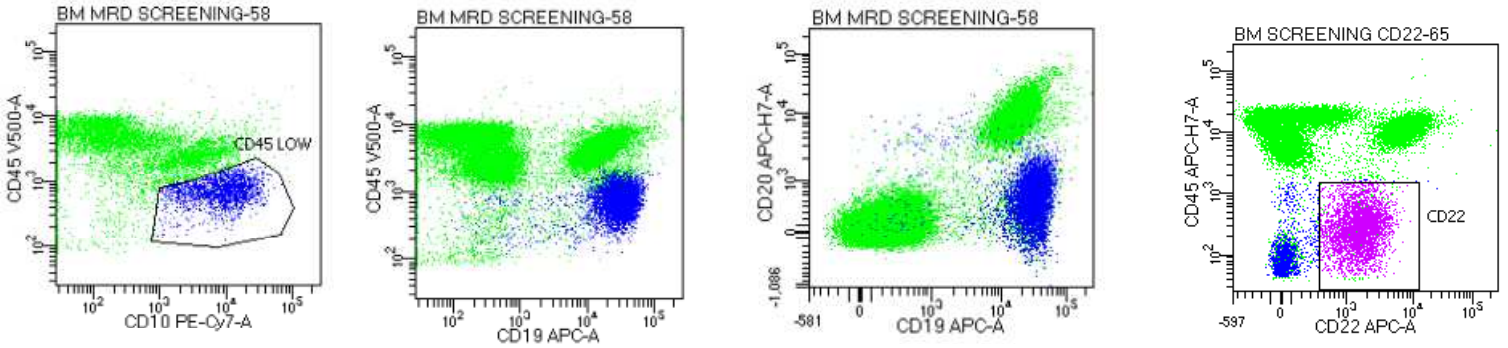


Combination of BAFFRCAR with CD19CAR demonstrate superior activity also towards CD19-negative B-ALL relapsed leukemia.

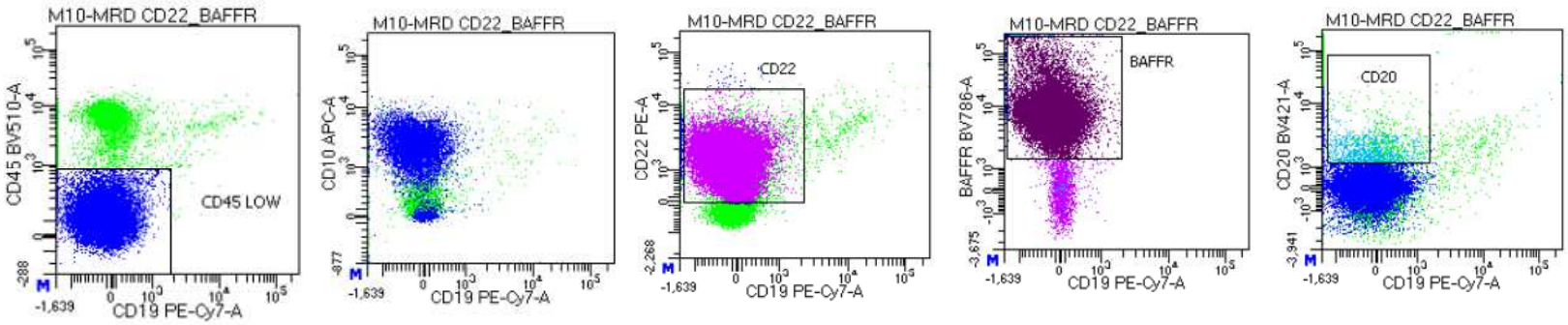
Turazzi N. *et al.* (2017); *British Journal of Haem.*

BAFFR and CD22 expression by a patient relapsed post anti-CD19 CAR T cells

Screening

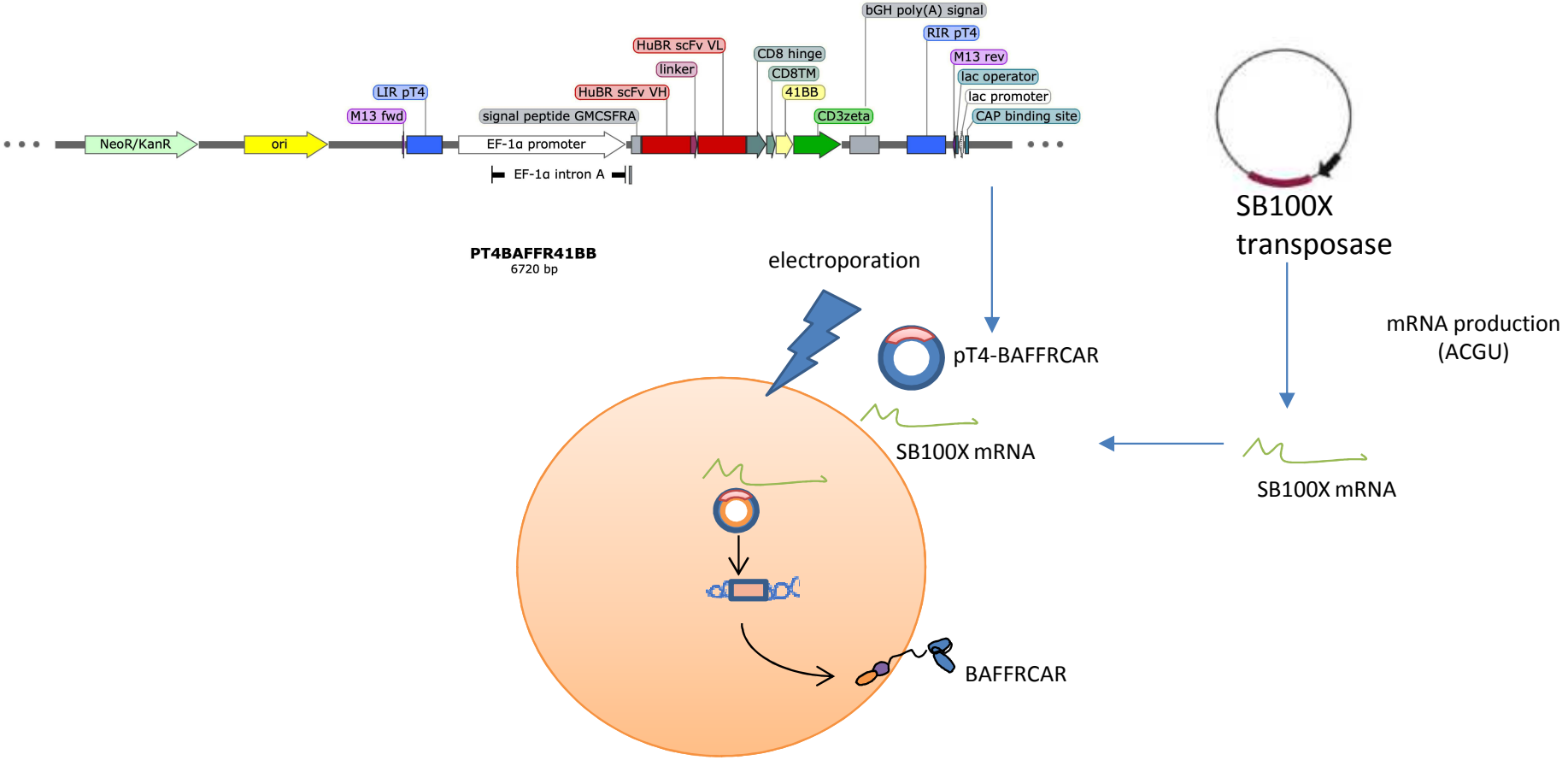


Relapse CD19neg (month 10)



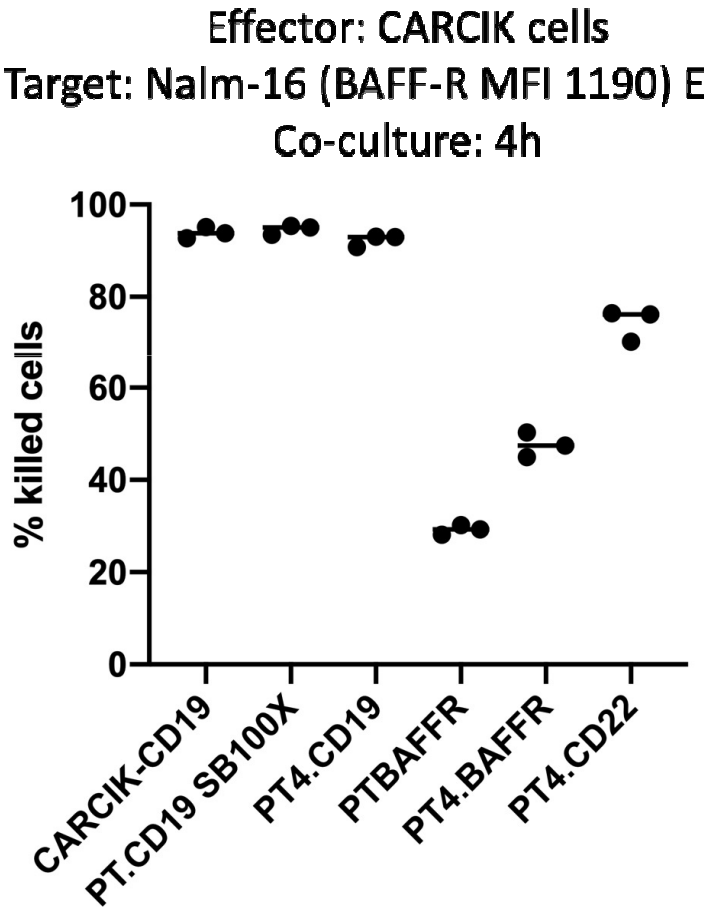
anti-BAFFR CAR T cells engineered with an optimized SB vector

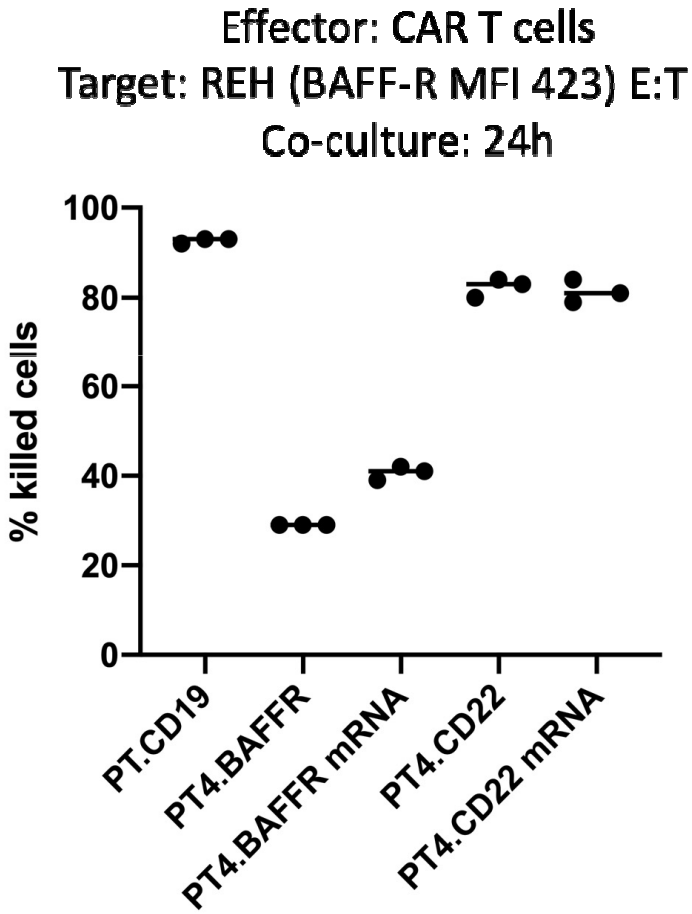
pT4 vector expressing anti-BAFFR CAR and SB100X mRNA



the transient SB100X expression given by mRNA ensures safety by avoiding transposon remobilization

SB-engineered BAFFRCAR T cells exhibited potent cytotoxicity





Non-viral genetic manipulation of CB-derived CIK cells to express the CD19.CAR for the targeting of ALL

Generation of CIK cells from Cord Blood

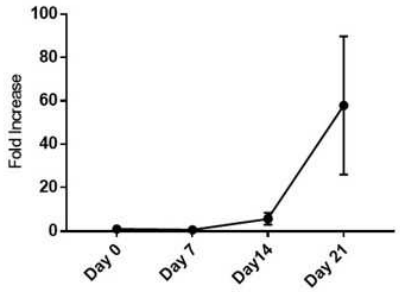
Cells recovered at the end of the expansion protocol (day + 21)

A' Total MNC × 10⁶	*B' Total CD3⁺ × 10⁶*	*C' Total CD3⁺/CD56⁺ × 10⁶*
990.0	949.0	872.0
768.0	744.0	570.0
360.0	350.0	264.0
1100.0	1066.0	816.0
804.5 ± 326.9	777.3 ± 314.4	630.5 ± 277.3
A'/A 53.5	B'/B 101.5	C'/C 1860
(24–66)	(70–152)	(1320–2180)

Introna M et al., Bone Marrow Transplantation (2006)

Generation of CAR CIK cells from Peripheral Blood

GMP production of CD19.CAR-CIK cells from 50 ml of fresh PB (n=25 batches)

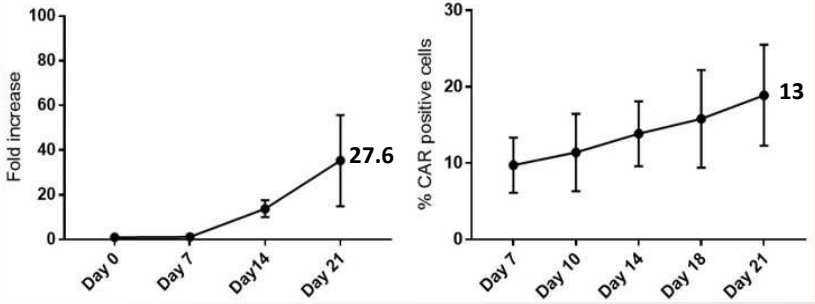


CAR expression

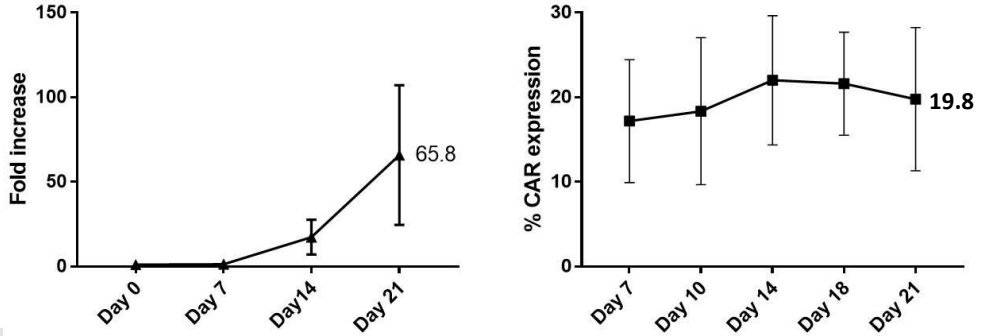
median	**38,30**
min	15,10
max	73,17
media	**40,98**
dev.std	15,73
n	25

Generation of CAR CIK from thawed CB bags

pT CD19.CAR + SB11 n=9



pT4 CD19.CAR + SB100X n=4



CAR-CIK-CD19 cells: summary

- Manufacture of allogeneic CARCIK cells by SB from 50 mL donor PB is a feasible, cost-effective and robust process
- CARCIK-CD19 are able to expand rapidly and efficiently and persist in r/r pediatric and adult B-ALL patients
- CARCIK-CD19 were characterized by a high profile of safety in all treated patients and in term of genotoxicity
- High CR rate was achieved in B-ALL patients treated with high doses of CARCIK-CD19

Future plans for CARCIKCD19 in pediatric and adult ALL and B-NHL

- Program for **compassionate use of CARCIK-CD19**, in pediatric and adult patients with relapsed/refractory B cell precursor ALL after (allo-HSCT) (approvato da AIFA 15/01/2021);

- Measurable residual disease driven strategy for **one or two infusions of autologous or allogeneic** non-viral, transposon-manipulated CARCIK-CD19 cells. A Phase II study in pediatric and adult patients with relapsed/refractory B cell precursor ALL (approved by AIFA and EC-Monza).

- Phase Ia-II trial to determine the safety of allogeneic **PBMNC or cord-blood derived** cytokine induced killer cells transduced with a transposon CD19-chimeric antigen receptor (CARCIK-CD19) gene in adult and pediatric patients with relapsed or refractory B-cell non-Hodgkin lymphoma (pending submission)

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