



GIORNATE EMATOLOGICHE VICENTINE

X edizione

12-13 Ottobre 2023

Palazzo Bonin Longare - Vicenza

**Cellule CARCIK generate tramite la piattaforma non virale Sleeping Beauty
per il trattamento delle malattie oncoematologiche**

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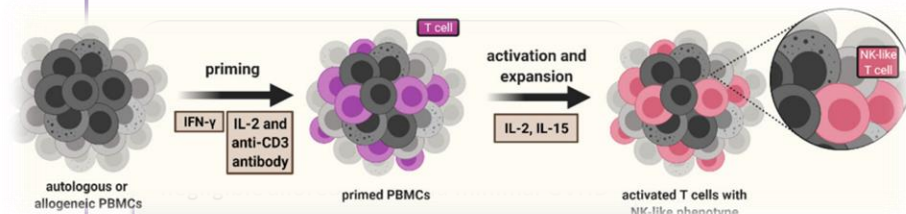
Disclosures of Sarah Tettamanti

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Our approach: simplification of the technology platform



Cytokine-Induced Killer cells as effector cells

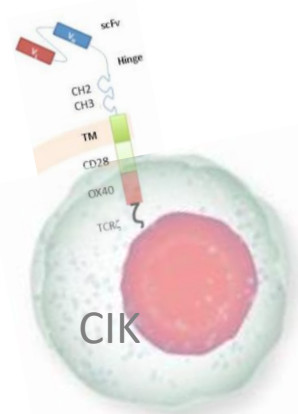


- intrinsic capability of **reaching leukemia-infiltrated tissues**

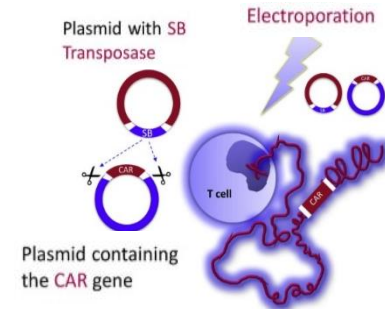
Clinical experience with allogeneic CIK:

feasible, safe and well tolerated with reduced risk of GVHD, included in 5 patients with CIK from haploidentical donors

(Introna et al. BBMT 2017, In press, EudraCT N°2008-003185-26)



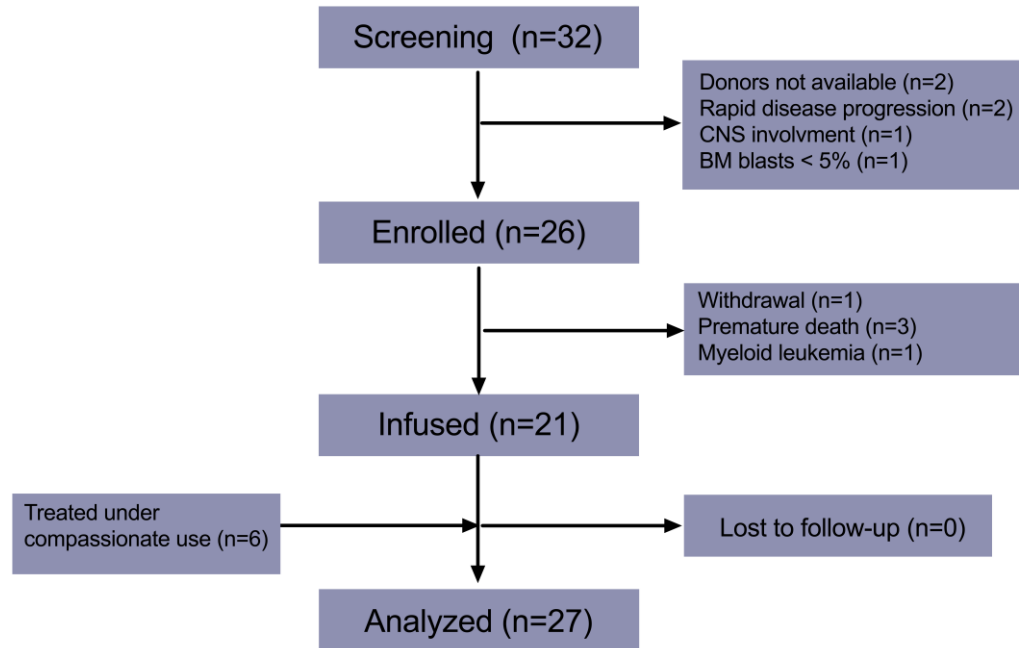
Non-viral gene transfer manufacturing



Sleeping Beauty TRANSPOSONS

Mobile DNA elements naturally occurring in the genome
Easy to purify, non-immunogenic, random pattern of integration

SB-engineered CARCIK-CD19 manufactured from the HSCT donor: eligibility and consort

**Patients characteristics:**

- No. of prior lines (median, range): 4(2-8)
- No. of previous alloHSCT, n (%):
 - 1 (18, 66.6%);
 - 2 (9, 33.3%)
- Type of transplant, n (%):
 - Haplo 10 (37%)
 - MUD 10 (37%)
 - Sib 7(26%)
- aGvHD post last tx:
 - Grade I and II (n=8, 29%), GIII % (n=1, 4%)
- cGvHD post last Tx:
 - Grade I and GII (n=4, 15%); GIII (n=0, 0%)
- BM blasts at the enrollment, median (range): 40 (0-100)
- Extramedullary disease, n (%): 7 (26%)

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

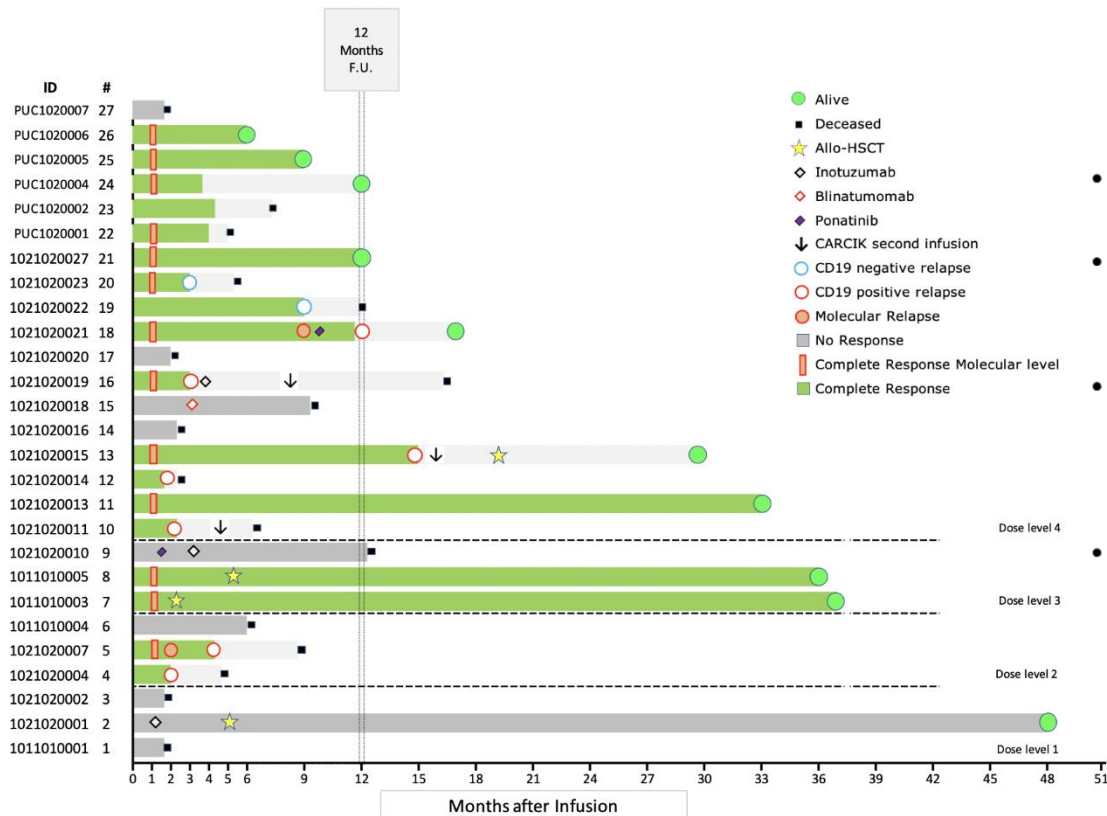
Events	Patients
CRS, n (%)	
• Grade 1	4 (15%)
• Grade 2	5 (19%)
• Grade 3	0 (0%)
ICANS, n (%)	
Grade 3	2 (7%)
GvHD, n (%)	
Grade I-IV	0 (0%)
Infection, n (%)	
• Grade 1-2	2 (7%)
• Grade \geq 3	7 (26%)
Prolonged cytopenia, n (%)	
Severe neutropenia, day 28	7 (32%)
Severe thrombocytopenia, day 28	17 (68%)

- 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 25 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/mm³; severe thrombocytopenia <50000/mm³

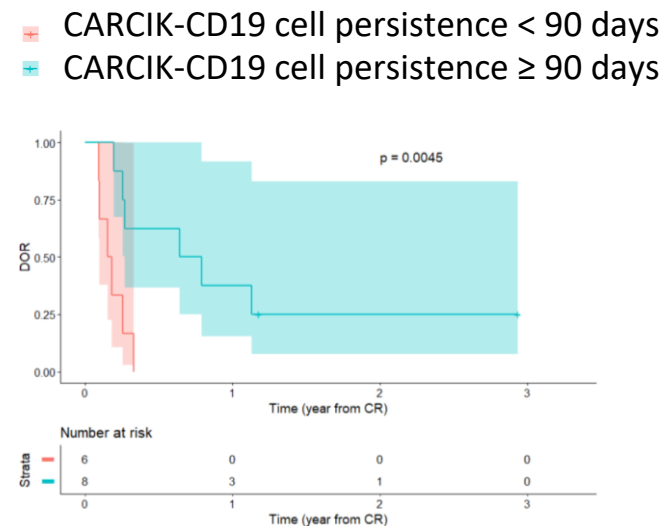
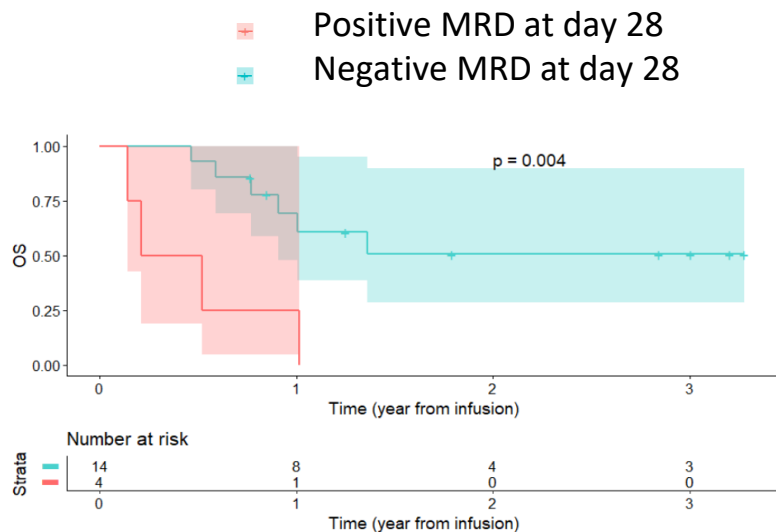


Response data



- 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

Minimal residual disease and CARCIK-CD19 persistence and clinical outcome



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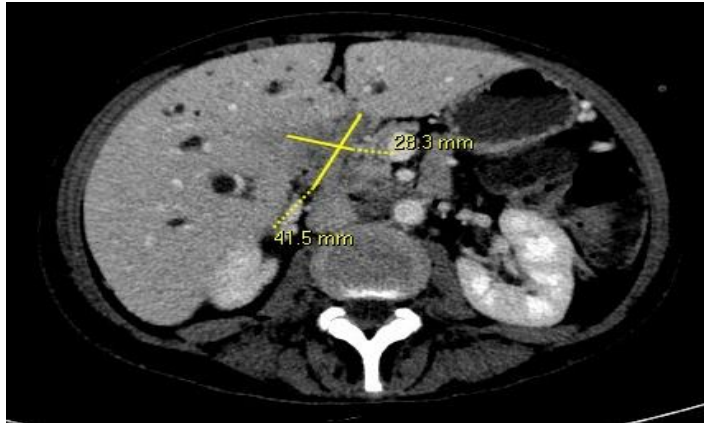


Regione
Lombardia



CAR-CIK mediated contraction of extramedullary disease

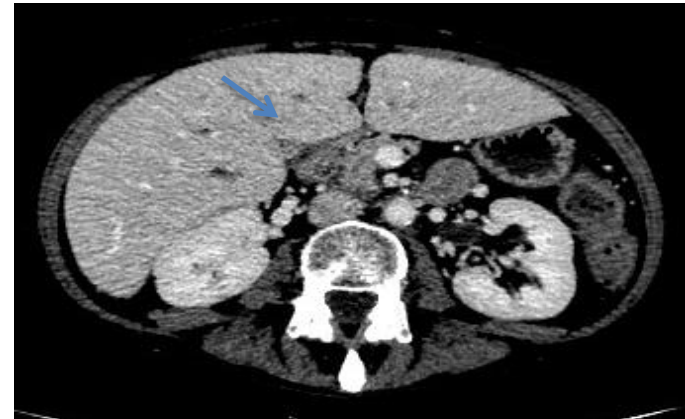
Patient #21020014: CT scan before and after CARCIK-CD19



07 June 2019: Relapse post Allo-HSCT presenting liver adenopathy

27 June 2019:

- AST/ALT: 157/287 UI,
- GammaGT: 1183 UI,
- Bil: 18.8 mg/dl



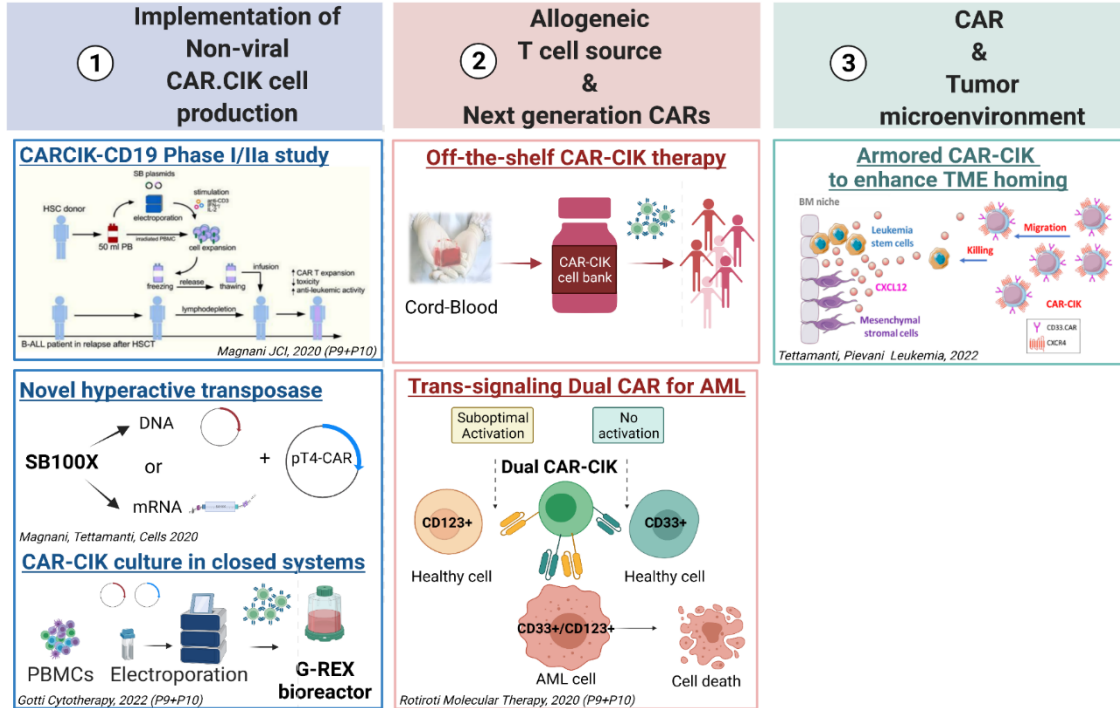
12 September 2019, day +44 after CARCIK-CD19 infusion:

- AST/ALT: 12/58 UI,
- Gamma GT: 82 UI,
- Bil 0,8 mg/dl

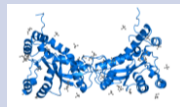
Implementation of CAR-Clk cell platform



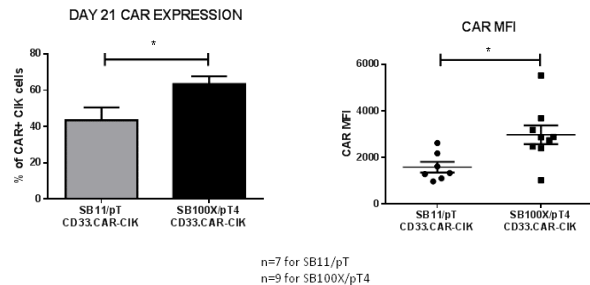
CAR-Clk cell platform



Implementation of the platform: use of a more efficient SB100X transposase

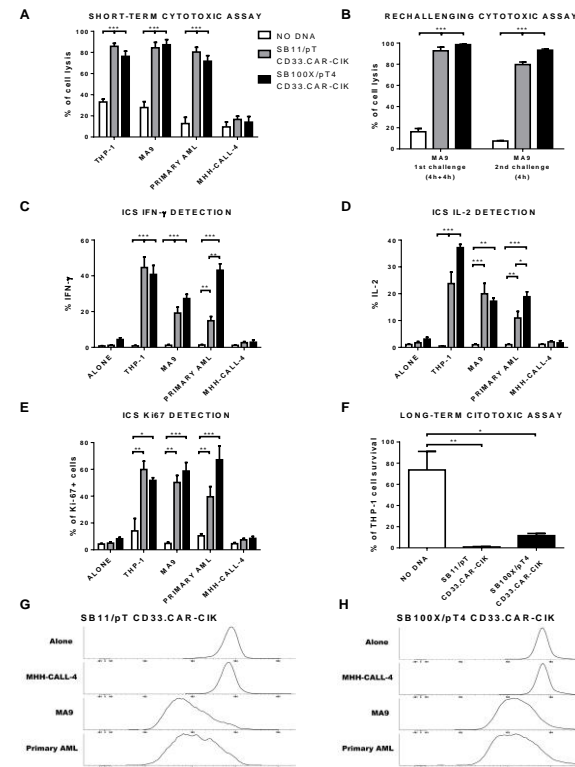


Low Amount of SB100X transposase & Increased CAR expression with pt4/SB100X



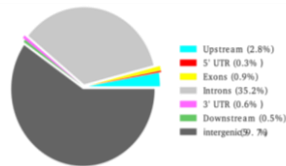
Cytotoxicity

Cytokine release



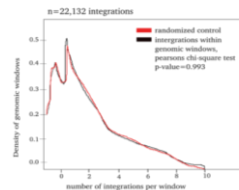
Safe pattern of integration of SB pt4/SB100X

GENOME-WIDE SLEEPING BEAUTY INTEGRATIONS IN RELATION TO RefSeq ANNOTATION



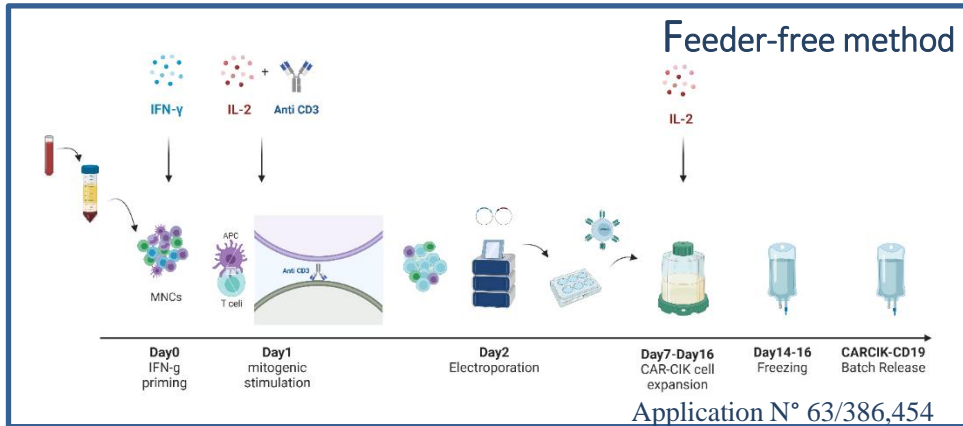
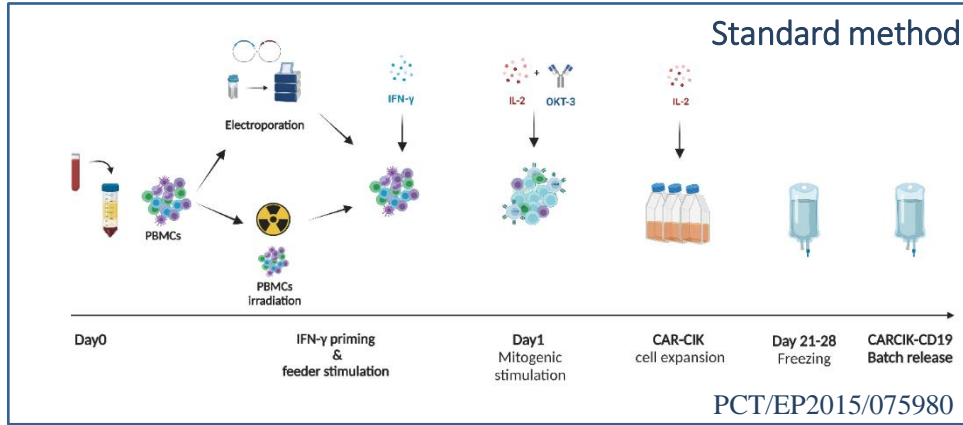
* Upstream = 1kb upstream from TSS, Downstream = 1kb downstream from 3' UTR

SLEEPING BEAUTY INTEGRATIONS PATTERN COMPARED TO RANDOMIZED CONTROL



Proliferation

Implementation of the platform: feeder-free method and use of the G-REX bioreactor



Co-filed with Colmune, Inc.,
Durham NC

Document Description: Power of Attorney

Approved for use through 03/10/2022 - 03/08/2023

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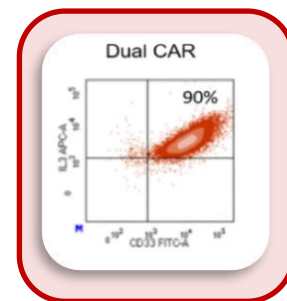
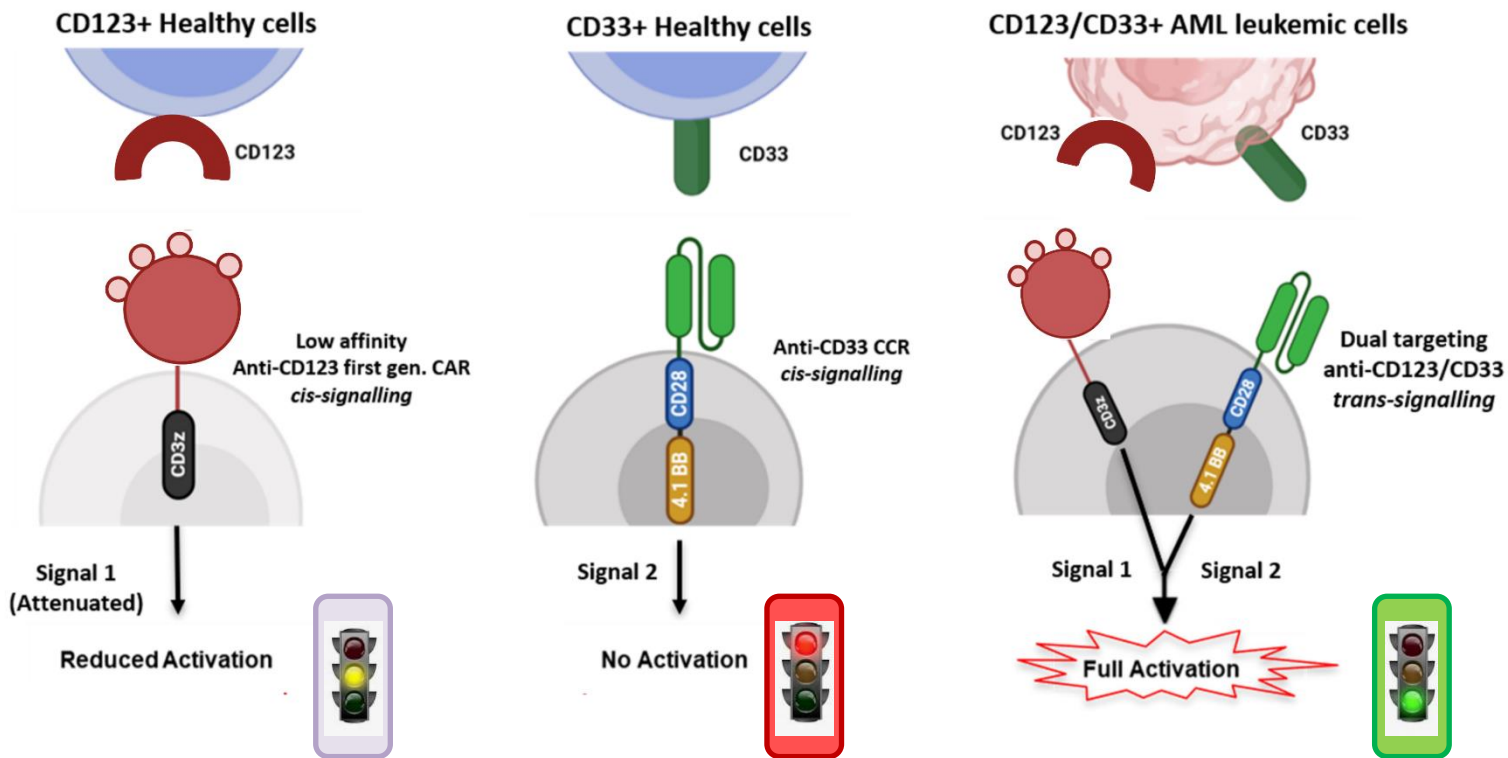
POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in 63/386,454 the attached transmittal letter or to access below.

Application Number	Filing Date
63/386,454	December 7, 2022

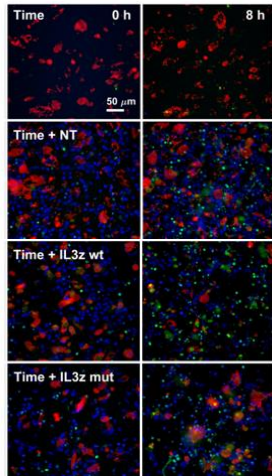
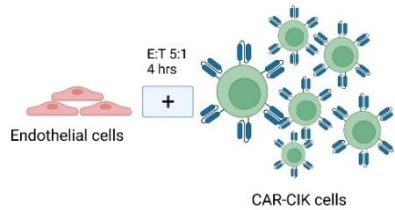
Phase I-II trial to determine the safety
of allogeneic CARClk-CD19 in adult and
pediatric patients with
R/R B-NHL

Trans-signaling Dual CAR CIK cells for AML



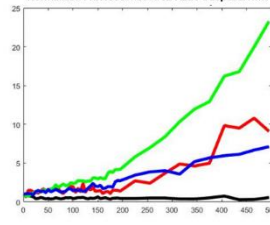
Safety profile evaluation: toxicity against endothelial cells & HSPCs

Endothelium

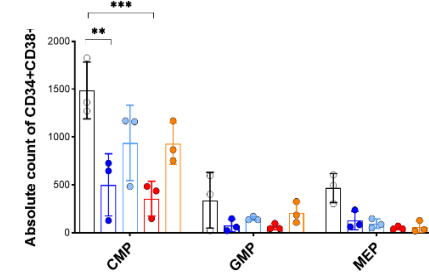
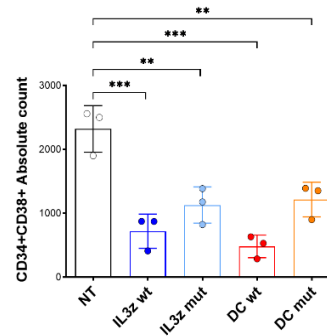
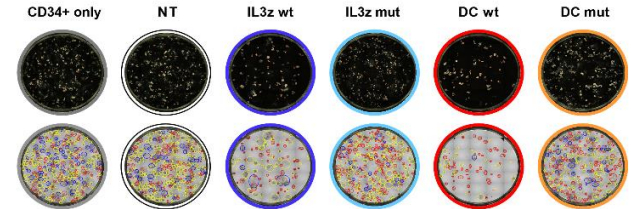
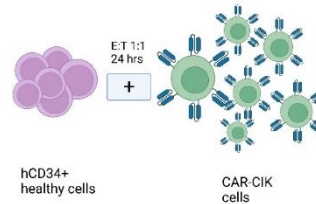


- Time alone
- Time + NT
- Time + IL3z wt
- Time + IL3z mut

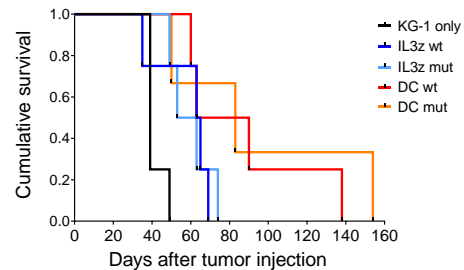
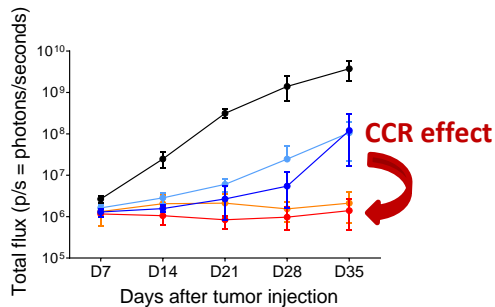
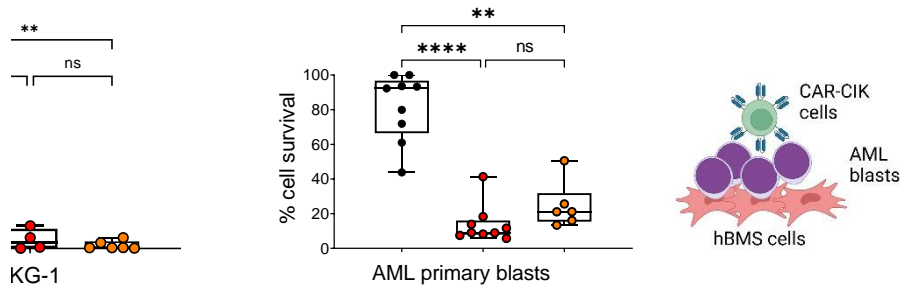
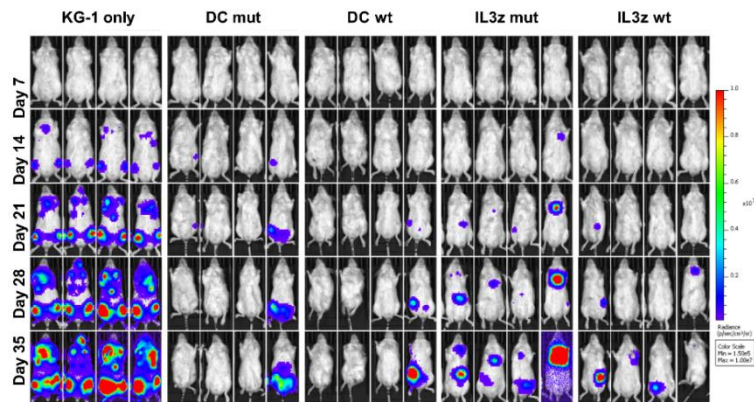
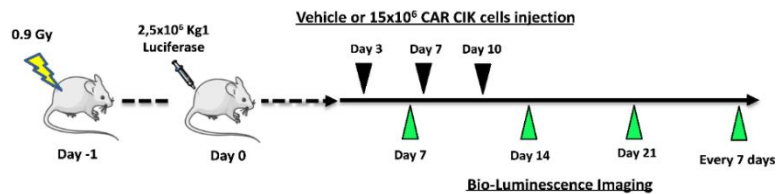
Normalized Fluorescence Area Ratio Caspase/Time



HSPCs

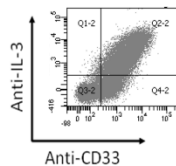


Anti-leukemic efficacy evaluation: *in vitro* and *in vivo*

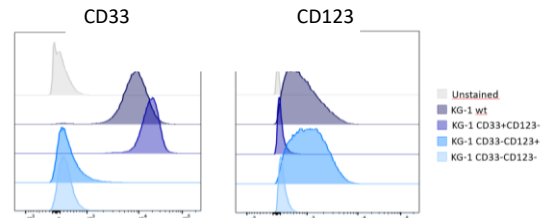


Dual CAR Trans-activation: *in vitro* evaluation

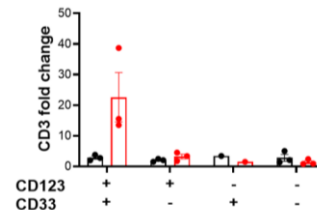
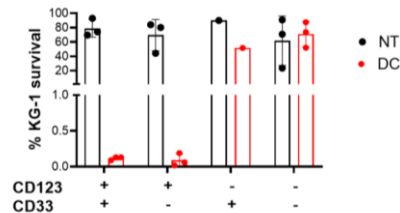
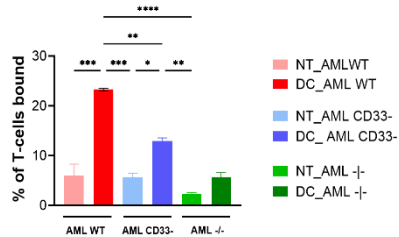
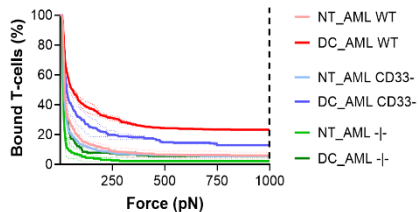
pT4-transposon



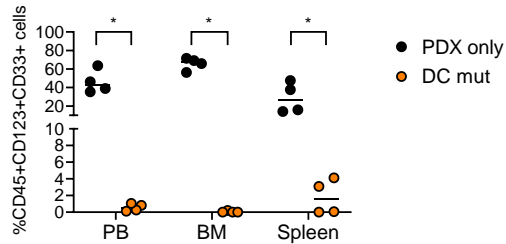
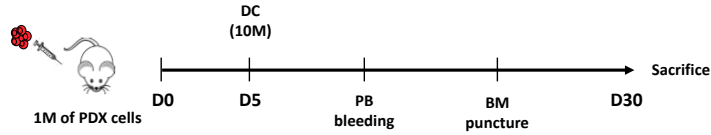
CRISPR-Cas9 KO cell lines



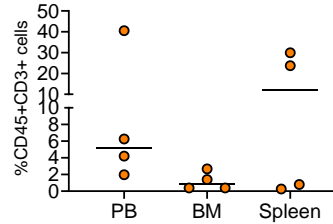
Cell avidity analysis- z-Movi® Analyzer Lumicks



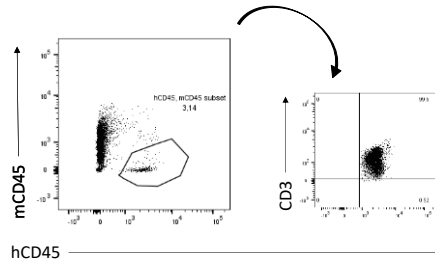
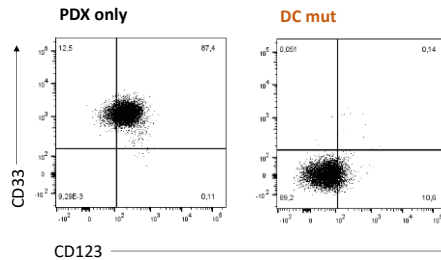
pT4-DualCAR-CIK cells: *in vivo* antileukemic activity



F



H



CONCLUSIONS and FUTURE PERSPECTIVES

- The **non-viral Sleeping Beauty-derived CAR CIK cells** are a **solid versatile CAR-T platform alternative to viral vectors**, with reduced CoG and simplified production processes → to be **further implemented** with mRNA SB100X transposase and more closed systems
- The non viral SB platform can be adopted to derive **“off-the-shelf” Cord Blood derived CAR CIK cells**
- The non-viral CAR CIK cell platform can be exploited to generate **next-generation CARs**, such as **Dual CARs** or **Armored CARs, beyond CD19-targeting**
- **Dual CD123/CD33 CAR-CIK** cells mediate high anti-leukemic efficacy against AML cells through trans-acting costimulation

Acknowledgements



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San Gerardo dei Tintori

Sistema Socio Sanitario



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