FONDAZIONE MONZA E BRIANZA
PER IL BAMBINO E LA SUA MAMMA

AZIENDA ULSS N. 6 - VICENZA
DIVISIONE DI EMATOLOGIA

FONDAZIONE PROGETTO EMATOLOGIA

GIORNATE EMATOLOGICHE VICENTINE

Gene transfer and cell expansion for engineering of anti-tumor T cells: ready for everyone?

Ettore Biagi, MD PhD, Ass. Prof., Molecular Therapy Unit,
Center for Cell and Gene Therapy "Stefano Verri",
"Matilde Tettamanti" Research Lab, Hem-Onc Department,
San Gerardo Hospital, Monza (Italy)

Vicenza, October 12, 2016

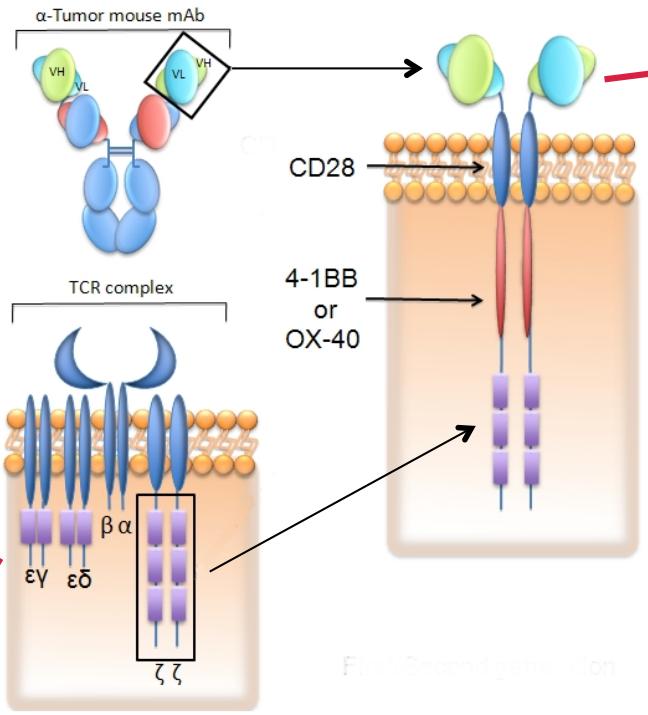
CAR: Breakthrough of the Year 2013

Chimeric Receptors for Immunotherapy of Acute Leukemias

Acute Lymphocytic leukemia (ALL) and Acute Myeloid leukemia (AML) in children and adults: still associated with a very **poor prognosis**

CHIMERIC ANTIGEN RECEPTORS

CARs



The RADAR

An extracellular domain recognizing tumor-associated antigens derived from mAb

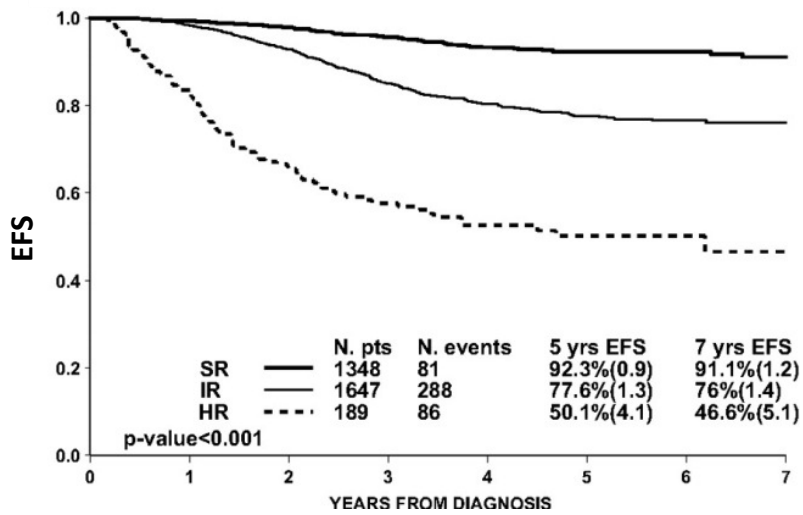
An intracellular signaling domain triggering T cell activation

The BAZOOKA

modified from Chekmasova AA, Brentjens RJ (2010), *Discov Med,* 9(44):62-70

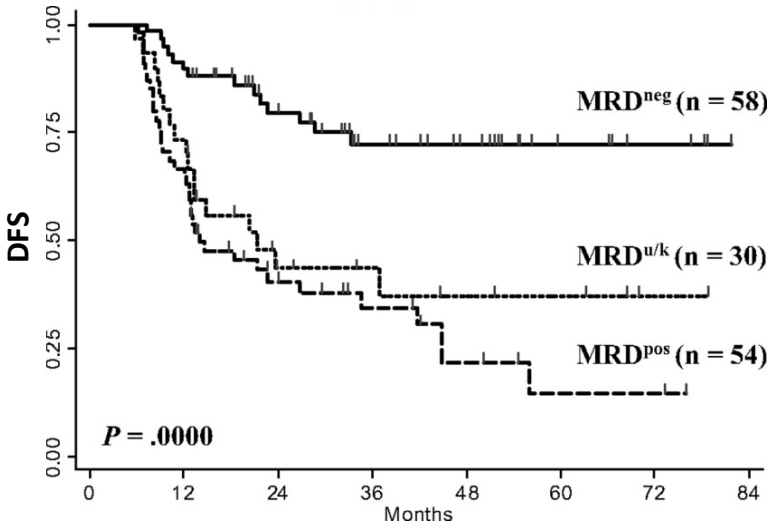
Outcome of childhood and adult BCP-ALL patients

Children in AIEOP-BFM ALL2000 frontline protocol



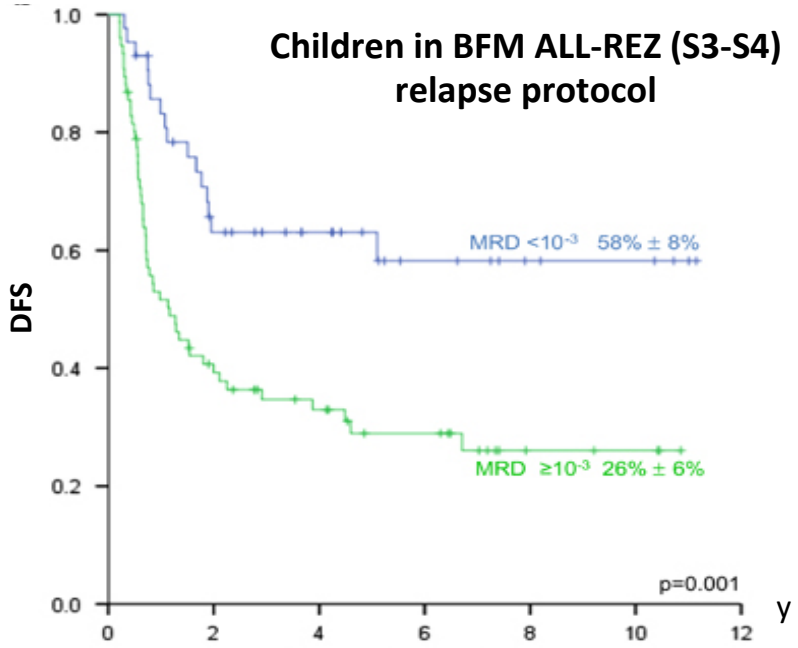
Conter V et al., Blood 2010;115:3206-14

Adult in NILG-ALL 09/00 frontline protocol



Bassan R et al., Blood 2009;113:4153-62

Children in BFM ALL-REZ (S3-S4) relapse protocol



- **20%** of young patient relapse (mostly high-risk pts). Cure rate after relapse is approximately **25%** to **40%.**

- refractory ALL (never achieving a CR) in children or adults has a dismal prognosis and these patients **do not benefit from HSCT**.

- relapsed or refractory (r/r) ALL patients, both pediatric and adult, have significant **unmet medical needs.**

Eckert C et al., Leukemia 2015;29:1648–55

Ongoing Clinical Studies using CAR T cells for hematologic malignancies

Table 2. Ongoing clinical trials using allogeneic CAR T cells for hematologic malignancies, as of May 2014

Disease	*Target antigen (CAR signaling domain)*	*Patient age*	*Vector*	*Sponsor*	*Clinical Trial.gov ID*
ALL	CD19 (4-1BB–CD3ζ)	⩾18 Years	Lentivirus	University of Pennsylvania	NCT01551043
ALL[a]	CD19 (CD3ζ)	⩽19 Years	Retrovirus	Memorial Sloan Kettering cancer center	NCT01430390
ALL, CLL, NHL[b]	CD19 (CD3ζ)	Pediatric and adult	Retrovirus	Baylor College of Medicine	NCT00840853
ALL, NHL[c]	CD19 (CD3ζ)	1–75 Years	Transposon	MD Anderson Cancer Center	NCT01362452
ALL, NHL		1–65 Years			NCT01497184
NHL, CLL	CD19 (CD3ζ)	18–75 Years	Retrovirus	National Cancer Institute	NCT01087294
ALL, DLBCL, MCL, NHL, CLL[d]	CD19 (CD3ζ)	18–75 Years	Lentivirus	Fred Hutchinson Cancer Research Center	NCT01475058
ALL[e]	CD19 (CD3ζ)	⩽18 Years	Retrovirus	University College, London	NCT01195480
ALL, CLL, NHL	CD19 (CD137-CD3ζ and CD3ζ)	5–90 Years	Retrovirus	Chinese PLA General Hospital	NCT01864889
AML	CD33 (CD137-CD3ζ and CD3ζ)				NCT01864902

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; NHL, non-Hodgkin's lymphoma. [a]Epstein–Barr virus (EBV)-specific donor-derived cytotoxic T lymphocytes (CTLs). [b]Trivirus-specific donor-derived CTLs (against cytomegalovirus (CMV), EBV and adenovirus). [c]Donor-derived cord blood T cells. [d]Donor-derived CMV- or EBV-specific CD62L$^+$ T_{CM}. [e]EBV-specific CTLs.

				Research Center	
MF, CTCL	CD30	18–70 Years	Retrovirus	University of Cologne	NCT01645293
ALL, CLL, NHL	CD19 (CD137-CD3ζ and CD3ζ)	5–90 Years	Retrovirus	Chinese PLA General Hospital	NCT01864889
AML	CD33 (CD137-CD3ζ and CD3ζ)	5–90 Years			NCT01864902
MM	CD138 (CD137-CD3ζ and CD3ζ)	18–80 Years			NCT01886976
ALL, NHL	CD20 (4-1BB–CD3ζ)	18–90 Years			NCT01735604
MCL	CD19 (CD137-CD3ζ and CD3ζ)	50–80 Years			NCT02081937
AML, MDS, MM	Lewis-Y (Anti–Lewis-Y-CD28-CD3ζ)	⩾18 Years	Retrovirus	Peter MacCullum Cancer Center	NCT01716364

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; HL, Hodgkin's lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MF, mycosis fungoides; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma. [a]Autologous Epstein–Barr virus (EBV)-specific cytotoxic T lymphocytes (CTLs). [b]Central memory-enriched CD8$^+$ T cells.

An Immune System Trained to Kill Cancer

Jessica Kourkounis for The New York Times

CLOSE-UP Dr. Carl June examined re-engineered T-cells last week in his Philadelphia lab.

By DENISE GRADY
Published: September 12, 2011

The New York Times

© 2012 The New York Times MONDAY, DECEMBER 10, 2012

In Girl's Last Hope, Altered Cells Beat Leukemia

By DENISE GRADY

PHILIPSBURG, Pa. — Emma Whitehead has been bounding around the house lately, practicing somersaults and rugby-style tumbles that make her parents wince.

It is hard to believe, but last spring Emma, then 5, was near death from leukemia. She had relapsed twice after chemotherapy, and doctors had run out of options.

Desperate to save her, her parents sought an experimental treatment at the Children's Hospital of Philadelphia, one that had never before been tried in a child, or in anyone with the type of leukemia Emma had. The experiment, in April, used a disabled form of the virus that causes AIDS to reprogram Emma's immune system genetically to kill cancer cells.

The treatment very nearly killed her. But she emerged from it cancer-free, and about seven months later is still in complete remission. She is the first child and one of the first humans ever in whom new techniques have achieved a long-sought goal — giving a patient's own immune system the lasting ability to fight cancer.

Emma had been ill with acute lymphoblastic leukemia since 2010, when she was 3, said her parents, Kari and Tom. She is their only child.

She is among just a dozen patients with advanced leukemia to have received the experimental treatment, which was developed at the University of Pennsylvania. Similar approaches are also being tried at other centers, including the National Cancer Institute and Memorial Sloan-Kettering Cancer Center in New York.

"Our goal is to have a cure, but we can't say that word," said Dr. Carl June, who leads the research team at the University of Pennsylvania. He hopes the new treatment will eventually replace bone marrow transplantation, an even more arduous, risky and expensive procedure that is now the last hope when other treatments fail in leukemia and related diseases.

Three adults with chronic leukemia treated at the University of Pennsylvania have also had

Continued on Page A16

JEFF SWENSEN FOR THE NEW YORK TIMES

Emma Whitehead, with her mother, Kari, is now cancer-free.

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Chimeric Antigen Receptor–Modified T Cells for Acute Lymphoid Leukemia

Stephan A. Grupp, M.D., Ph.D., Michael Kalos, Ph.D., David Barrett, M.D., Ph.D., Richard Aplenc, M.D., Ph.D., David L. Porter, M.D., Susan R. Rheingold, M.D., David T. Teachey, M.D., Anne Chew, Ph.D., Bernd Hauck, Ph.D., J. Fraser Wright, Ph.D., Michael C. Milone, M.D., Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.

RESEARCH ARTICLE

CANCER IMMUNOTHERAPY

CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia

Renier J. Brentjens,[1,2,3*†] Marco L. Davila,[1†] Isabelle Riviere,[1,2,3,4†] Jae Park,[1] Xiuyan Wang,[3,4] Lindsay G. Cowell,[5] Shirley Bartido,[4] Jolanta Stefanski,[4] Clare Taylor,[4] Malgorzata Olszewska,[4] Oriana Borquez-Ojeda,[4] Jinrong Qu,[4] Teresa Wasielewska,[4] Qing He,[4] Yvette Bernal,[1] Ivelise V. Rijo,[6] Cyrus Hedvat,[6] Rachel Kobos,[7] Kevin Curran,[7] Peter Steinherz,[7] Joseph Jurcic,[1] Todd Rosenblat,[1] Peter Maslak,[1] Mark Frattini,[1] Michel Sadelain[1,2,3*]

3/2013

CARs as innovative clinical option

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

University of Pennsylvania, Philadelpia

Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Shannon L. Maude, M.D., Ph.D., Noelle Frey, M.D., Pamela A. Shaw, Ph.D., Richard Aplenc, M.D., Ph.D., David M. Barrett, M.D., Ph.D., Nancy J. Bunin, M.D., Anne Chew, Ph.D., Vanessa E. Gonzalez, M.B.A., Zhaohui Zheng, M.S., Simon F. Lacey, Ph.D., Yolanda D. Mahnke, Ph.D., Jan J. Melenhorst, Ph.D., Susan R. Rheingold, M.D., Angela Shen, M.D., David T. Teachey, M.D., Bruce L. Levine, Ph.D., Carl H. June, M.D., David L. Porter, M.D., and Stephan A. Grupp, M.D., Ph.D.

N ENGL J MED 371;16 NEJM.ORG OCTOBER 16, 2014

www.thelancet.com Published online October 13, 2014 http://dx.doi.org/10.1016/S0140-6736(14)61403-3

NCI, Bethesda

T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial

Daniel W Lee, James N Kochenderfer, Maryalice Stetler-Stevenson, Yongzhi K Cui, Cindy Delbrook, Steven A Feldman, Terry J Fry, Rimas Orentas, Marianna Sabatino, Nirali N Shah, Seth M Steinberg, Dave Stroncek, Nick Tschernia, Constance Yuan, Hua Zhang, Ling Zhang, Steven A Rosenberg, Alan S Wayne, Crystal L Mackall

Investors have demonstrated significant interest in CAR / TCR

Significant Investments in CAR / TCR Therapies

	Q1 2013	Q2 2013	Q3 2013	Q4 2013	Q1 2014	Q2 2014	Q3 2014	Q4 2014
ADAPTIMMUNE transforming T cell therapy					Series A $104M			
Bellicum PHARMACEUTICALS					Series B $34M		Series C $55M	*IPO filed*
cellectis					€20.5M *private placement*			
JUNO THERAPEUTICS						Series A $176M	Series B $134M	*IPO filed*
Kite Pharma		Series A $35M*				Mezzanine $50M	*IPO complete*	
NOVARTIS								

July 2012: *$20M investment in CAR therapy @ UPENN*
December 2012: *$42M investment to purchase Dendreon US plant*

PERSPECTIVE


Bruce L. Levine


Carl H. June

Assembly line immunotherapy

Bruce L. Levine and **Carl H. June** explore how to make engineered immune cells that can eradicate cancer widely available.

Many scientists have raised legitimate **concerns about the perceived complexity** of this type of therapy and its **broad** applicability… **impossible to commercialize?**

BY MAKING USE OF EXISTING EQUIPMENT AND FACILITIES, AND BY AUTOMATING PRODUCTION, IT WILL BE POSSIBLE TO MAKE THESE THERAPIES WIDELY AVAILABLE

Developing engineered T-cell therapies in large numbers will be **challenging,** but it is **justified given their power to treat cancer**.

Limitations and challenges of CAR T-cell approaches

Manufacturing challenges

Complex manufacturing

Regulatory complexities, impacting product development, logistics and timelines

Gene transfection related scale-up

Cellular stress associated with non-viral transfection hinders cell expansion and scale-up manufacturing

Viral transfection methods may impose commercial scale-up hurdles

Current CAR-T Challenges

Clinical challenges

Non-response and relapse

Early relapses, despite high levels of initial complete remissions*

fety and toxicity

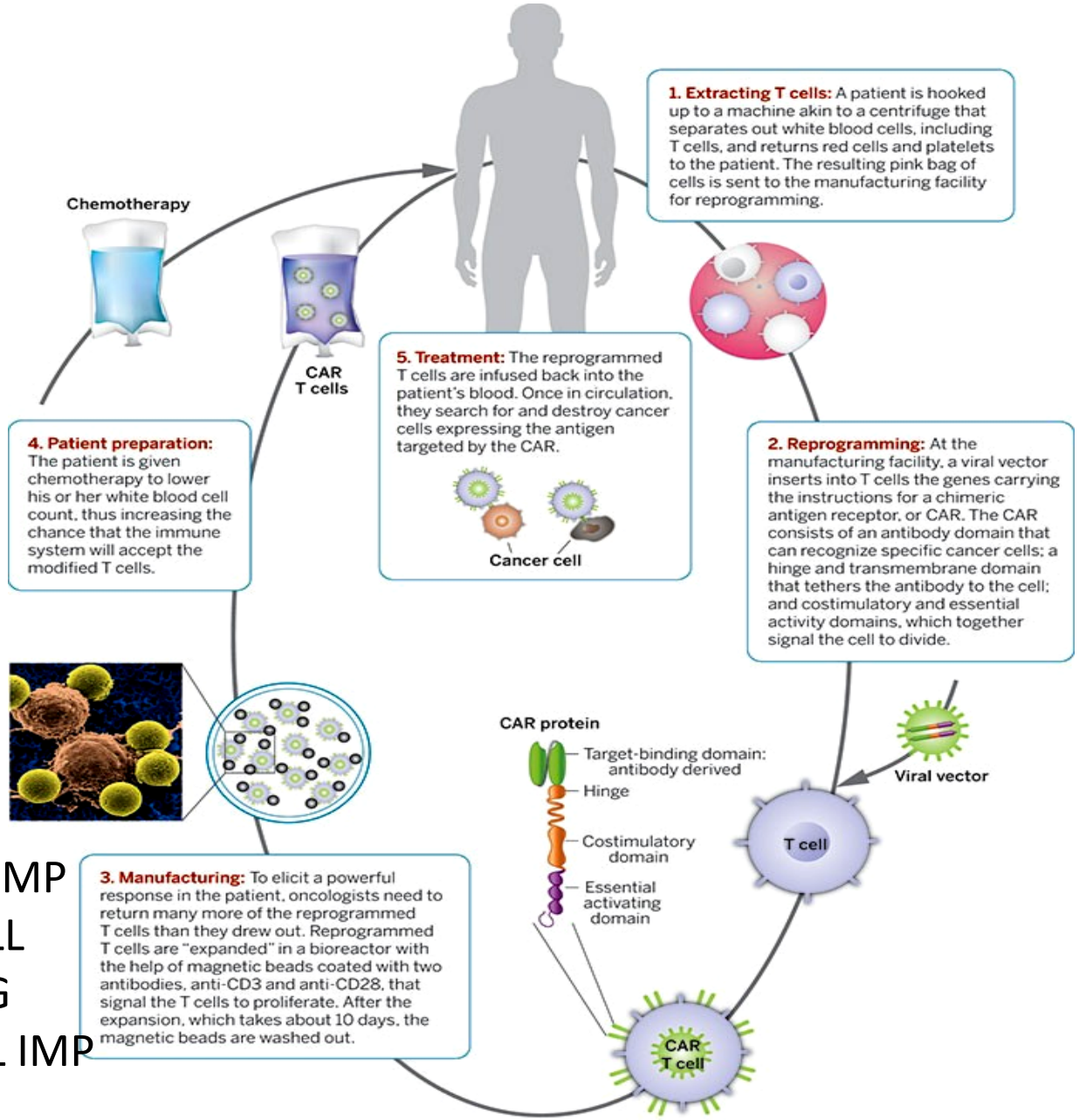
sk of Graft versus Host Disease vHD)

Many patients unable to get access to optimal CAR therapy

Inability to generate sufficient autologous PBMCs for optimal dosing

*Immunologically the reasons for the lack of optimal response are poorly understood

Complex manufacturing: HURDLES ON THE WAY TO CLINIC…



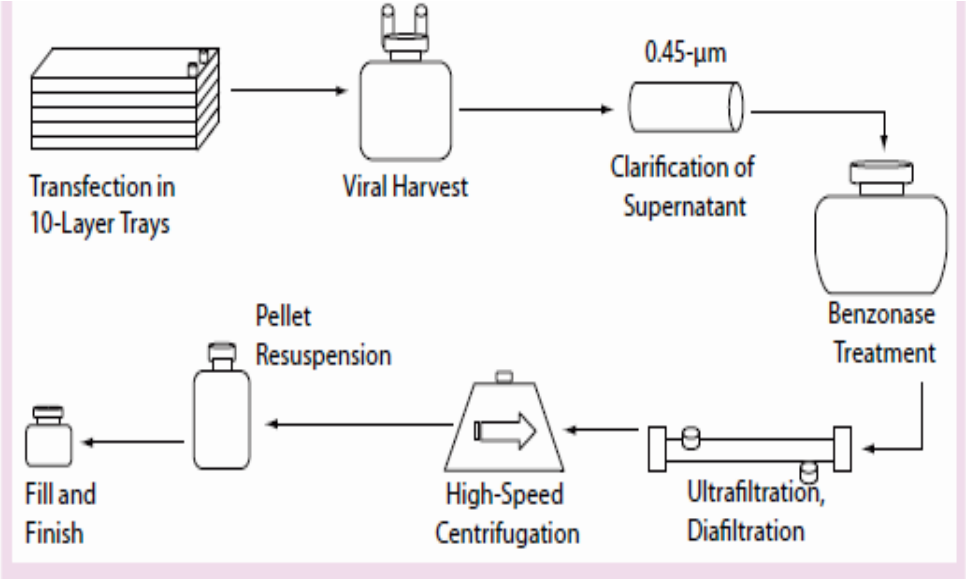
1. VIRAL DESIGN AND GMP PRODUCTION

2. LARGE-SCALE GMP TRANSDUCED-CELL MANUFACTURING AND QC ON FINAL IMP

Production of viral vectors

Current downside of viral vectors for CAR expression:

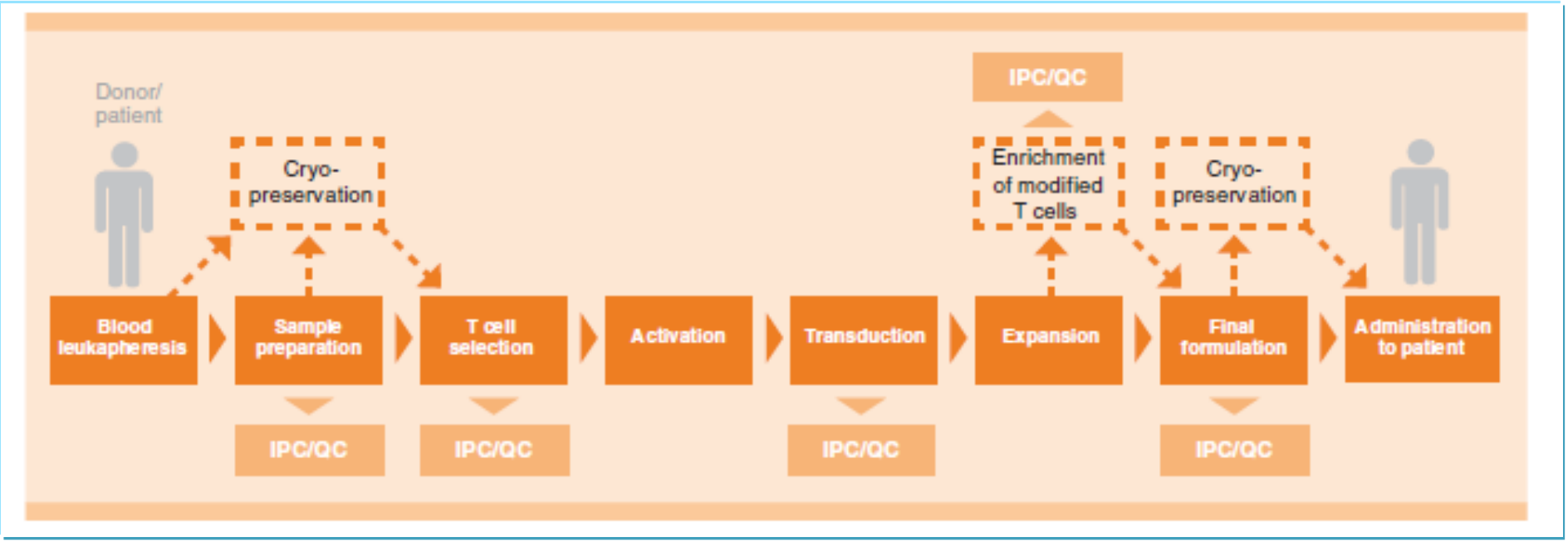
- Time-consuming (6 to 9 months)
- Skilled trained staff
- High Costs (600 th. up to 1 mil $) associated with a GMP-compliant production run of vector



from Ausubel L.J. (2012), *Bioprocess Int*, 10(2), 32-34

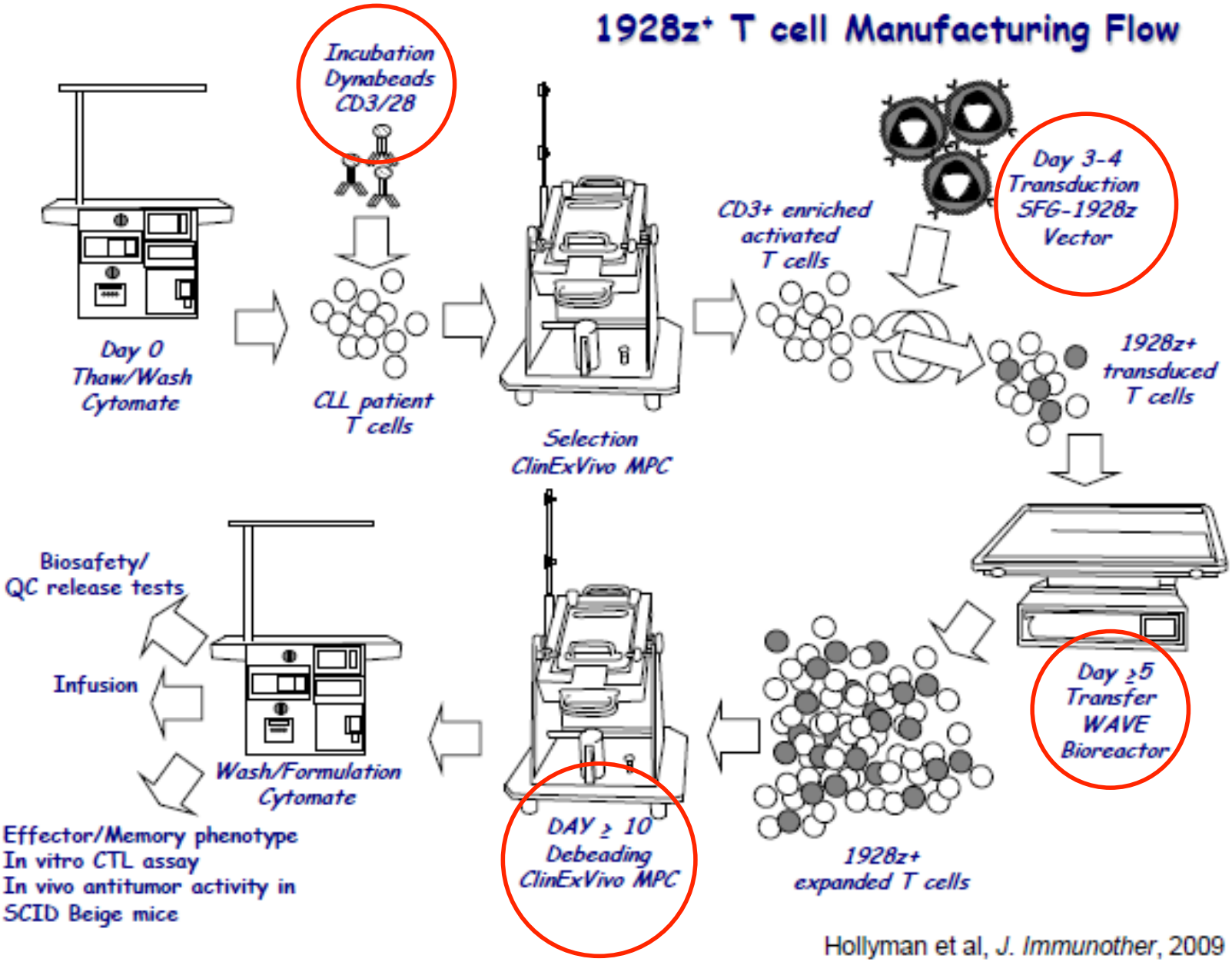
- Multiple and complex steps of manipulations using suitable cell lines to produce lentiviral vectors
- Large volume of viral sup to be harvested and finally ultrafiltered and filled and finished
- Complex QC testing on final cell product (Recombinant viral particles)

Work-flow for gene-engineered T-cell production



Leukapheresis: autologous, allogeneic, how many cells? How good?
T-cell selection: truly necessay? How easy and expensive?
Transduction: how? viral? Non viral?
Expansion: how easy? How long?
Final Formulation: cryopresevation! Transport! Bedsite infusion!

Expansion and transduction

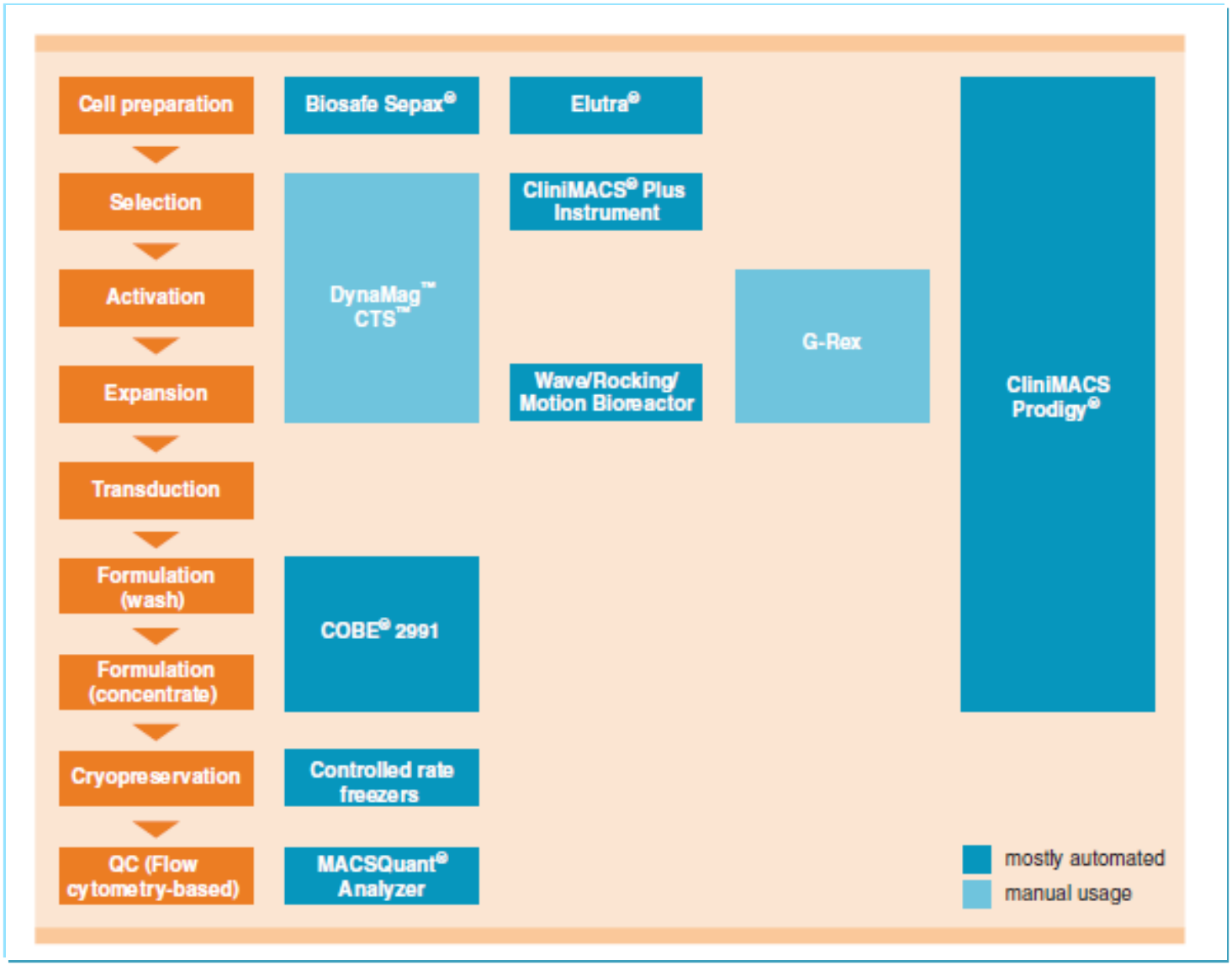


Hollyman et al, *J. Immunother*, 2009

Bioreactors for Viral Production, Transduction and for large Cell Expansion in suspension

1. Simplified approach
2. Less human handling
3. Fully automatic
4. Easily adaptable
5. Sterile
6. Authomatized cell final batching
7. COSTS!!??

Devices facilitating the clinical-grade manufacturing of engineered T cells



Closed viral transduction and expansion method

2043 Automated Lentiviral Transduction of T Cells with Cars Using the Clinimacs Prodigy
Gene Therapy and Transfer
Program: Oral and Poster Abstracts (ASH 2015)

Ulrike Mock*, PhD, Andrew Kaiser, PhD, Martin Pule, PhD, Adrian Thrasher, MD, PhD and Waseem Qasim, MBBS PhD
Cancer Institute, University College London, London, United Kingdom
CANCER INSTITUTE, UCL, London, United Kingdom
Research & Development, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Limitations and challenges of CAR T-cell approaches

Manufacturing challenges

Complex manufacturing

Regulatory complexities, impacting product development, logistics and timelines

Gene transfection related scale-up

Cellular stress associated with non-viral transfection hinders cell expansion and scale-up manufacturing

Viral transfection methods may impose commercial scale-up hurdles, due to complexity, time consuming manipulations and high costs

Current CAR-T Challenges

Clinical challenges

Non-response and relapse

Early relapses, despite high levels of initial complete remissions*

fety and toxicity

k of Graft versus Host Disease HD)

Many patients unable to get access to optimal CAR therapy

Inability to generate sufficient autologous PBMCs for optimal dosing

***Immunologically the reasons for the lack of optimal response are poorly understood**

NON VIRAL DNA PLASMID-BASED METHODS

Characteristics:

Non-immunogenic

Largely inexpensive to purify

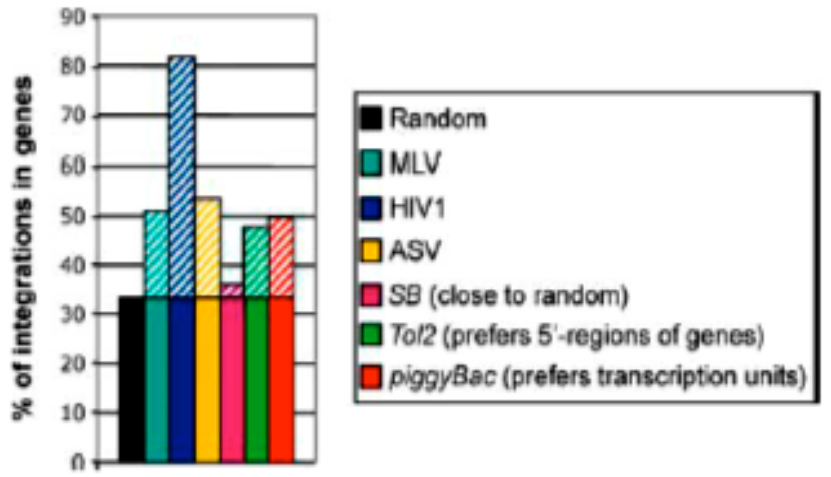
No hard constraints on sequences

No risk of contamination by infect

Random pattern of integration

Disadvantages:

Genomic insertion preferences of integrating vector systems



modified from Izsvak Z. (2010), *BioEssays*, **32**, 756-767

Low rates of integration of transgenes

Low rates of delivery to target-cell nuclei

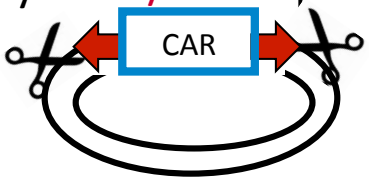
Needs "help" to get in to the nucleus (**transposase and nucleofection**)

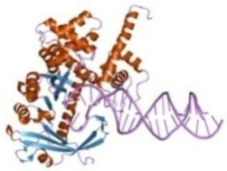
Transposons: an “easy” alternative to viral vectors for gene therapy

(collaboration with L. Cooper, MD Anderson, Houston, TX, USA)

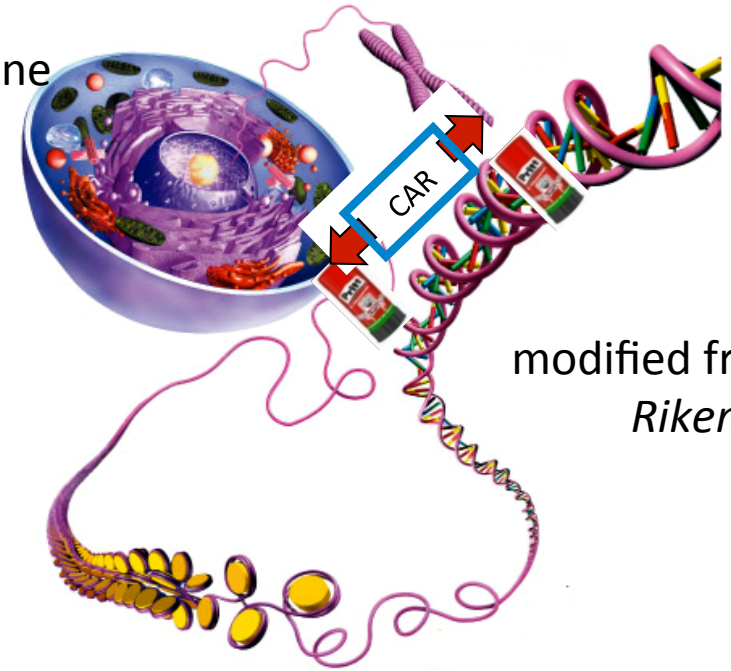
Sleeping Beauty (SB) transposon

The 1th plasmid contains CAR gene enclosed by SB IR/DR sequences





A 2nd plasmid contains the SB11 Transposase that cuts IR/DR allowing integration



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

Electroporation (**GMP-grade Amaxa Nucleofector**) uses an electrical pulse to create temporary pores in cell membranes

OPTIMIZATION: NUCLEOFECTOR

AMAXA Nucleofector™ technology

VARIABLES:

- VOLTAGE
- BUFFER
- DNA AMOUNT
- TARGET CELLS AMOUNT

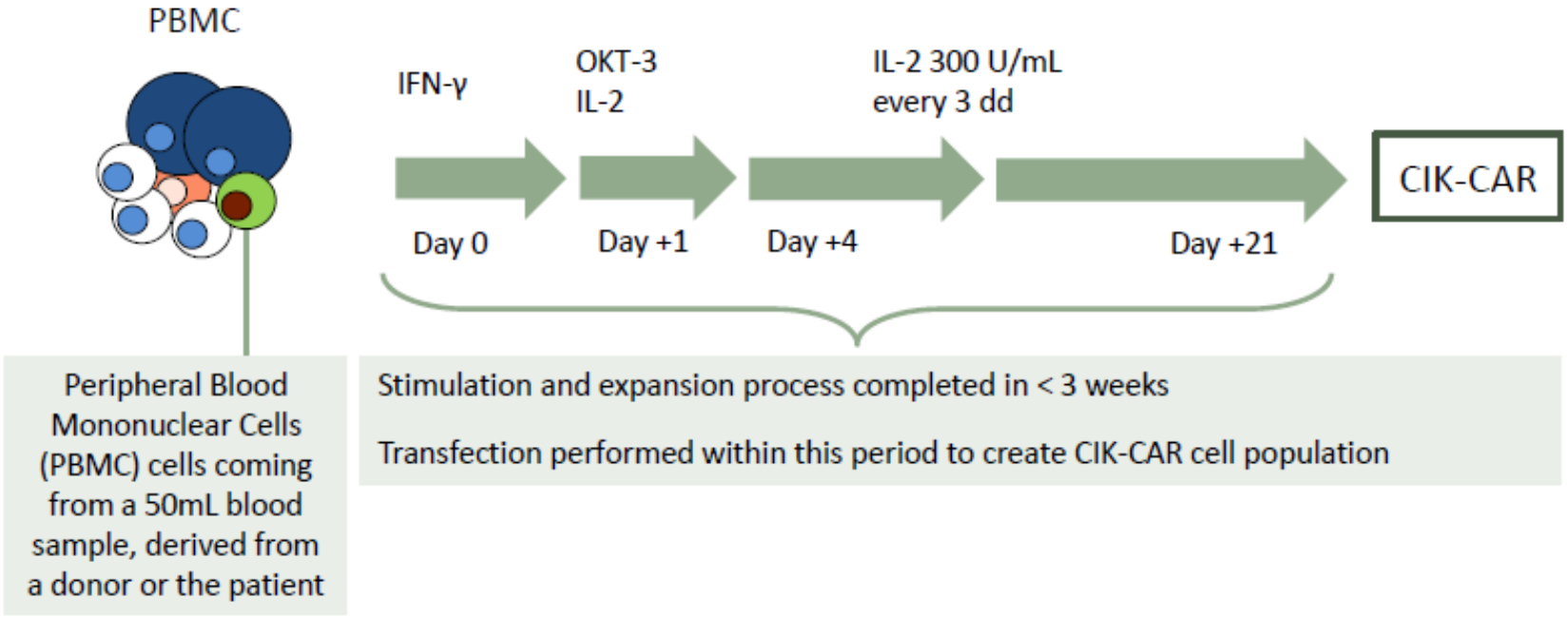
Clinical grade expansion of CIK cells modified by SB system

Effector T lymphocytes with acquired NK-like cytotoxicity,

produced *in vitro* under GMP conditions from PBMC in 21 days using only OKT3 antibody, IFN-g, IL-2.

enriched in $CD3^+CD56^+$CD1d-unrestricted NKT-T cells, which arise from $CD3^+CD56^-$ CIK cell progenitors

(Rambaldi *Leukemia* 2014)



- a non MHC-restricted NK-like cytotoxicity, negligible alloreactivity and minimal GVHD
- intrinsic capability of reaching leukemia-infiltrated tissues

(Linn *Journal of Biomed and Biotech* 2010, Sangiolo *Journal of Cancer* 2011)

Clinical experience with allogeneic CIK cells: feasible (even from the washouts of the bags containing the CB unit), safe and well tolerated (Rambaldi A, Biondi A , Biagi E, *Leukemia* 2014)

Immunotherapy for AML and ALL by a non-viral gene transfer

Clinical-grade modification of CIK Cells With CAR by non-viral SB gene transfer

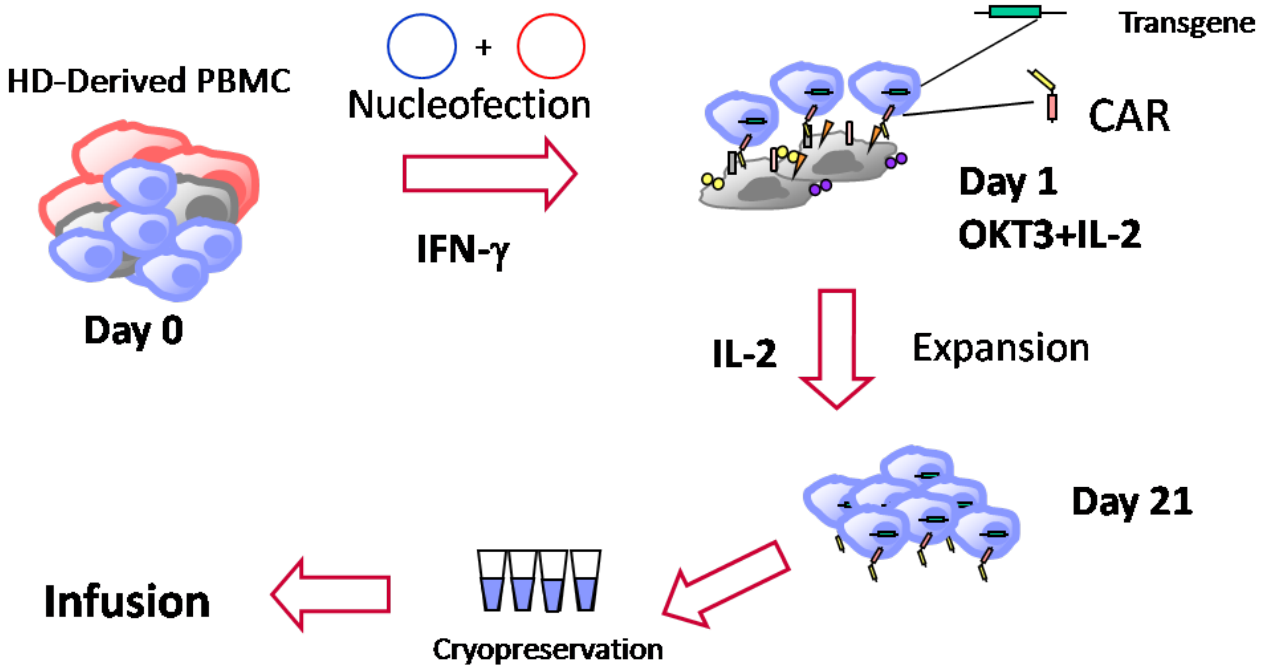
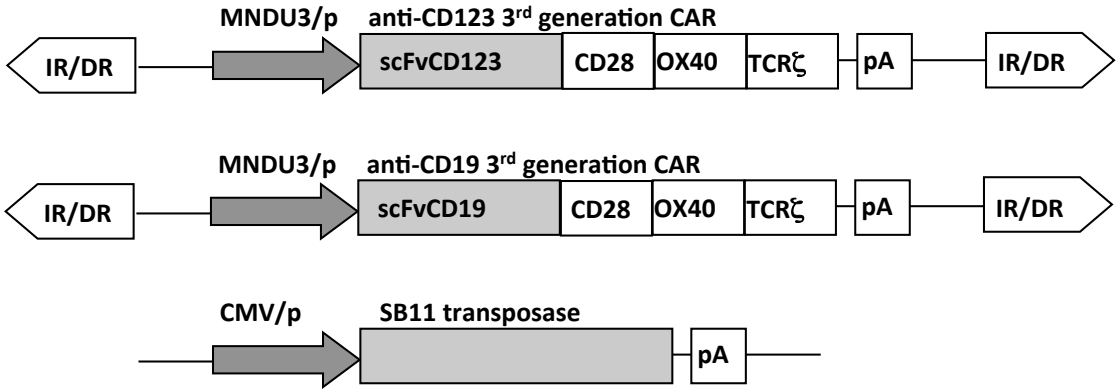


Diagram of the SB transposon and transposase constructs used in this study



Pizzitola I , Biagi E, Leukemia 2014,
Tettamanti S, Biagi E, BJH, 2013
Giordano G, Biagi E, Blood, 2011
Marin, V, Biagi E Haematologica, 2010
Marin V, Biagi E, Exp Haem, 2007

Significant potential manufacturing advantages

Simplified Approach

- No need for apheresis; 50mL donor blood or cord blood sample suffices for PBMCs
- Single-step cell stimulation method
- No purification step needed

Reduced Regulatory Complexity

- Less regulatory complexities for non-viral transfection processes
- Potentially less expensive and less complex handling procedures

Overcomes Cellular Stress

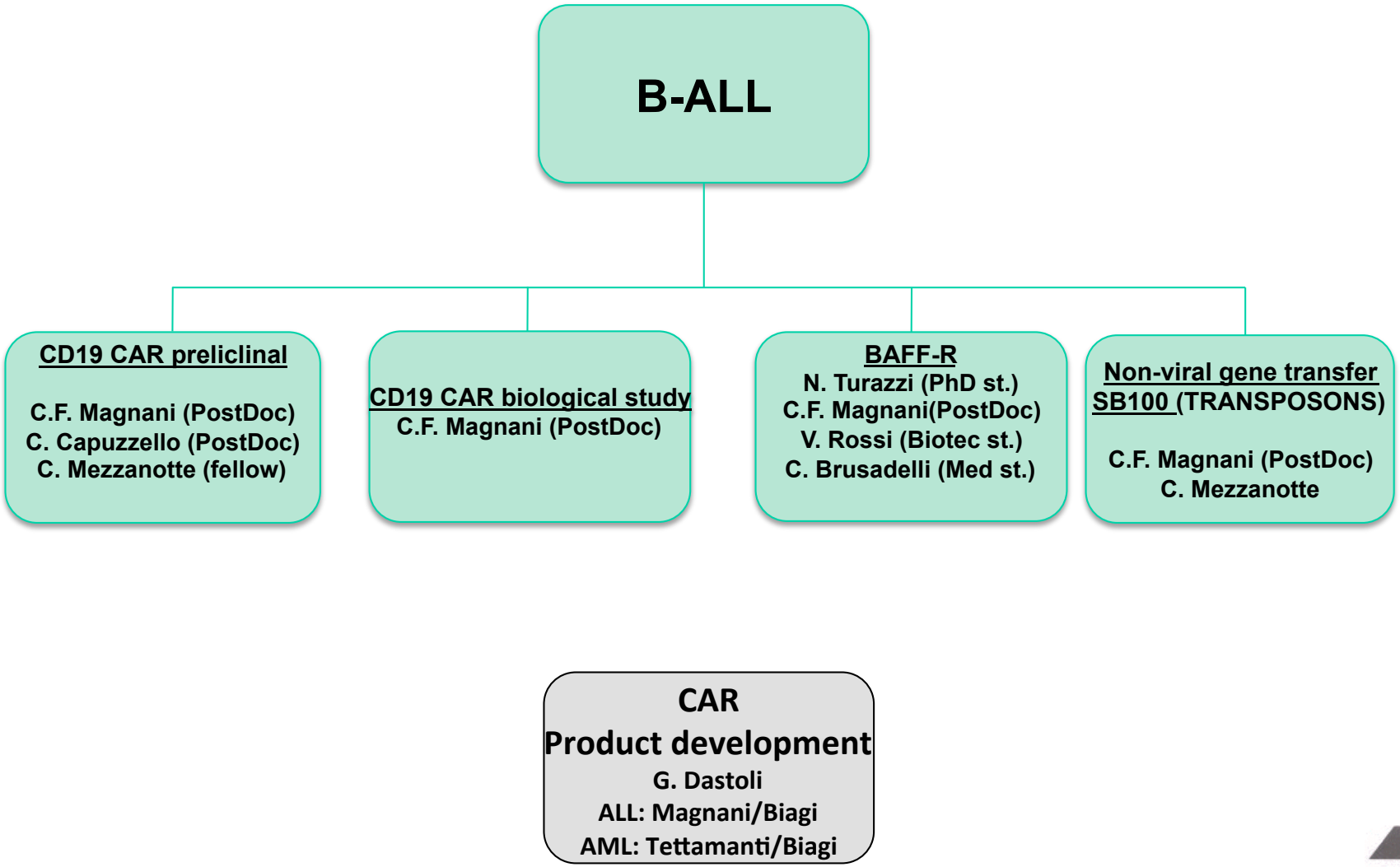
- Technology rescues cells from cellular stress, generally caused by non-viral transfection methods
- Approach optimizes cell expansion for commercial scale-up manufacturing

Reduced Mutagenesis Risk

- Safer compared to viral vectors that may display undesired insertion-site preferences
- Viral transfection has an increased probability to deregulate targeted genes expression

Our unique manufacturing process provides a simple, efficient, and effective alternative to viral-vector based CAR-T technologies

CARS in ALL: state of the art and future perspectives



Aim of the work

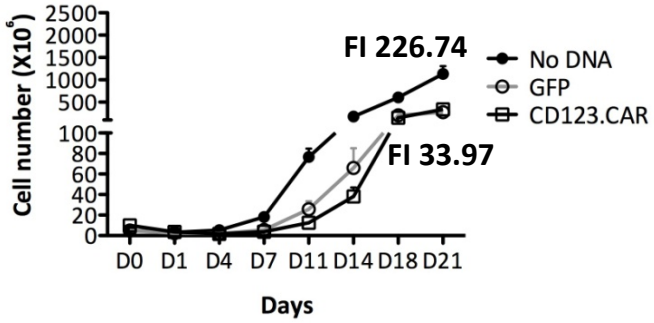
Pre-clinical evaluation of CD19.CAR CIK cell therapy

- The impact of clinical-grade production process on the functionality of CD19.CAR CIK cells
- Efficacy of the treatment in patient-derived xenograft model of ALL
- General toxicity and biodistribution

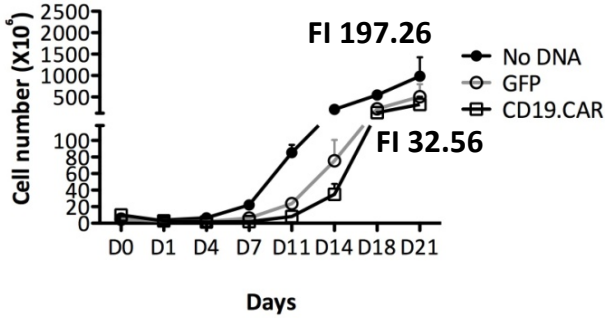
Expansion and phenotype of CIK cells modified by SB system

Proliferation of CIK cells nucleofected in the absence of DNA, with GFP, and with transposon encoding CD123.CAR or CD19.CAR

CD123.CAR

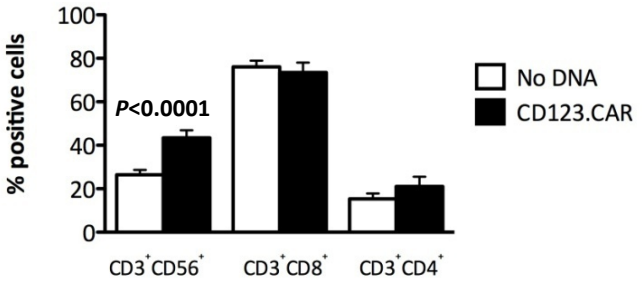


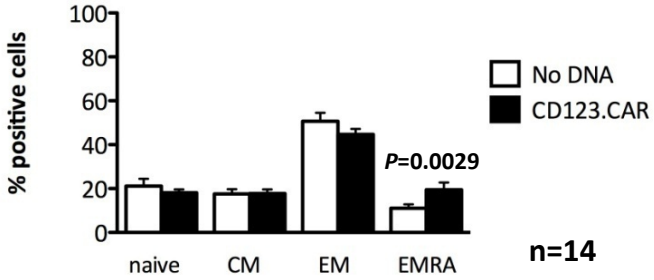
CD19.CAR



CD56/CD8/CD4 and memory phenotype of CD3⁺ CIK cells

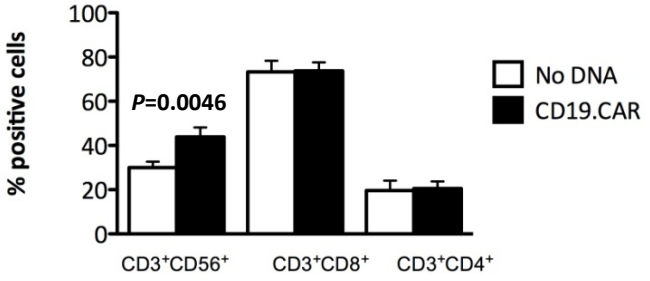
CD123.CAR

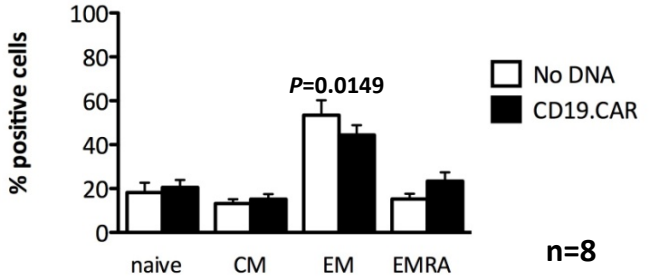




n=14

CD19.CAR



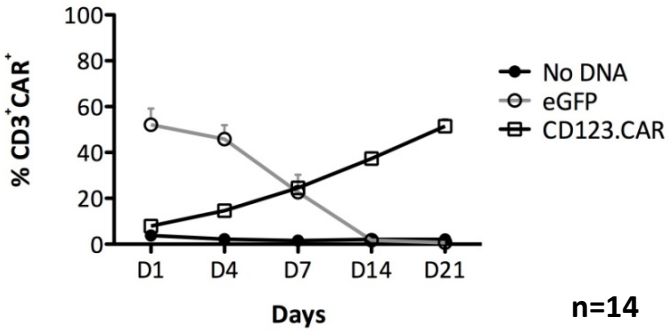


n=8

Expansion and phenotype of CIK cells modified by SB system

Modification of CIK cells determined overtime by flow cytometry

CD123.CAR



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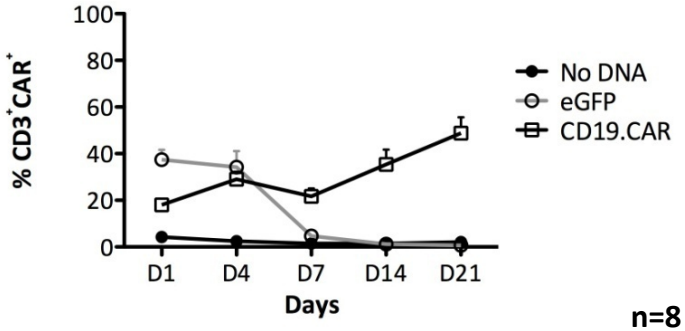
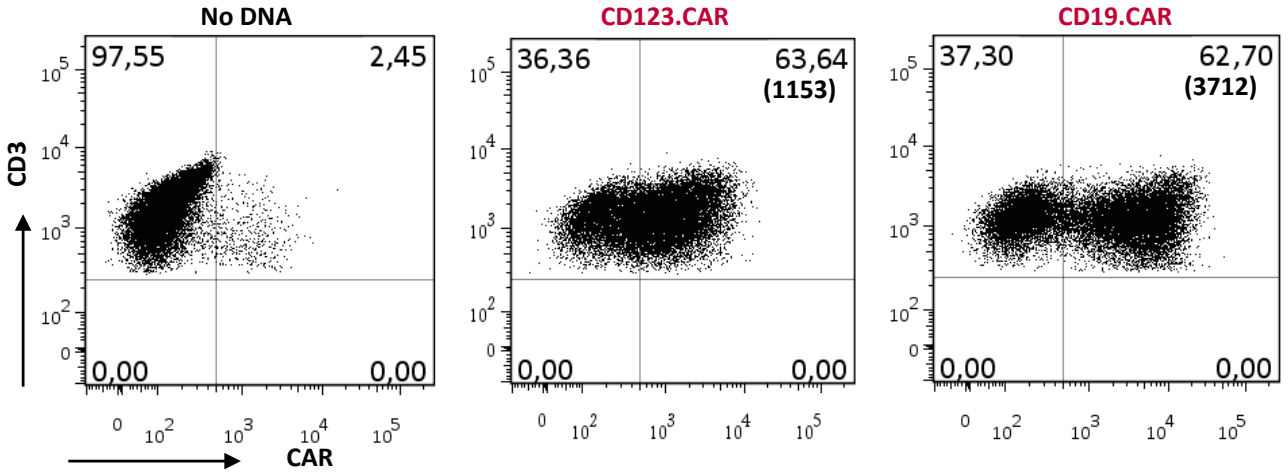
CD19.CAR



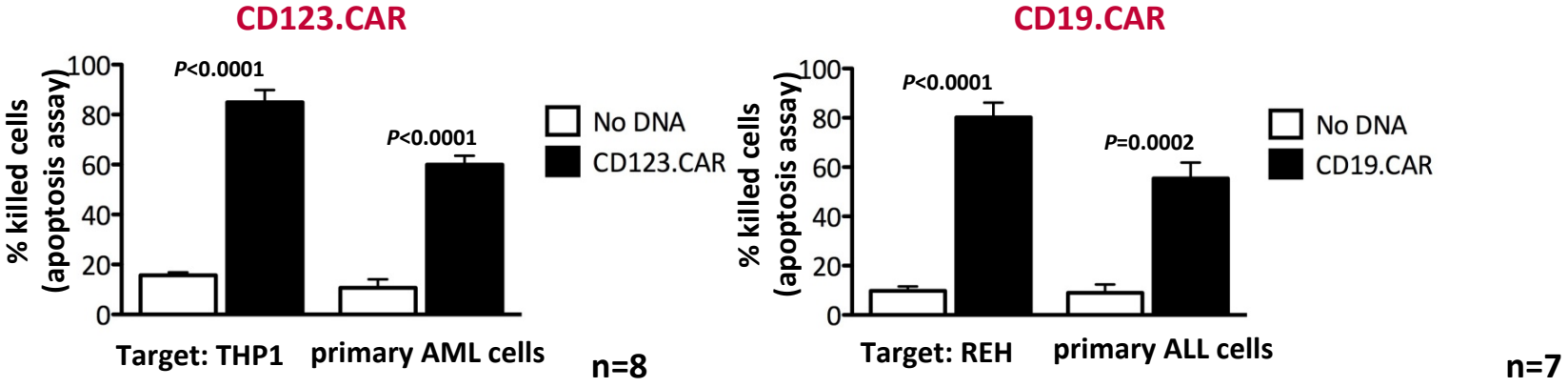
n=8

CAR expression of CD3+ CIK cells (d21)

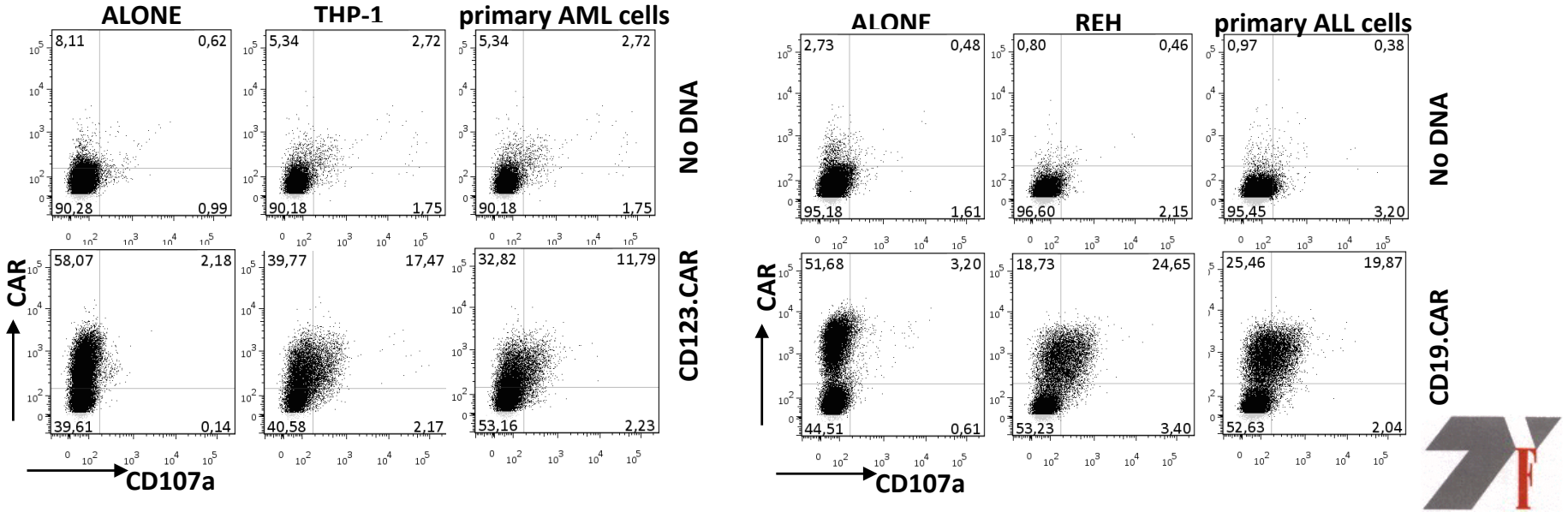


SB encoding CARs redirects CIK cells towards leukemia

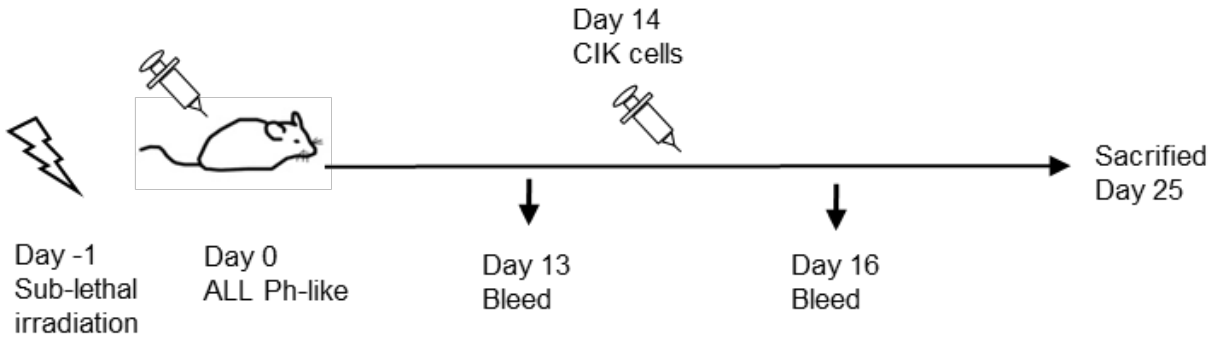
Cytotoxic activity determined by apoptosis detection

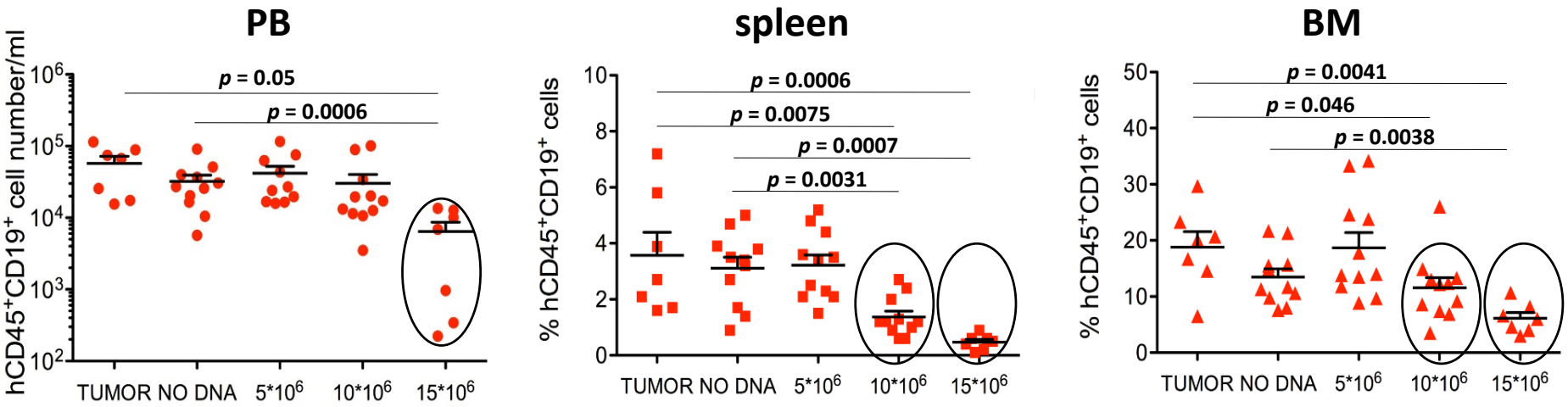


Cytotoxic degranulation measured by expression of CD107a/LAMP1



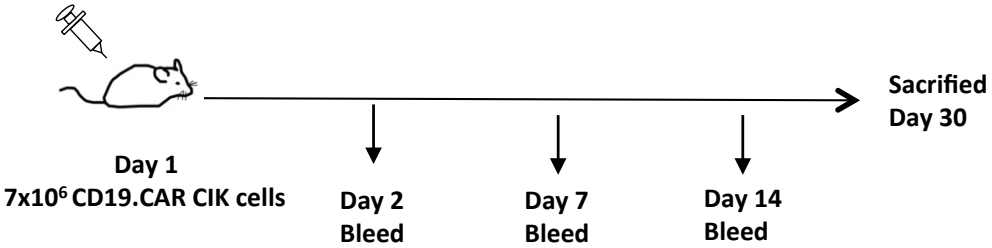
Anti-leukemic effector function of CD19.CAR CIK cells in Patient-Derived Xenograft model (dose dependent)

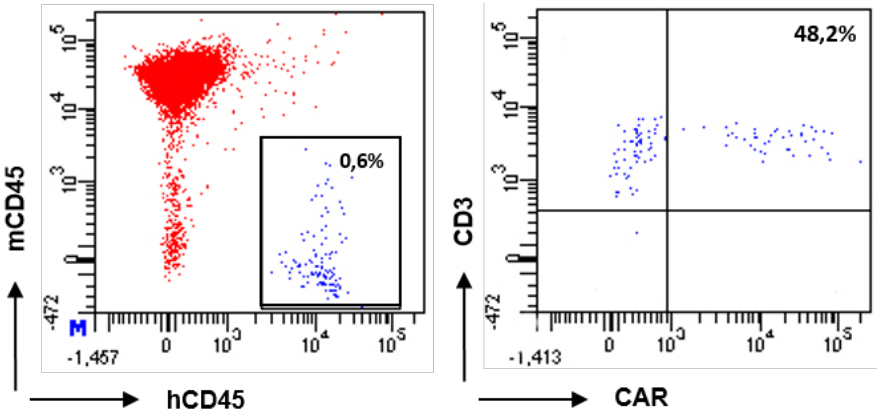




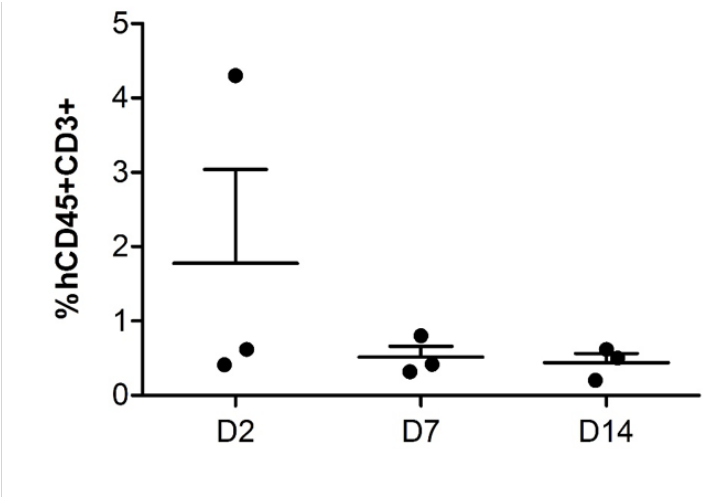
Dose of 15x10^6 CARCIK-CD19 CAR+ is the most active by dose escalation

Biodistribution and Toxicity in GLP conditions: 1 single dose 15x106 CARCIK-CD19 CAR+, 2 months study

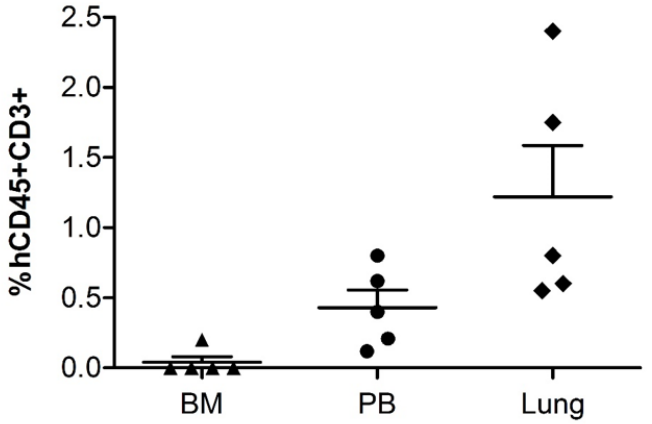




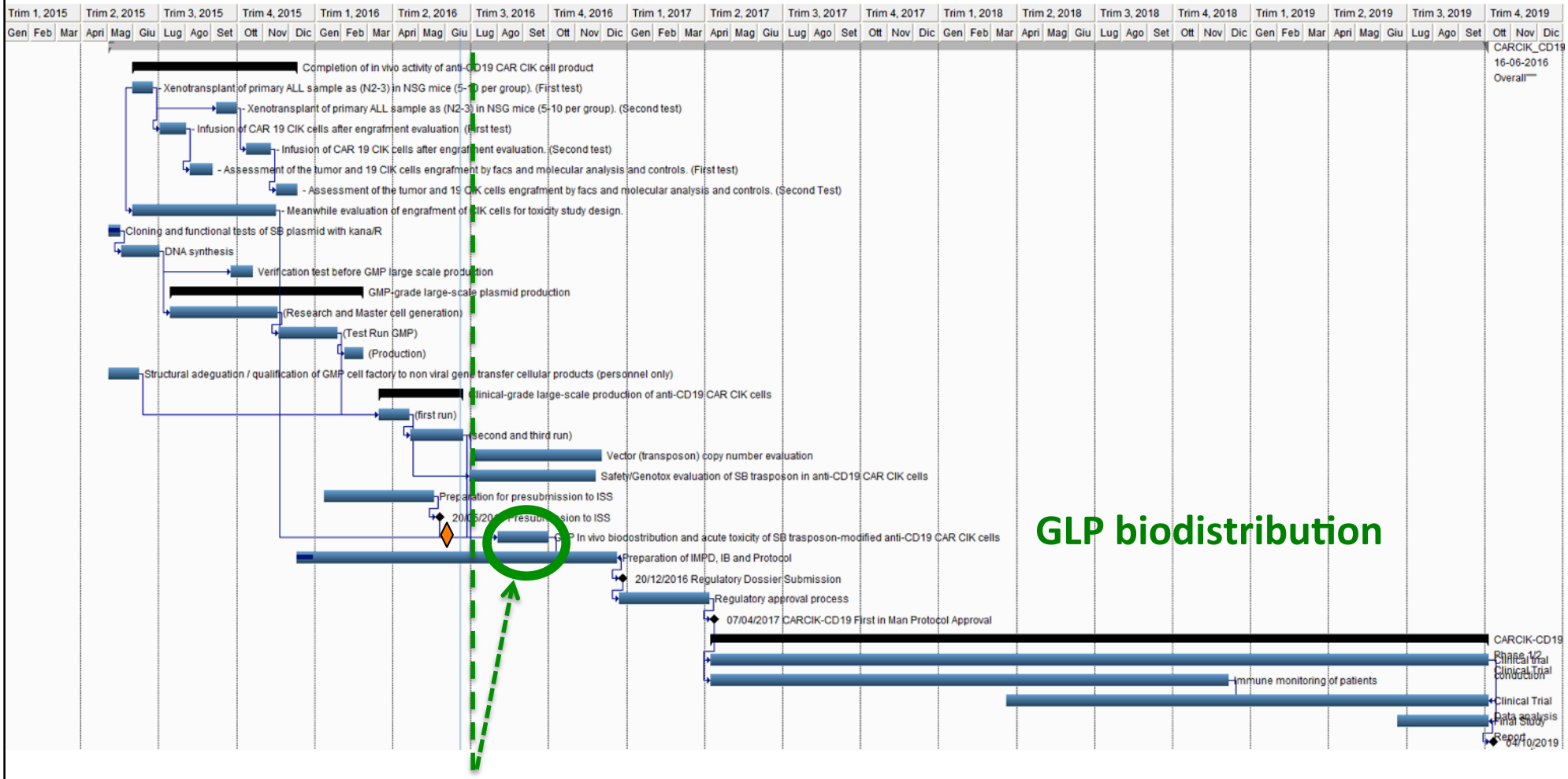
Peripheral Blood



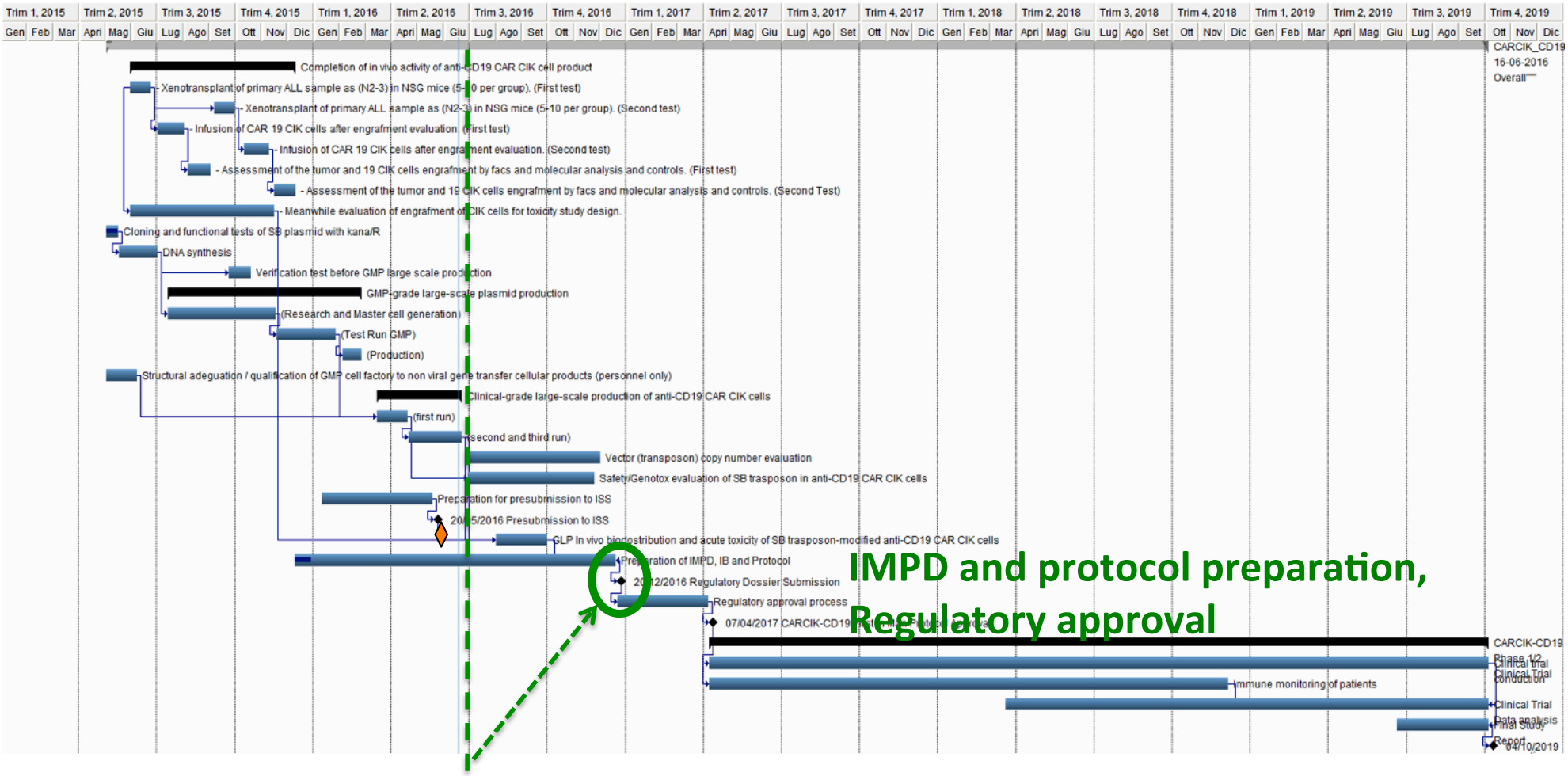
Sacrifice (Day 30)



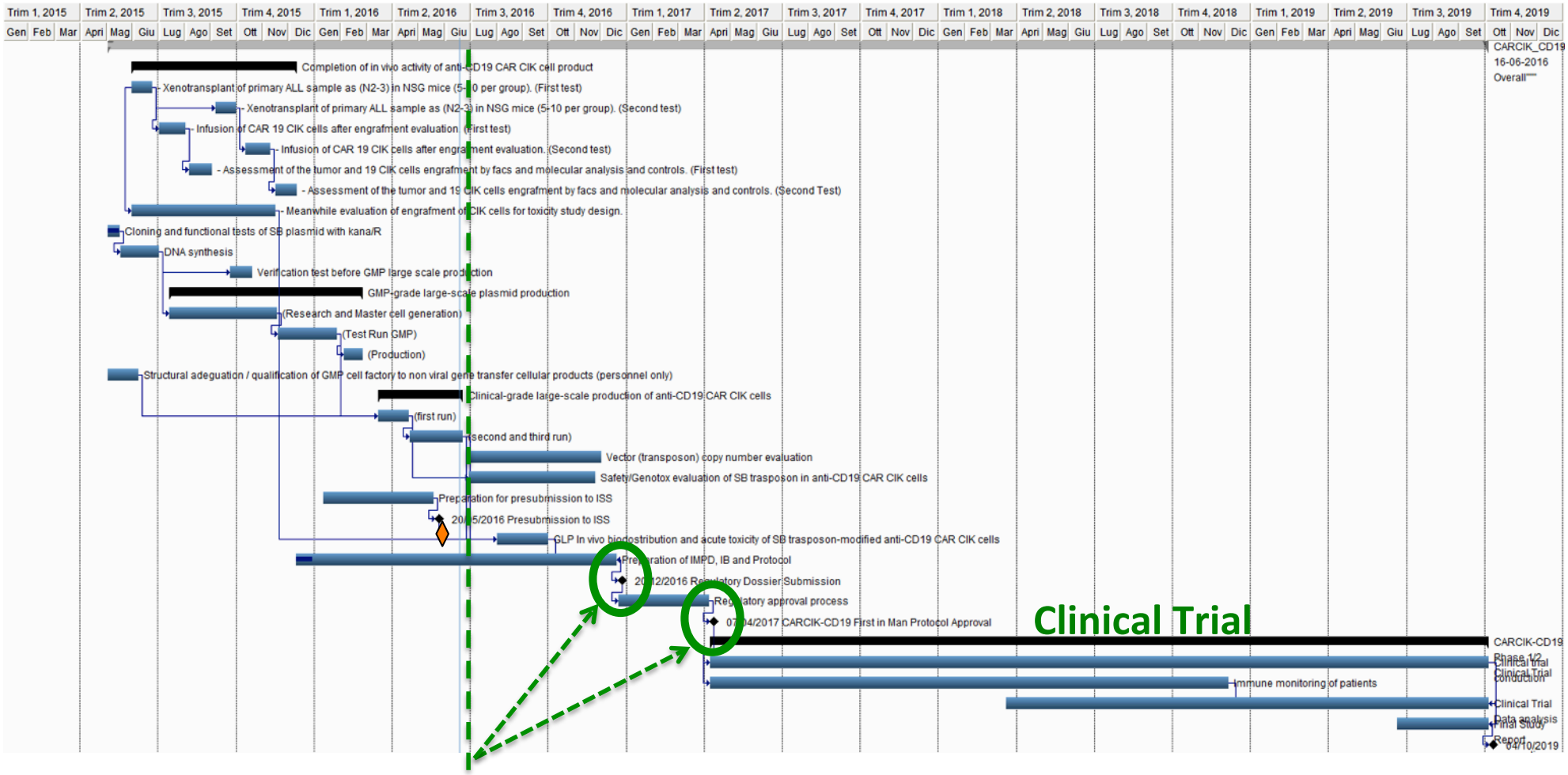
CARCIK-CD19 Development: action plan



CARCIK-CD19 Development: action plan



CARCIK-CD19 Development: action plan

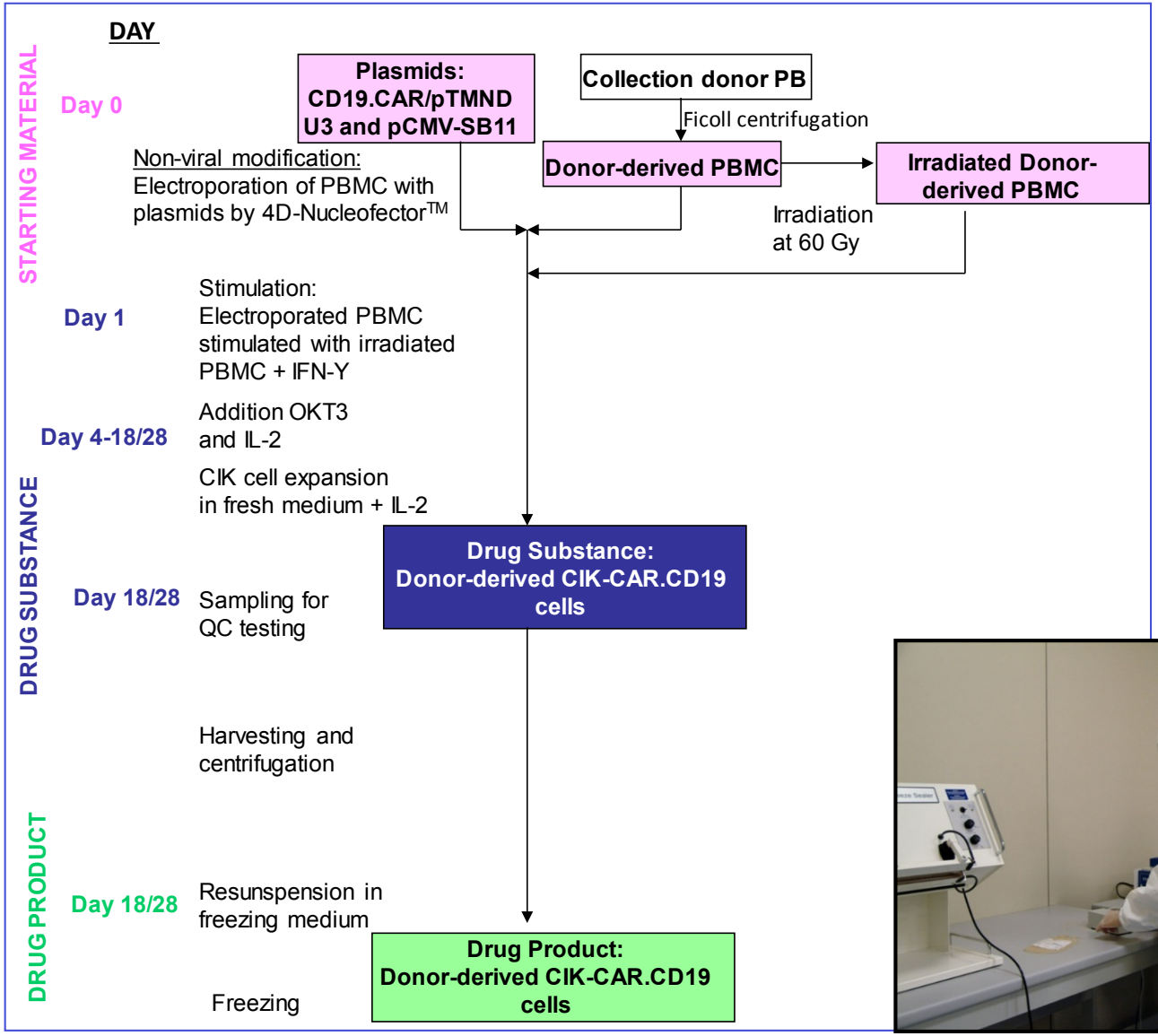


Cell and non-viral gene therapy factory
"Stefano Verri" ASST Monza- Ospedale San Gerardo

Sistema Socio Sanitario
Ospedale San Gerardo
Regione Lombardia
ASST Monza

GMP MANUFACTURING OF PTG-CARCIK-CD19

In collaboration with Gaipa G. and Verri's staff



Agenzia Italiana del Farmaco
AIFA

ALLEGATO 2

SCOPO DELL'AUTORIZZAZIONE

Denominazione ed indirizzo del sito: AZIENDA OSPEDALIERA S. GERARDO DI MONZA-LABORATORIO PER LA TERAPIA CELLULARE E GENICA STEFANO VERRI - VIA PERGOLESI, 33 , 20052 MONZA(MB)

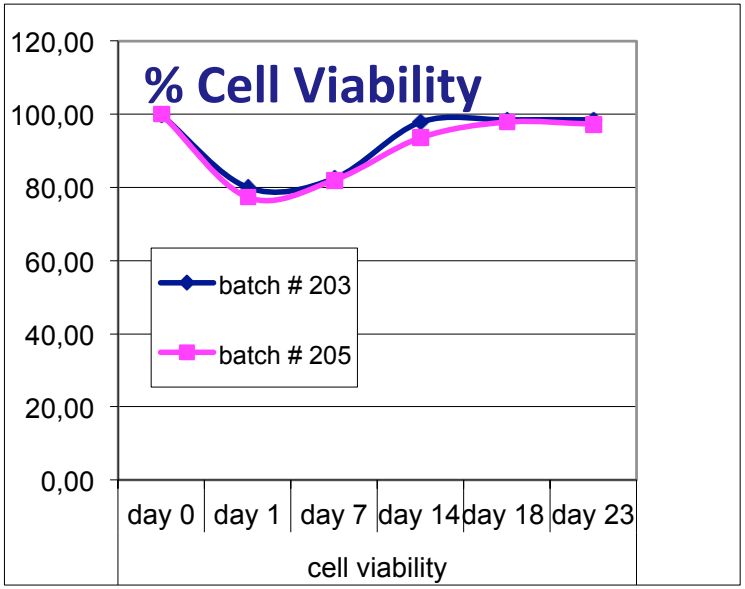
Prodotti Medicinali Umani

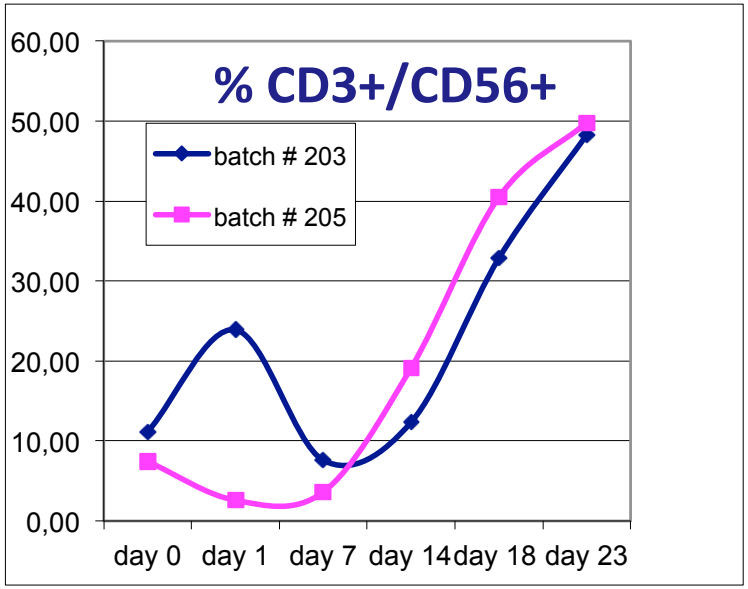
Attività Autorizzate
Attivita' di Produzione (Parte 1)
Parte 1 - ATTIVITA' DI PRODUZIONE PER MEDICINALI SPERIMENTALI

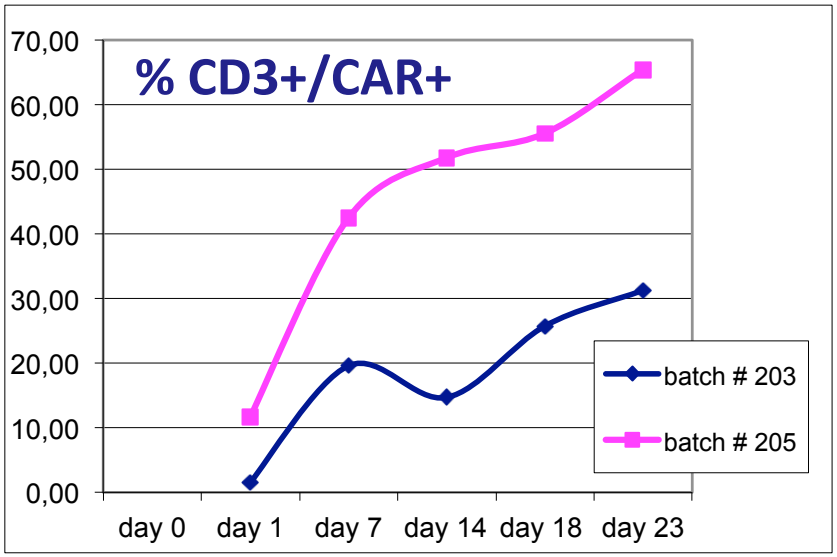
1.1	Prodotti sterili	
	1.1.1 *Preparati in asepsi*	
		1.1.1.4 Liquidi di piccolo volume
1.3	Prodotti medicinali biologici	
	1.3.1 *Prodotti medicinali biologici*	
		1.3.1.3 Prodotti per terapia cellulare
		1.3.1.4 Prodotti per terapia genica

Restrizioni o chiarimenti inerenti le operazioni di produzione
1.1.1.4 Liquidi di piccolo volume: Solo prodotti per terapia cellulare e per terapia genica;
1.3.1.3 Prodotti per terapia cellulare: Linfociti e cellule staminali;
1.3.1.4 Prodotti per terapia genica: Solo cellule geneticamente modificate con vettori non virali;

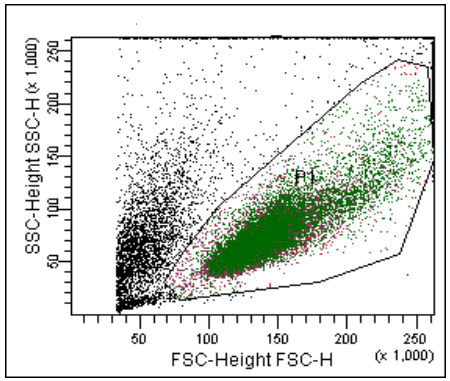
Summary results of two Large GMP production runs of PTG-CARCIK-CD19 (third run still ongoing)

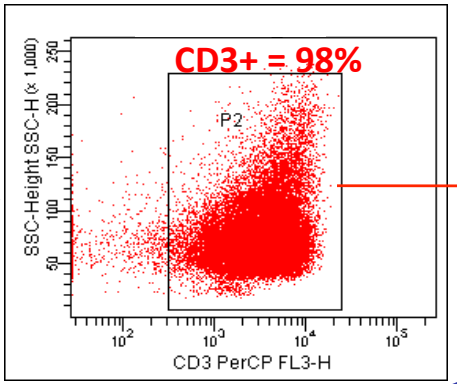


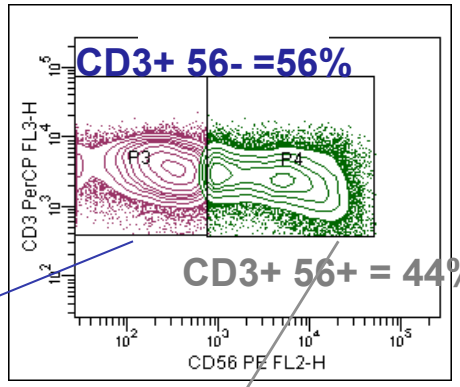


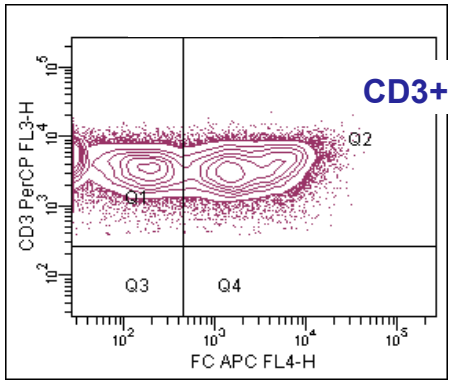


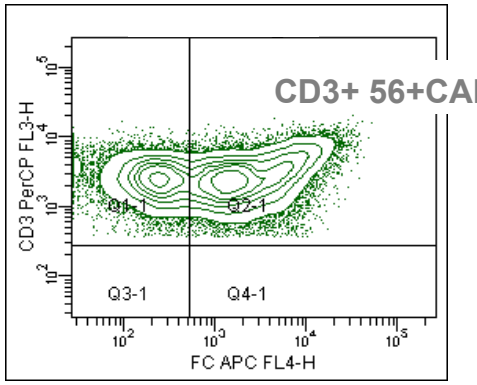
Composition of PTG-CARCIK-CD19 Drug Product

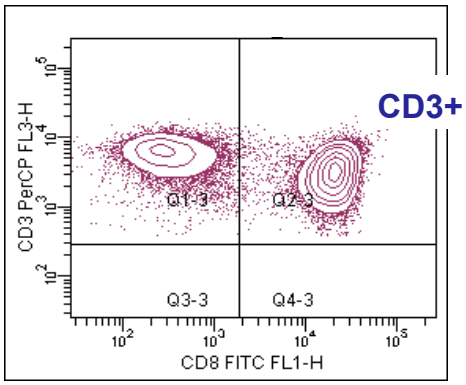


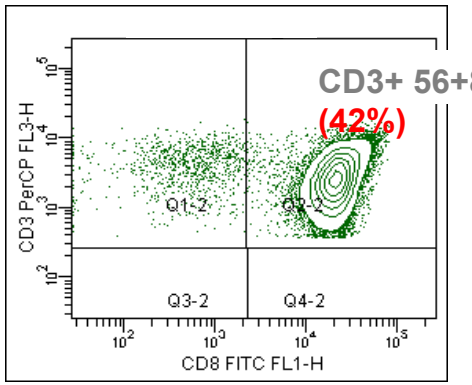












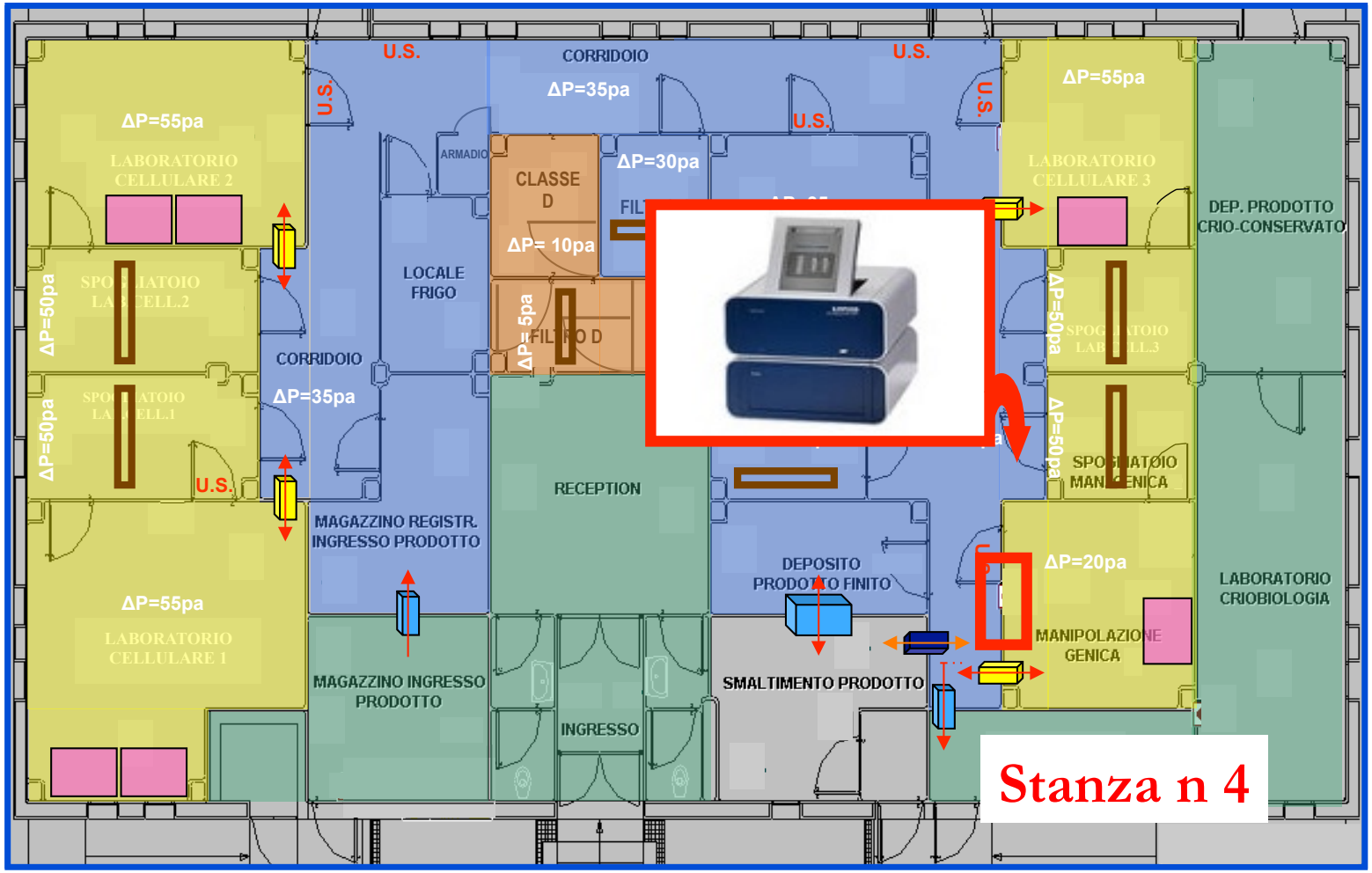
Release criteria for PTG-CARCIK-CD19

TEST	SPECIFICATION
Sterility - Bacterial and fungal*	Sterile
Absence of endotoxin*	< 0.5 EU/mL
Mycoplasma*	Absent
Viability	≥ 80%
Immunophenotype: CD3+/CAR + CD3+/CD56+	 ≥ 20% of the $CD3^+$ cells ≥ 30% of the $CD3^+$ cells
Cytotoxicity (Apoptosis/ Necrosis)	≥ 25% lysis of the $CD19^+$ cell lines (E:T ratio 5:1)
Vector Copy Number (Q-PCR)	<5
SB11 Detection (Q-PCR)	Under limit of detection

…Cells as “Biological Drugs”…

From cell therapy to non viral gene therapy...

Cell factory Stefano Verri



Non classificato Classe D Classe C Classe B Classe A

Conclusions and Future Directions

Limitations and challenges of CAR T-cell approaches: the implementation of manufacturing, transfection method and clinical feasibility will increase the availability of these therapies

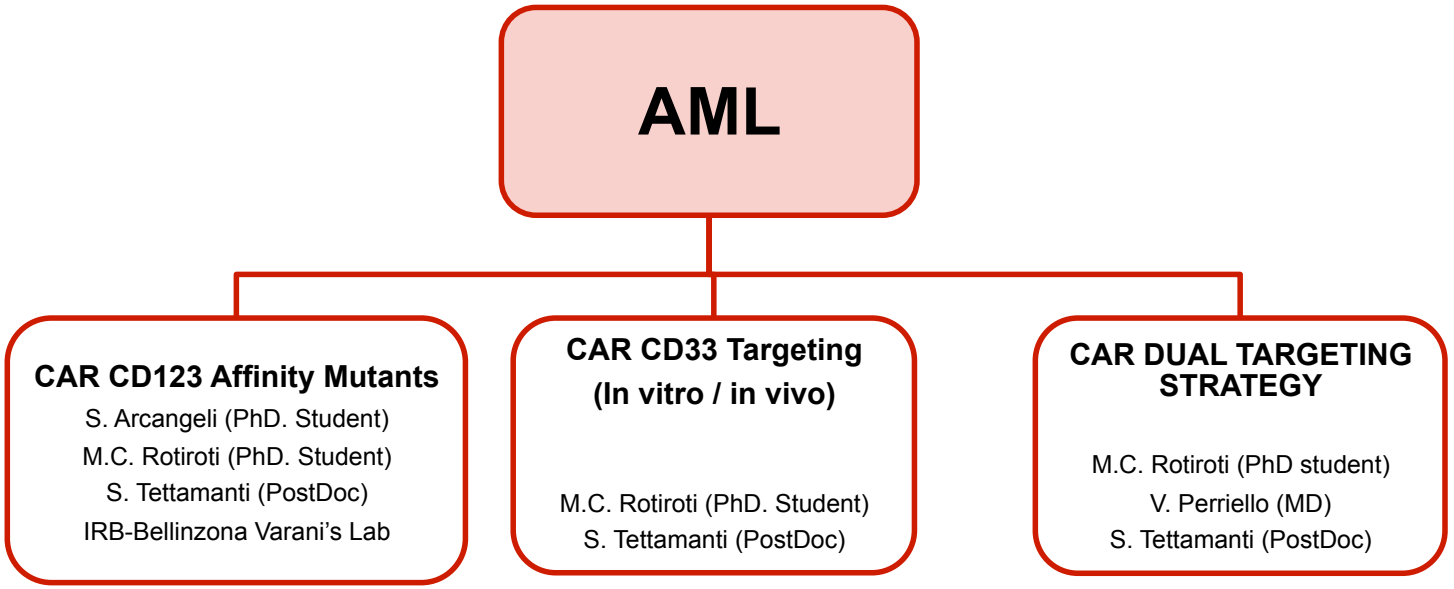
Conclusions

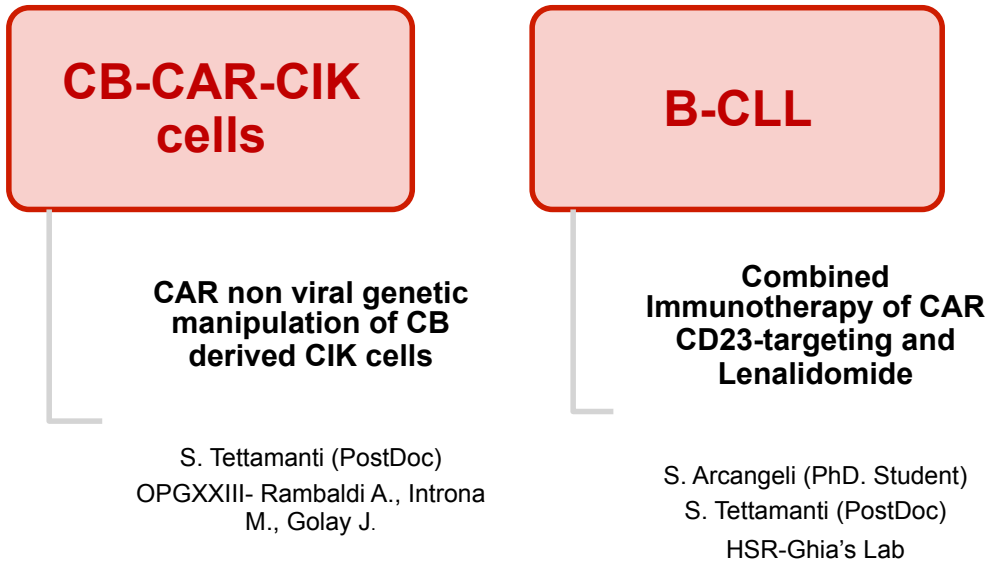
- **Non-viral** CIK-CAR approach may provide therapeutic benefit to a **broader patient population** than CAR-T approaches
- CIK-CAR **manufacturing** process as a **simple, efficient and effective** alternative to viral-vector CAR-T cells
- CIK-CAR cells exhibited **stable CAR expression, efficient cell expansion, tumor cell killing**
- **CD19.CIK-CAR platform** as a phase 1 **proof of concept** within 1 year (**sponsored research agreement**)

Other platform

- **AML targeting**: Insertion of iCasp9 suicide gene in CD123.CAR//CD33.CAR construct; additional new target antigens (TIM-3); affinity mutants
- **CLL targeting**: to apply non-viral platform to CD23.CAR

CARS in AML: state of the art and future perspectives





Acknowledgments

Claudia Capuzzello

Claudia Brusadelli

Valentina Rossi

COMITATO STEFANO VERRI
per lo studio e la cura
della leucemia

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

LA Cooper

Prof. Andrea Biondi

Grazia Fazio, PhD

Gianni Cazzaniga

UNI BASEL

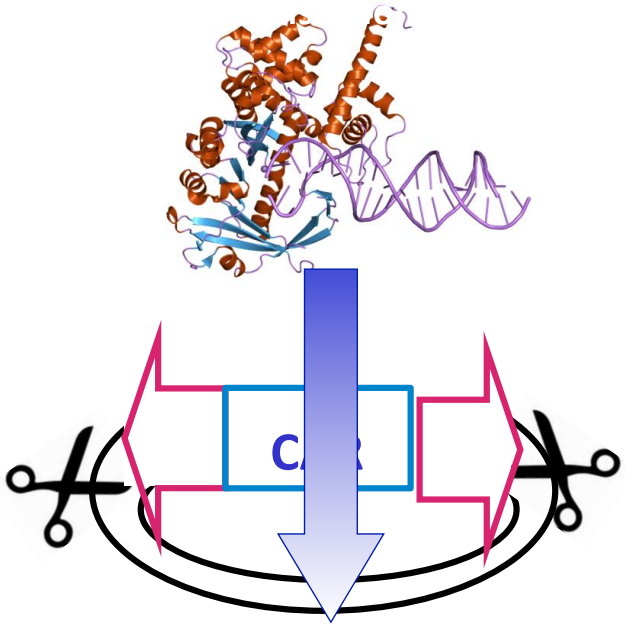
Prof. Dr. Antonius G. Rolink

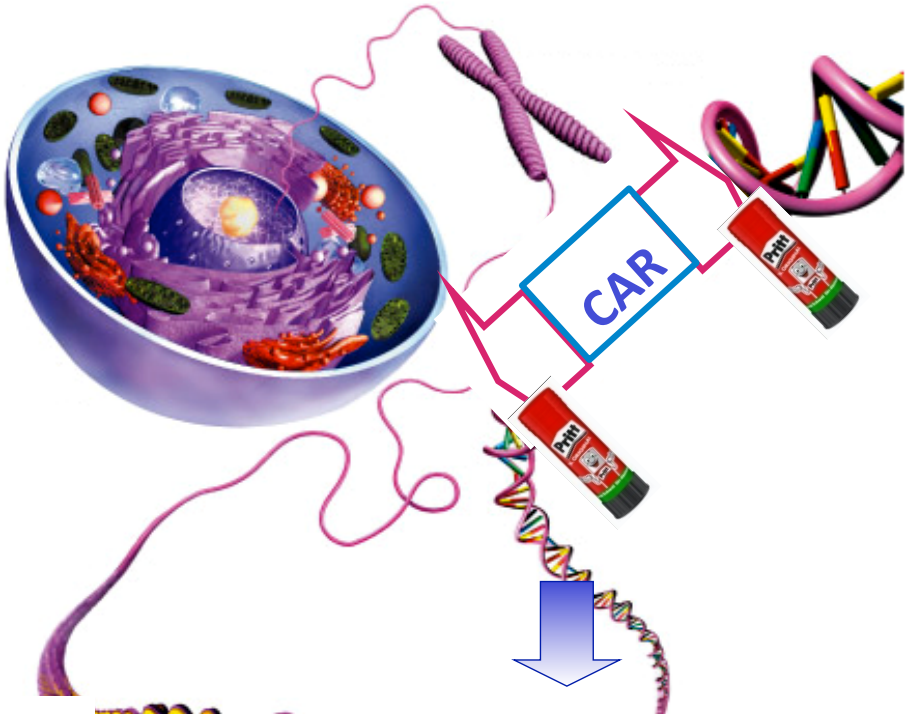
Montini Eugenio
Benedicenti Fabrizio
Andrea Calabria
Biasco Luca
Aiuti Alessandro

Daniela Belotti
Benedetta Cabiati
Stefania Cesana
Giada Matera
Valentina Colombo
Arianna Incontri
Giuseppe Gaipa (QP and QA)
Ettore Biagi (Medical Director)

How Sleeping Beauty works: the SAFETY issues

Transposase





Assessment of loss of expression of episomal transposase at day 21

Q-PCR analysis of expression of transposase

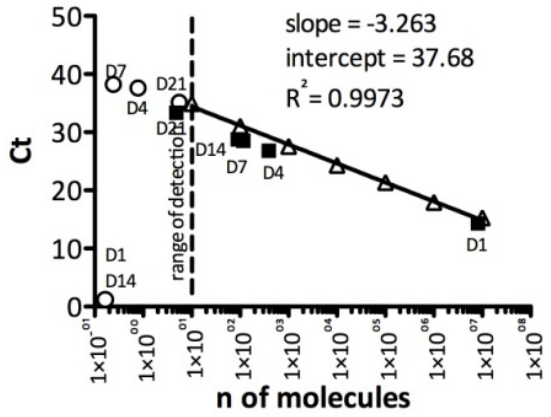
Identification of pattern of transposon insertion near cancer genes

Integration analysis

Safety profile of gene therapy by SB: transposase evaluation

Assessment of loss of transposase during the differentiation

Donor 1



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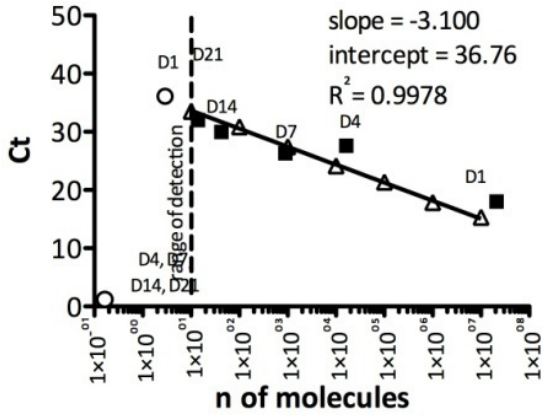

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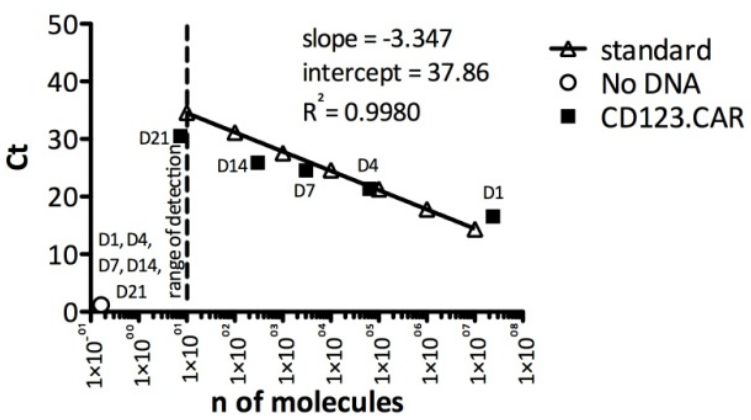
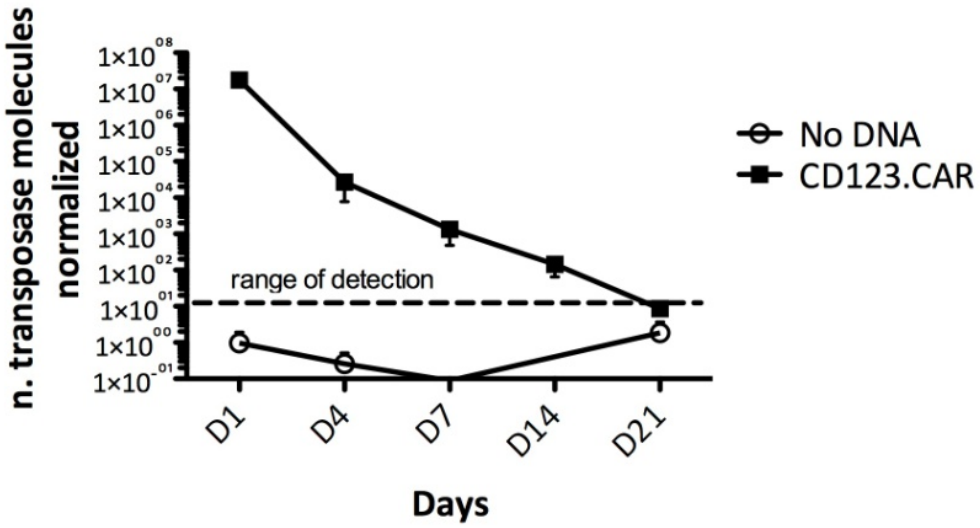
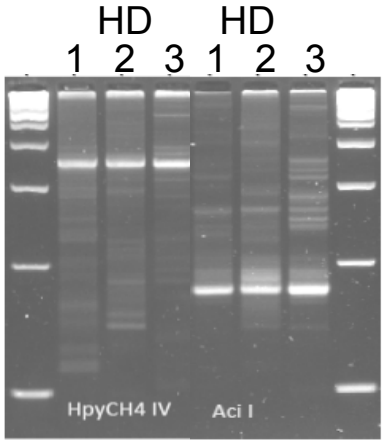


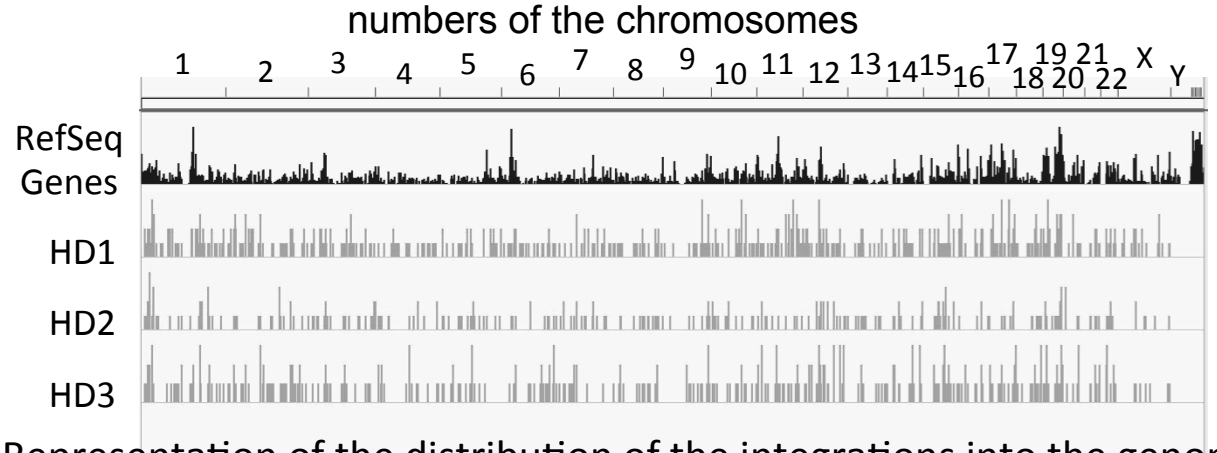


Safety profile of gene therapy by SB: Integration analysis

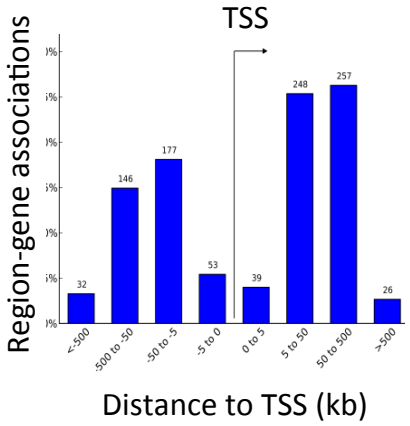
LAM-PCR and Next-generation Sequencing (collaboration with Montini E., TIGET)



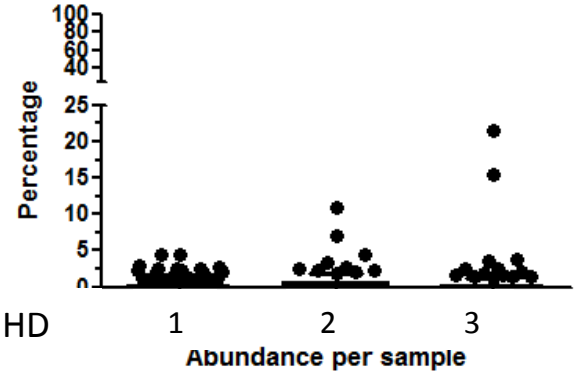
Spreadex gels



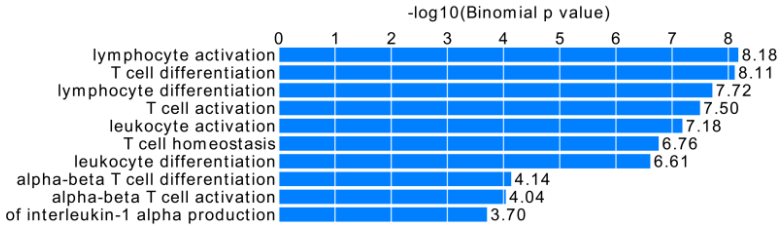
Representation of the distribution of the integrations into the genome



% of the sequence reads per integration site



Gene Ontology
Biological process



The integration sites (IS) are **distributed along the whole genome** with comparable frequency, **without preferences for gene dense regions and gene promoters and no common integration sites (CIS)** → safety