GIIMA
GRUPPO ITALIANO INTERDISCIPLINARE MANIPOLAZIONE E AFERESI PER TERAPIE CELLULARI

IX CONGRESSO NAZIONALE GIIMA

30 NOVEMBRE 2022
Aula San Raffaele
Ospedale San Raffaele - Milano

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Università Vita Salute San Raffaele
SR-Tiget, IRCCS Ospedale San Raffaele

Terapia genica delle emoglobinopatie: focus sulla beta-talassemia

UNIVERSITÀ VITA-SALUTE SAN RAFFAELE UniSR QUID EST HOMO?
OSPEDALE SAN RAFFAELE
1996-2021 25 YEARS SAN RAFFAELE TELETHON INSTITUTE FOR GENE THERAPY
SR-Tiget

ß-Thalassemia: cause and hallmarks

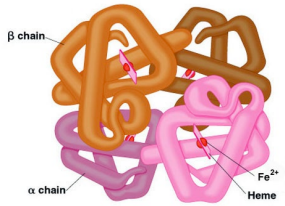
Described by Cooley in 1925

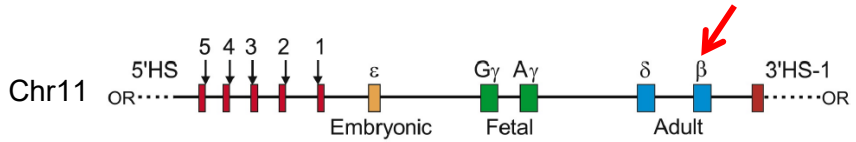
Genetic anemia

Autosomal recessive

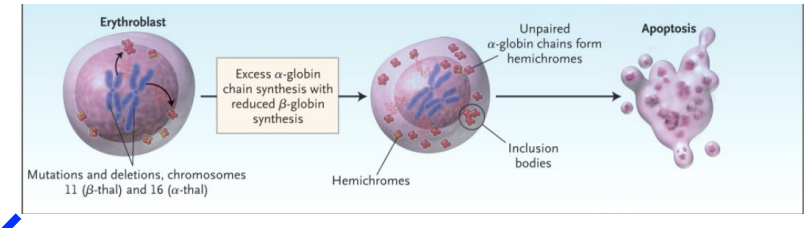
Severity based on genotype

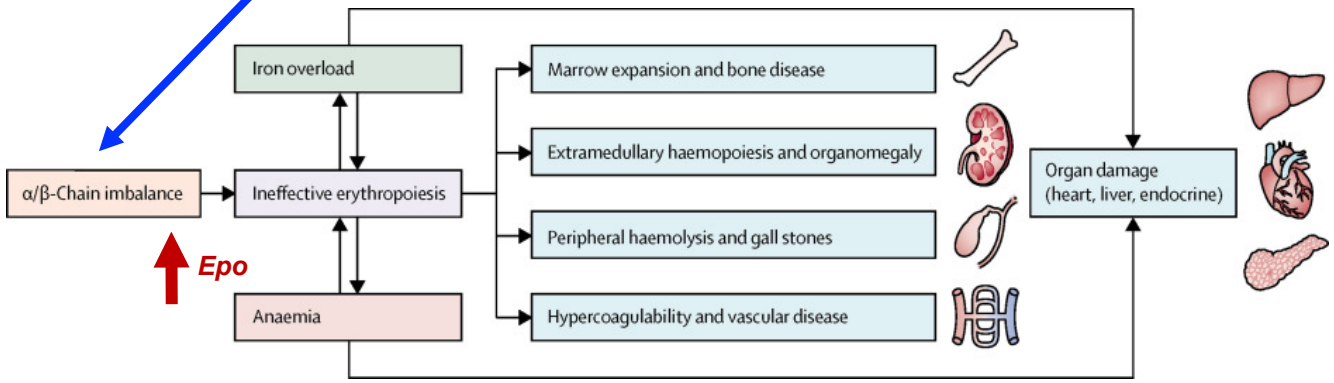
Hb content 20pg/cell



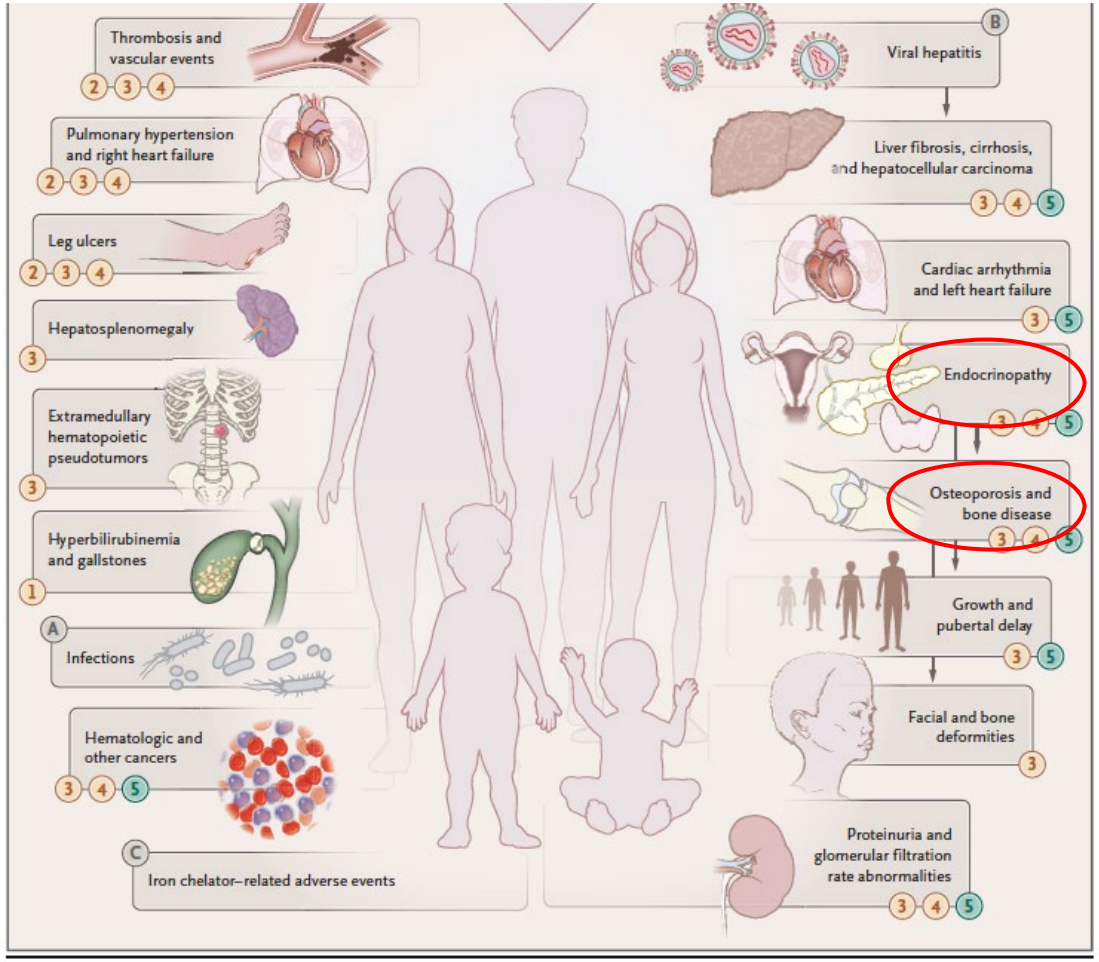


> 350 mutations





Not just anemia…



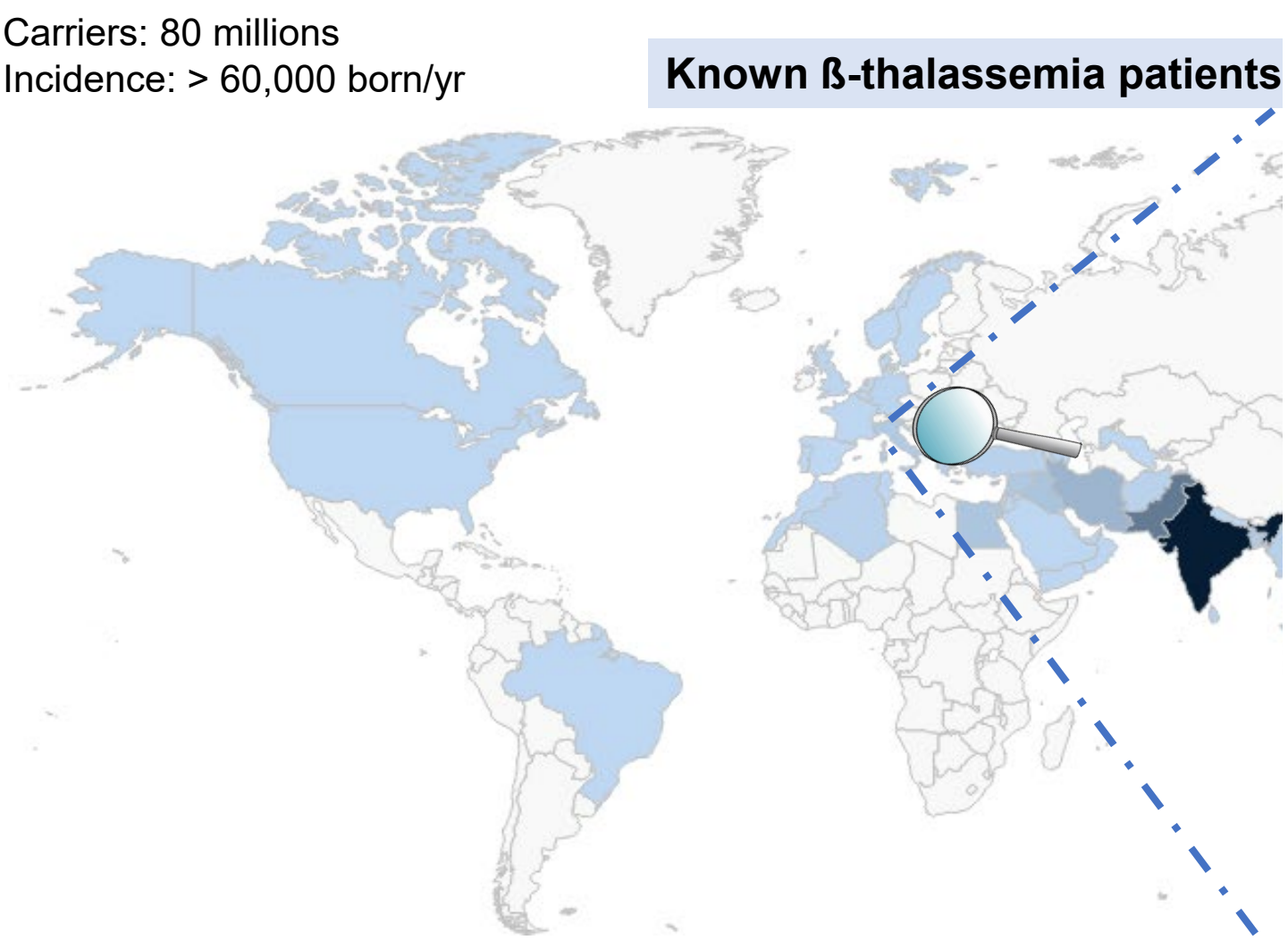
from Taher et al.NEJM, 2021

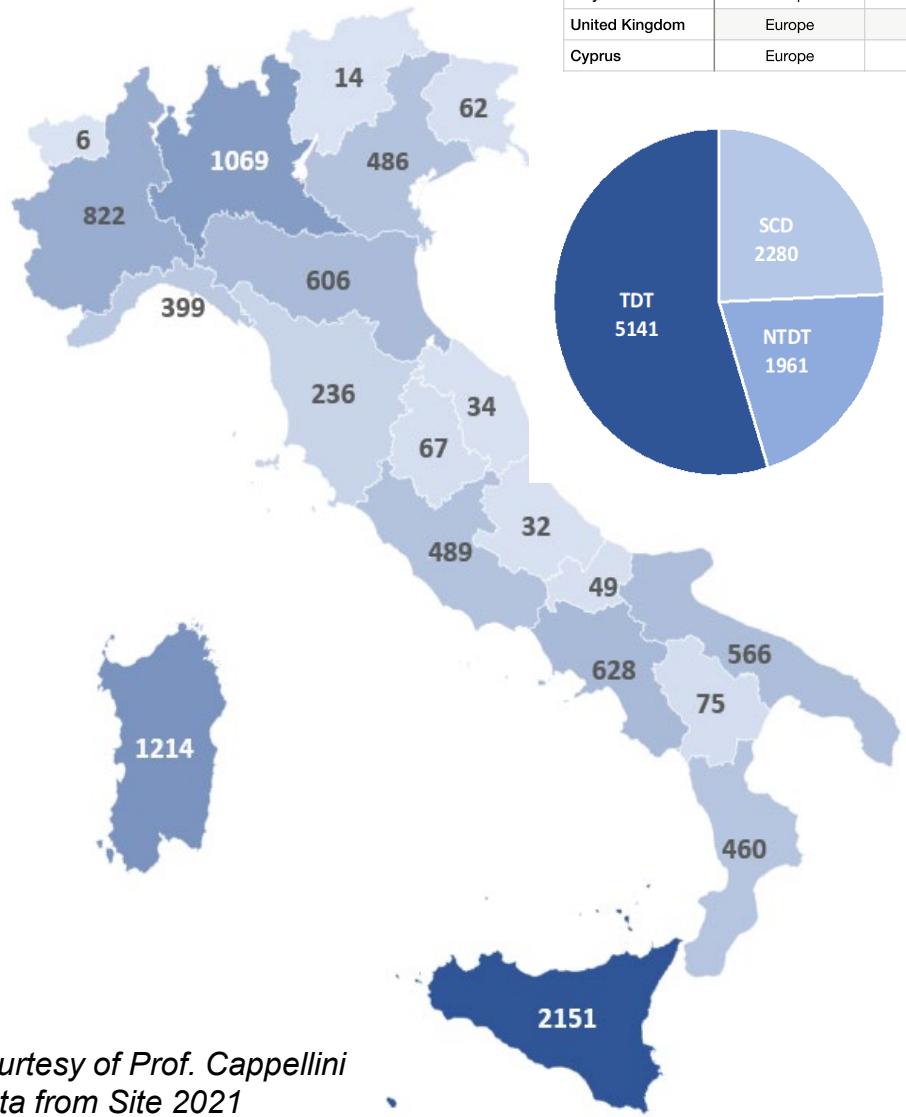
Beta-thalassemia worldwide

COUNTRY	CONTINENT	Patients
Cyprus	Europe	592
France	Europe	680
Germany	Europe	1500
Greece	Europe	2759
Italy	Europe	7000
United Kingdom	Europe	1168
Cyprus	Europe	592

Carriers: 80 millions
Incidence: > 60,000 born/yr

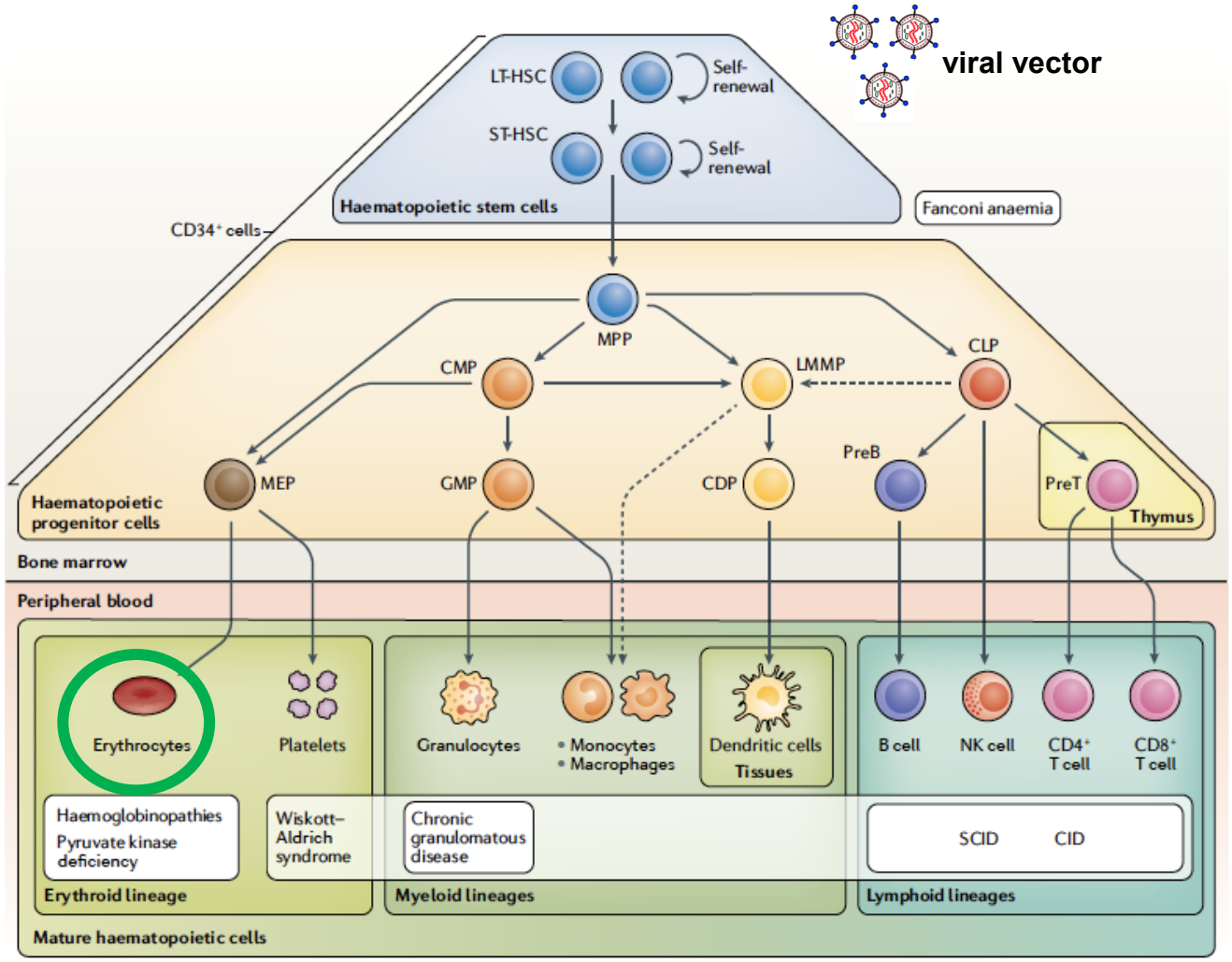
Known ß-thalassemia patients





courtesy of Prof. Cappellini
data from Site 2021

Rationale for HSC gene therapy of Bthal



- Correction of Hb production by HSC gene therapy comes from the reconstitution of the whole hematopoiesis

- The status of HSC (and the composition of CD34⁺ population) and of BM niche are crucial for clinical outcome in the setting of autologous (and allogeneic) transplantation

modified from Ferrari et al. *Nat. Rev. Genet. 2020*

The success of *ex vivo* gene therapy is based on:

- Efficient gene transfer/gene editing into target cells
- Efficient engraftment of modified HSCs and maintenance of “stemness”
- Adequate and persistent level of transgene expression
- Correction of the disease
- Safety

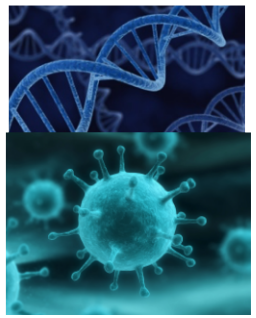
- An intense collaboration between researchers and clinicians
- A deep knowledge of the disease
- Infrastructures
- Grants for research
- Sponsor for trials

Gene therapy for BTHAL at SR-TIGET

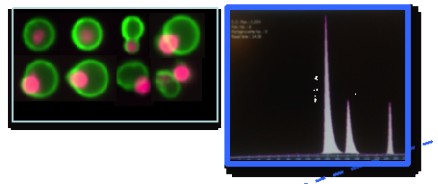
GMP LV manufacturing

2005 - - - - 2010 | 2010 GSK Alliance | 2012 | 2013 | 2014 | 2015

Vectors development



Preclinical studies (PoC)
- th3 mice
- thal human cells



GLP Preclinical studies (safety)

ISS pre-enquiry

Regulatory steps

Phase I/II trial

New trial

- LTFU study
- Improved protocol

2022

Apr 2014 — Scientific Advice (EMA)

Nov 2014 — Submission CTA (AIFA-ISS,EC)

Apr 2015 — Approval EC/AIFA-ISS

2016 EMA ODD

2018 Licensed to Orchard therapeutics

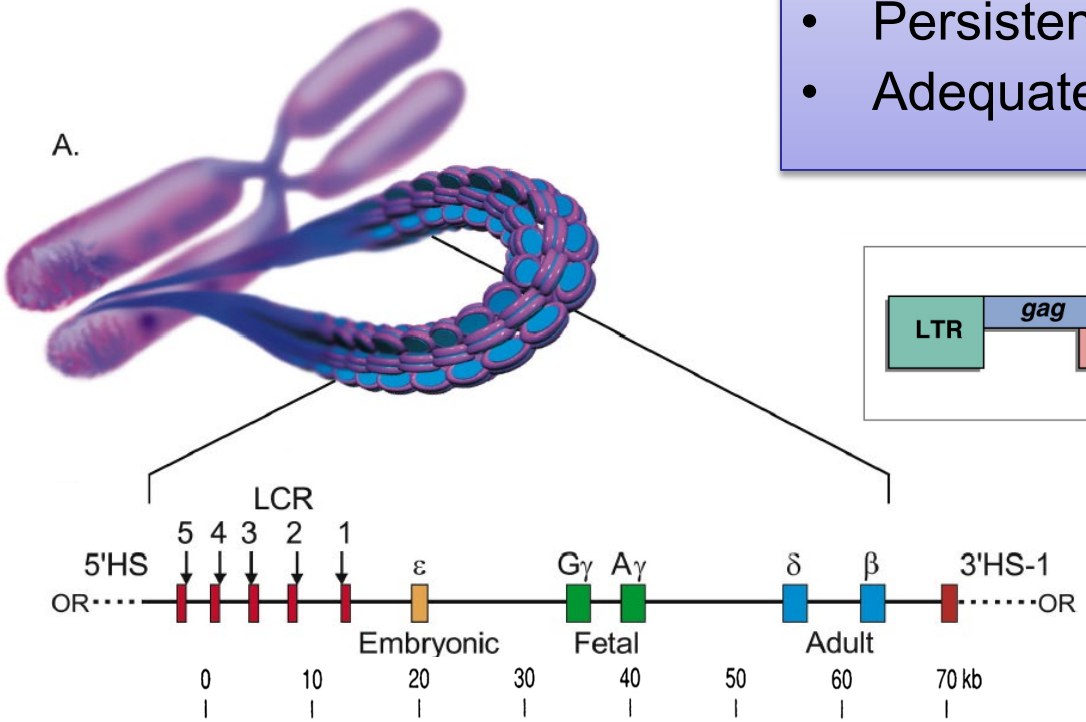
2018 EMA PRIME designation

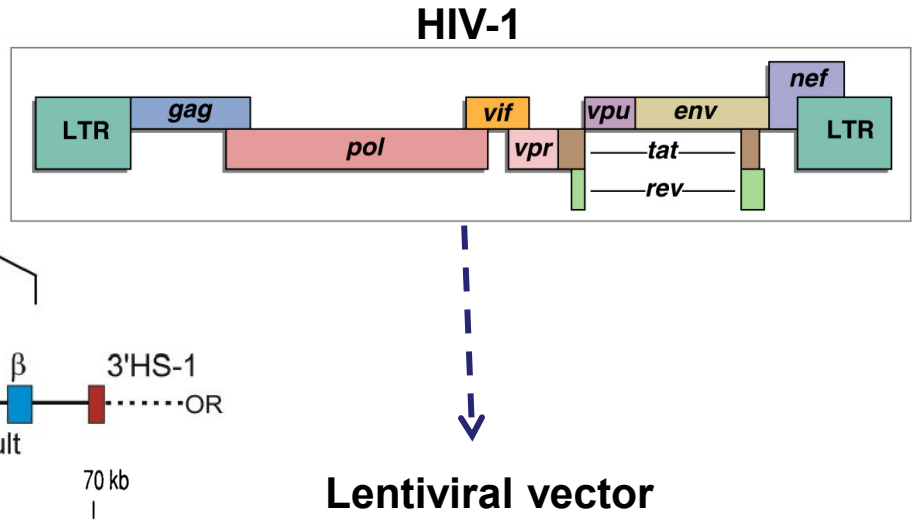
Gene transfer of ß-globin
The challenge of making efficient vectors

- Production of high-titer vectors
- Efficient gene transfer in primary cells

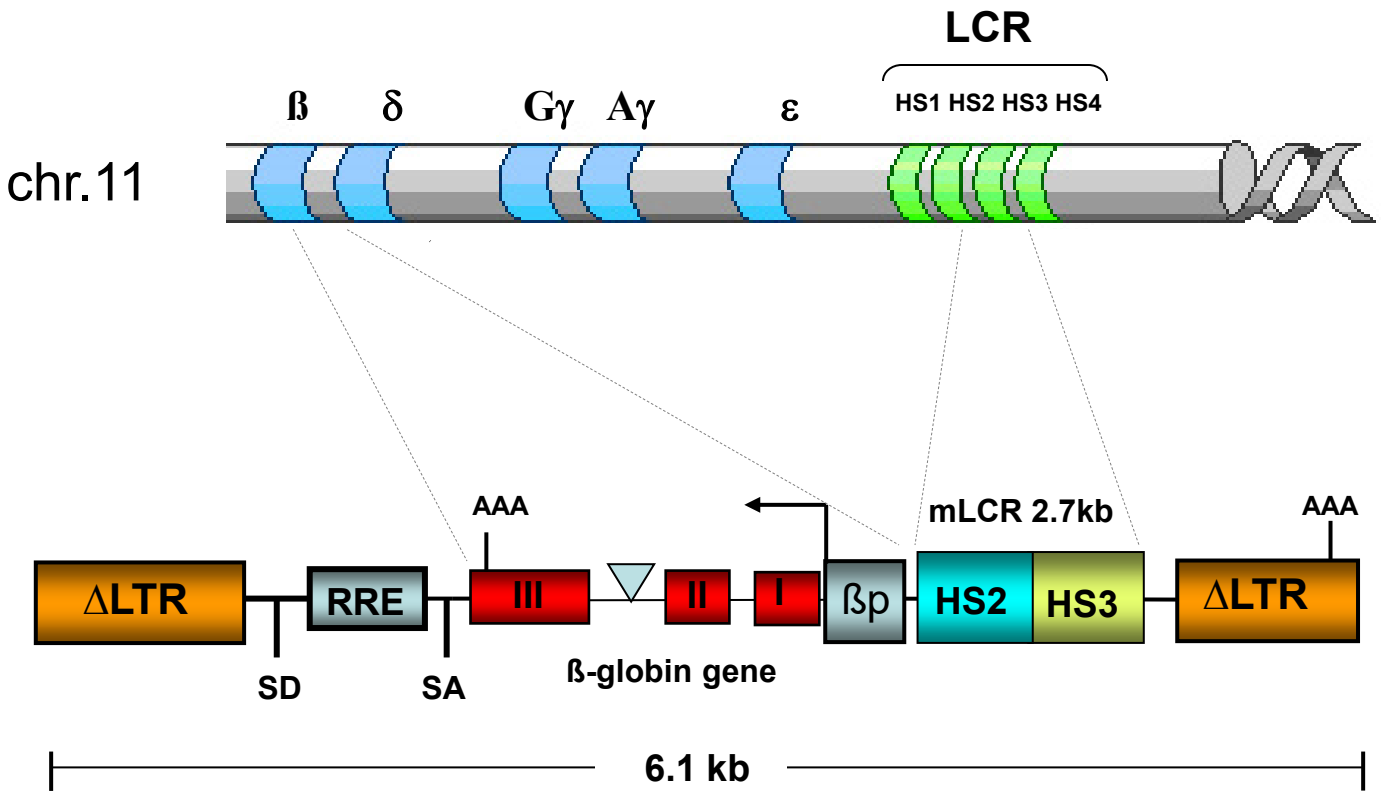
Correction is achieved if:

- Absence of rearrangements
- Persistence of transgene expression
- Adequate level of transgene expression



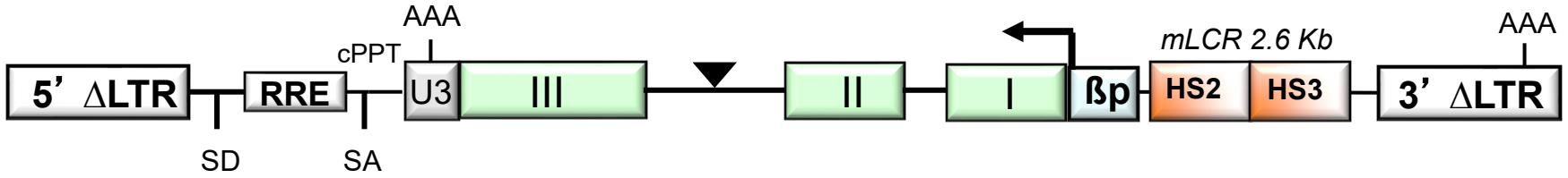


GLOBE lentiviral vector



Miccio et al., PNAS 2008

GLOBE vector and preclinical studies: **proof of concept and safety**



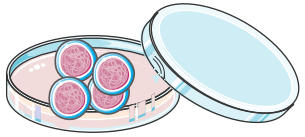
Gene **correction** and **safety** of GLOBE-LV transduced HSCs in the best available models:

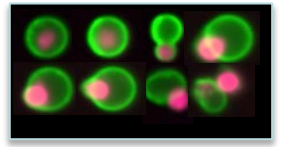
- **PoC in thalassemic mouse**

(Miccio et al., PNAS 2008; Miccio et al. PlosONE, 2011)

- **GLP toxicology and tumorigenicity study**

(Lidonnici et al. 2018)





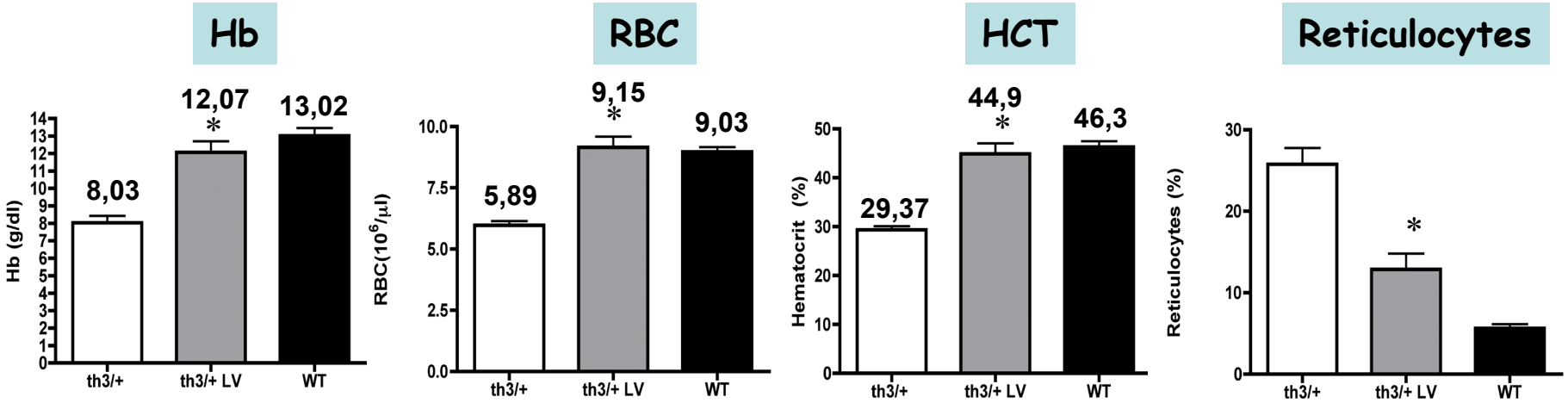
- **PoC in human thalassemic cells**

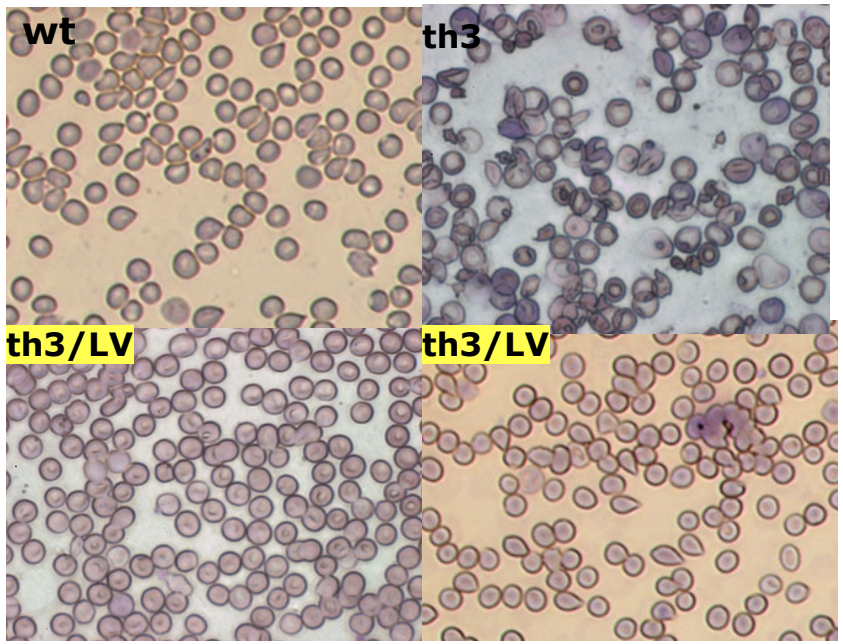
(Roselli et al., EMBO MolMed 2010)

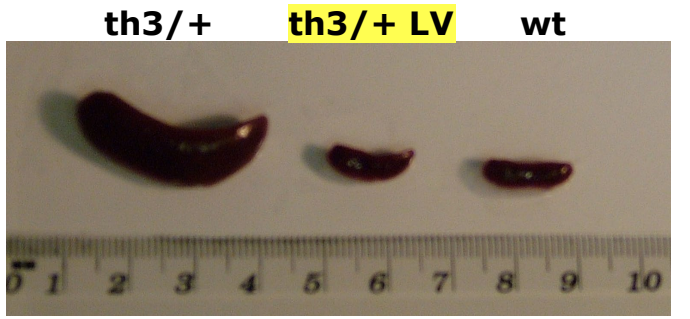
- **GLP biodistribution study in NSG mice**

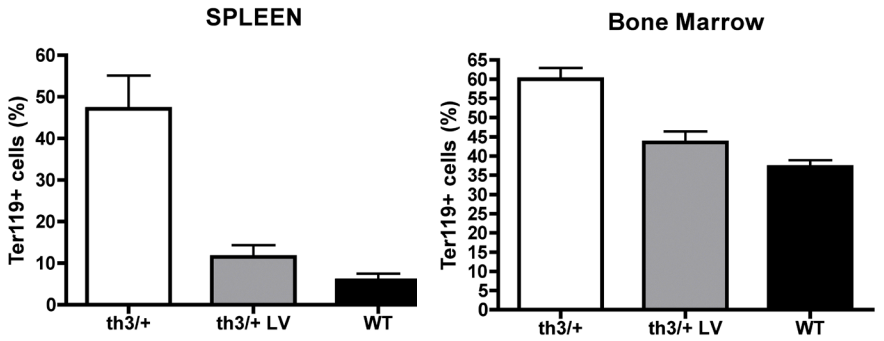
(Lidonnici et al. 2018)

Long term correction of thalassemia intermedia









Miccio et al. PNAS, 2008

Gene therapy for ß-thalassemia

To validate the therapeutic potential of LV transduced HSCs in the best available preclinical models:

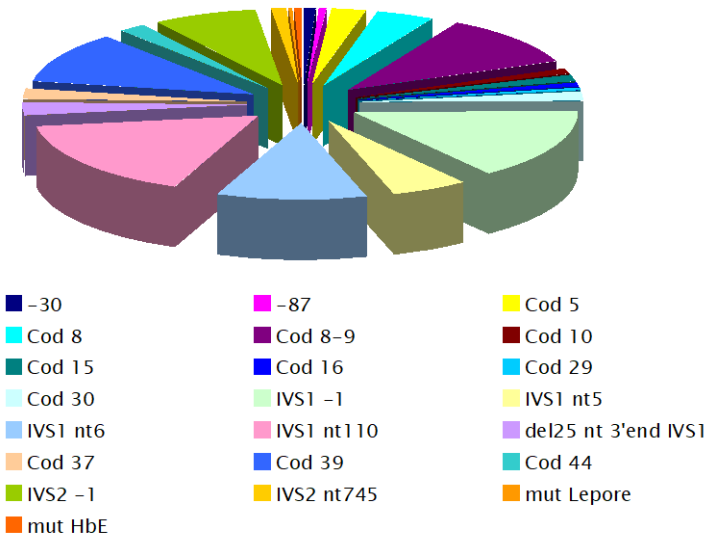
thalassemic mouse
human thalassemic cells

Studies on **BM-CD34+** cells from Thal patients

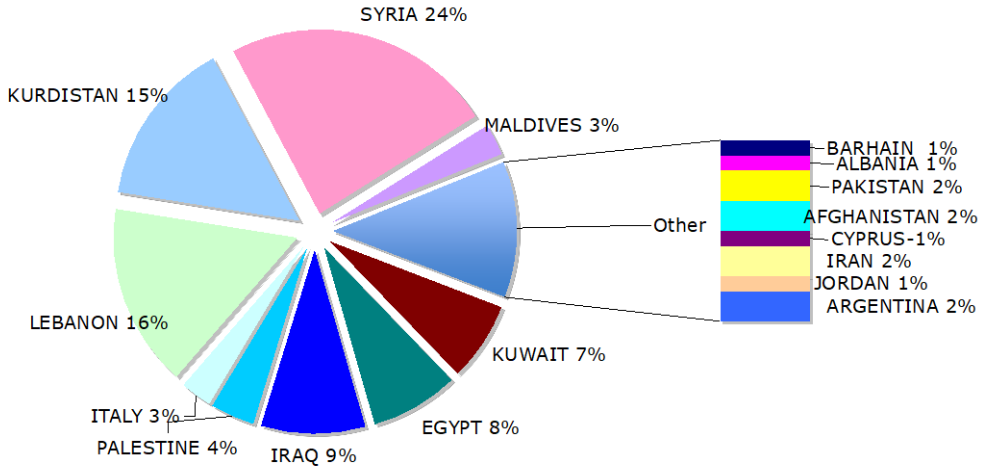
2004-2008
Program of allogeneic BMT
in thalassemia: HSR-IME network

No. patients		102
Gender		
	Male	56
	Female	46
Age (yr)		
	Range	2-24
	Median	8
Genotype		
	ß0	49
	ß+	53

GENOTYPE

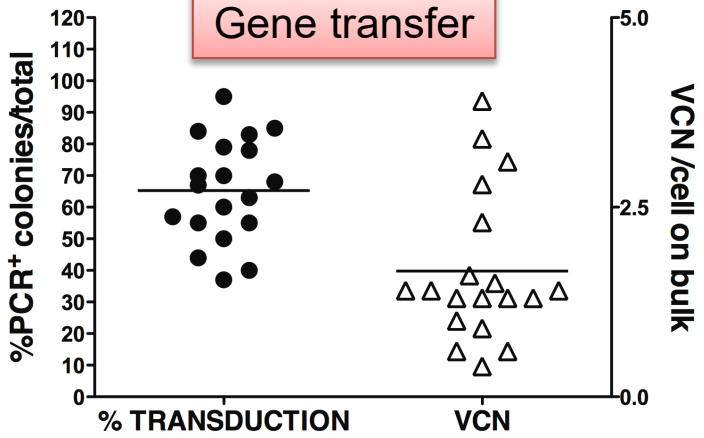


PATIENTS' ORIGIN

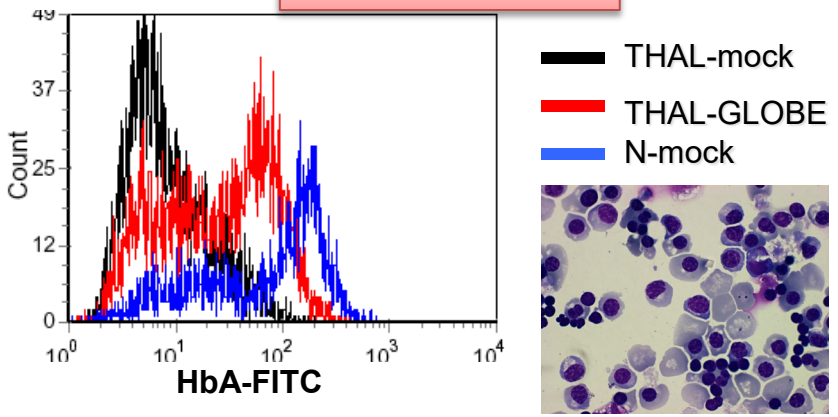


High gene transfer efficiency and rescue of HbA expression in Thal cells

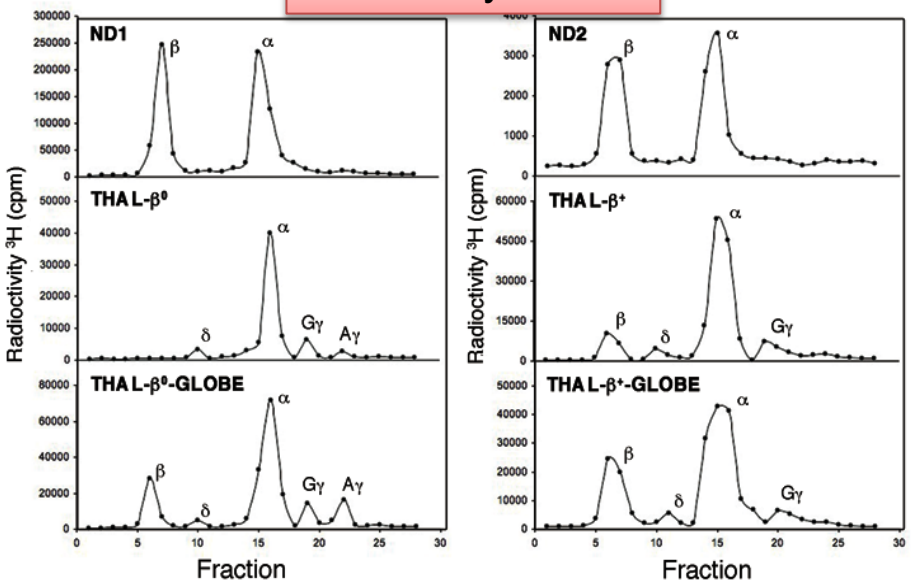
Gene transfer



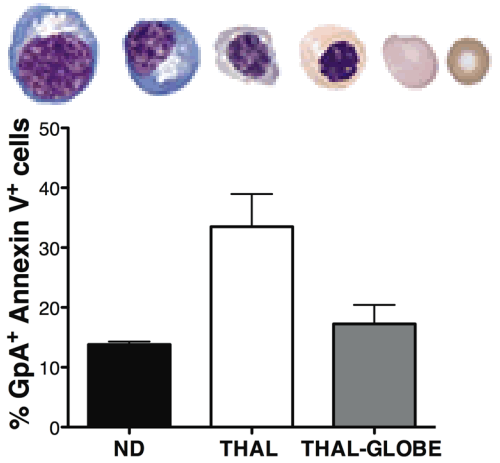
HbA expression



ß-chain synthesis



Rescue of ineffective erythropoiesis



Roselli et al., EMBO MolMed 2010

Gene therapy of BTHAL

Pre-clinical studies: **proof of safety**

Demonstration of **safety** of GLOBE-LV transduced HSCs in pre-clinical **GLP studies**:

- Toxicology and tumorigenicity (th3/+ mice)
- Biodistribution of huCD34+ cells (NSG mice)

European Medicines Agency

London 30 May 2008
EMEA/CHMP/GTWP/125459/2006

GUIDELINE ON THE NON-CLINICAL STUDIES REQUIRED BEFORE FIRST CLINICAL USE OF GENE THERAPY MEDICINAL PRODUCTS

HSR-TIGET
GLP Test Facility

CERTIFICATE OF COMPLIANCE WITH THE PRINCIPLES OF GOOD LABORATORY PRACTICE

(Legislative Decree Nr. 50, March 2nd 2007 – Directive 2004/9/EC)

Certificate: 2014/9
Date of issuance: 28/03/2014

In accordance with article 4(1) of Legislative Decree nr. 50 of March 2nd, 2007, articles 3, 4 and 5 of Italian Ministry Decree of July 4th, 1997, and in consideration of the positive result from the inspection conducted on 25/02/2014 – 26/02/2014

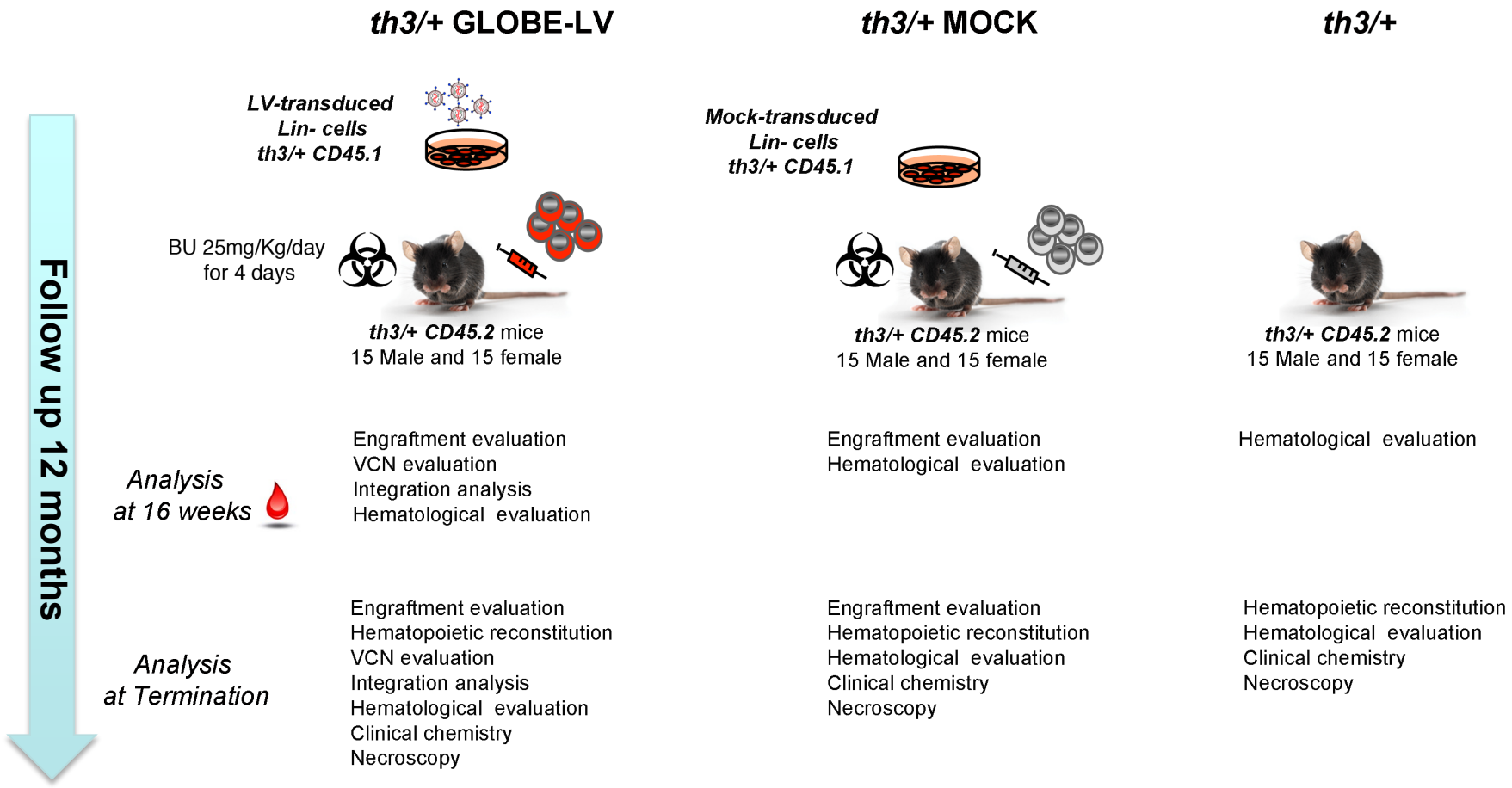
IT IS HEREBY CERTIFIED

that the Test Facility: **Istituto San Raffaele-Telethon per la Terapia Genica (HSR-TIGET)** – Ospedale San Raffaele S.r.L - Via Olgettina,60 – 20132 – Milano (MI)

is eligible to carry out tests in compliance with the principles of Good Laboratory Practice laid down in the Annex II of mentioned Legislative Decree, within the following areas of expertise:

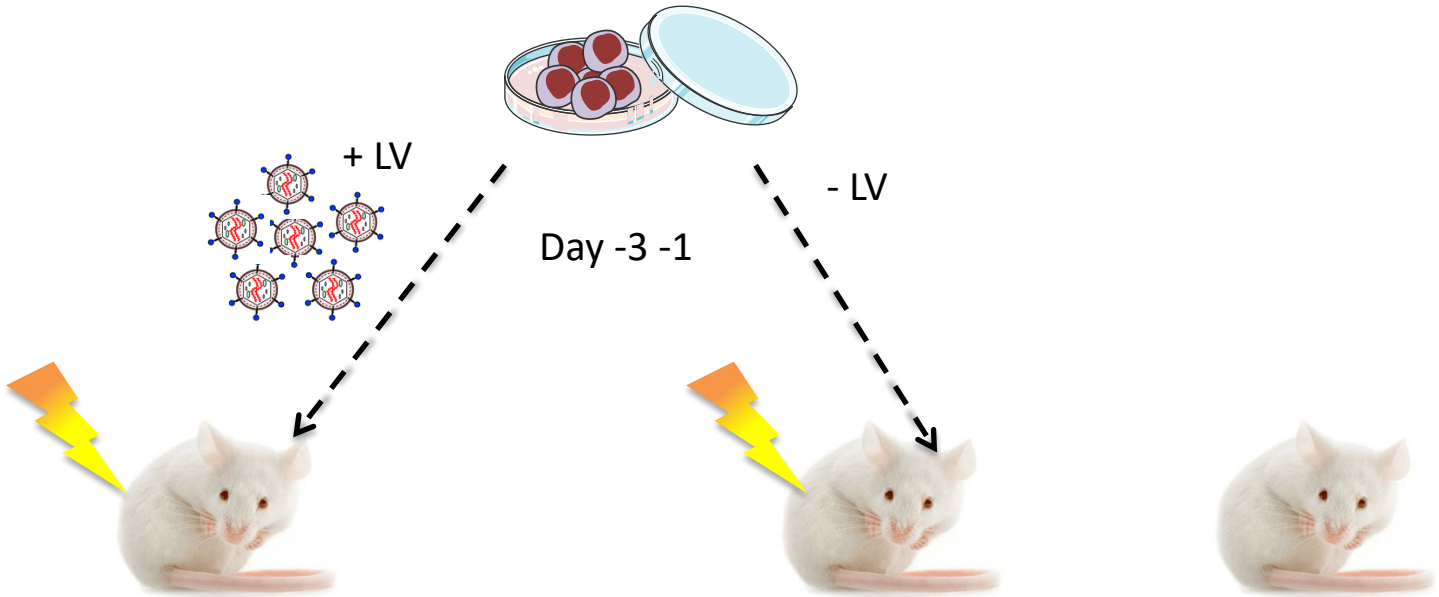
2) TOXICITY STUDIES
9) OTHER (SPECIFY)
9.4) BIOTECHNOLOGY AND MOLECULAR BIOLOGY STUDIES

Toxicity and tumorigenicity study for BTHAL GT
GLP Multisite Study



Lidonnici et al. 2018

Biodistribution of transduced **huCD34+** cells in **NSG** mice - **GLP STUDY**



FOLLOW UP 12 WEEKS w daily monitoring, clinical signs and BW record weekly

11 wks bleeding

Engraftment huCD45
Phenotype CD19,CD3,CD13
VCN analysis in PB

12 wks
Termination with perfusion

BM, spleen:
CD45,CD34,CD19, CD3,CD4, CD8, CD13
p24 assay (serum)
qPCR VCN analysis:
BM, spleen, thymus, brain, gonads

GLP Preclinical studies
Conclusions

- No tumorigenicity or toxicity associated with the transplantation of HSPCs transduced with LV GLOBE in thalassemic mice
- Confirmation of efficacy of gene therapy (i.e. correction of anemia, reduction of EMH)
- Normal biodistribution of genetically modified CD34+ cells
- Normal engraftment and differentiation in blood and lympho-hematopoietic organs
- Absence of vector in gonads

TIGET-BTHAL Clinical trial

NCT02453477

A phase I/II study evaluating safety and efficacy of autologous HSCs genetically modified with **GLOBE LV** encoding for the human ß-globin gene for the treatment of patients affected by transfusion dependent ß-thalassemia

Promoter: Ospedale San Raffaele, Milan
Sponsor: Telethon Foundation

Project Leader: Giuliana Ferrari

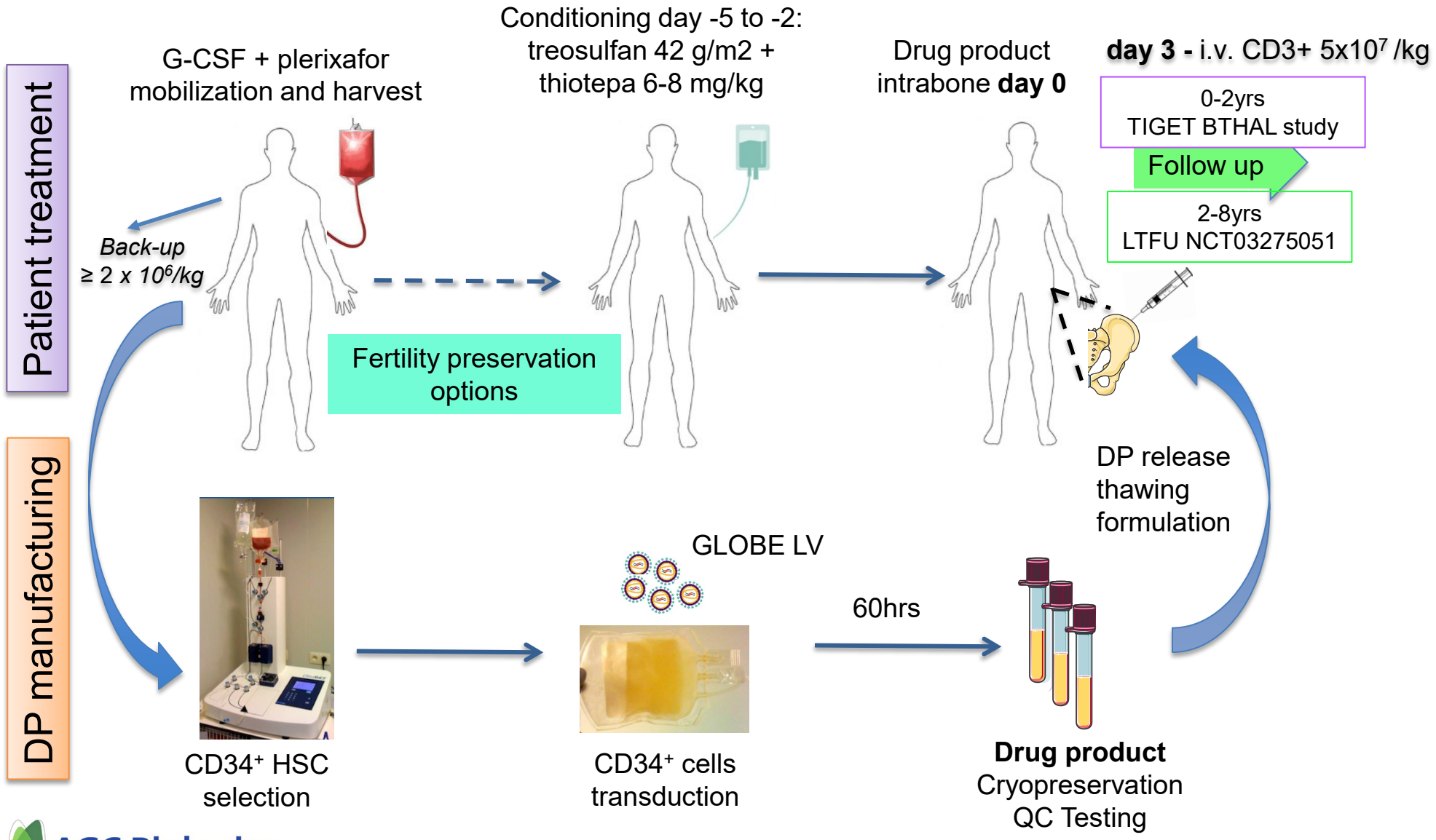
Principal Investigator:
Alessandro Aiuti
Co-Principal Investigators:
Fabio Ciceri
Sarah Marktel
Maria Domenica Cappellini

Primary safety endpoints:
- survival
- hematological engraftment
- overall safety and tolerability
- polyclonal engraftment of transduced cells

Primary efficacy endpoint:
- reduction of transfusion requirement up to transfusion independence

Research endpoints

TIGET-BTHAL Phase I/II trial - *NCT02453477*



AGC Biologics

TIGET-BTHAL: *intrabone infusion* RATIONALE

Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: a phase I/II study

Lancet Oncol. 2008

Francesco Frassoni, Francesca Gualandi, Marina Podestà, Anna Maria Raiola, Adalberto Ibatici, Giovanna Piaggio, Mario Sessarego, Nadia Sessarego, Marco Gobbi, Nicoletta Sacchi, Myriam Labopin, Andrea Bacigalupo

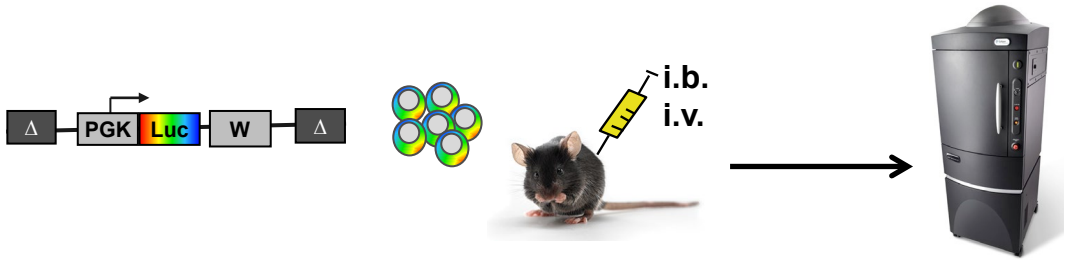
Unrelated Cord Blood Transplantation: Outcomes After Single-Unit Intrabone Injection Compared With Double-Unit Intravenous Injection in Patients With Hematological Malignancies

Vanderson Rocha,[1,2,3,17] Myriam Labopin,[4] Annalisa Ruggeri,[1,2,5] Marina Podestà,[6] Andrea Gallamini,[7] Francesca Bonifazi,[8] Fermin M. Sanchez-Guijo,[9] Montserrat Rovira,[10] Gerard Socie,[11] Ioannis Baltadakis,[12] Mauricette Michallet,[13] Eric Deconinck,[14] Andrea Bacigalupo,[15] Mohamad Mohty,[16] Eliane Gluckman,[1,2] and Francesco Frassoni[6]

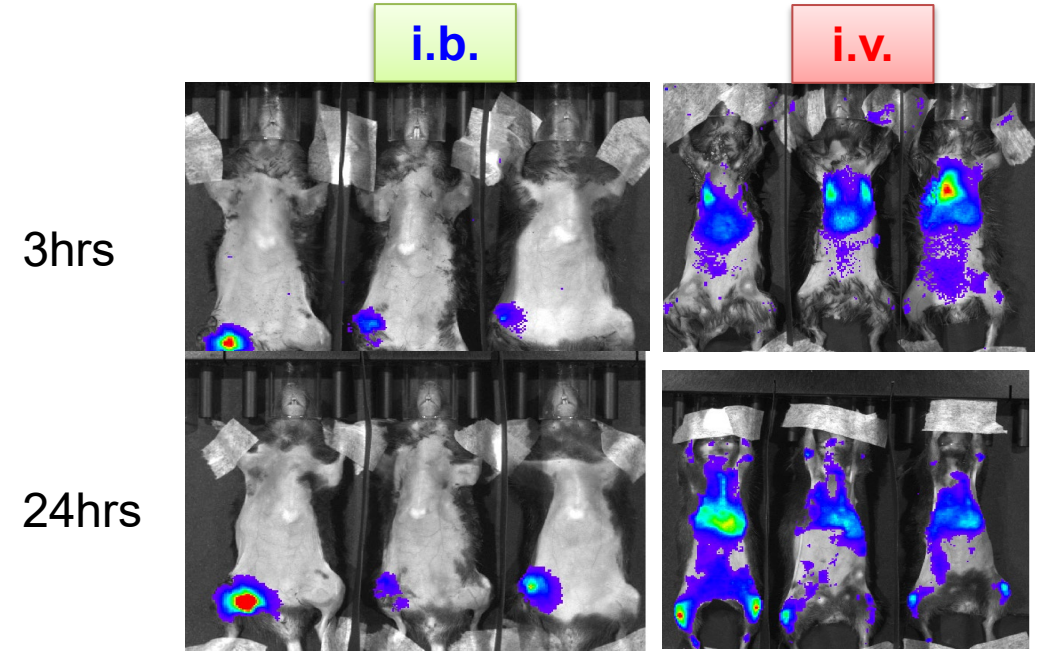
Advantages of <u>intrabone</u> *vs* <u>intravenous</u> injection:

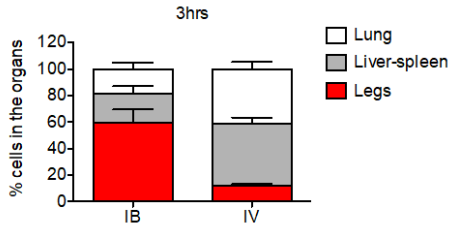
- bypass filter organs cell trapping
- faster myeloid and PLT recovery
- better engraftment
- polyclonal engraftment
- experience in CBT

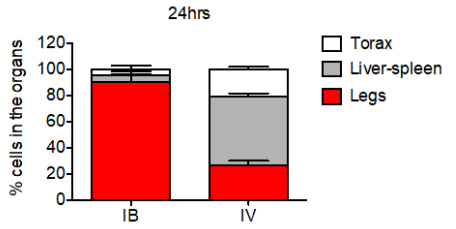
In vivo imaging: homing of lin$^-$ HSPCs: **intrabone** *vs* **intravenous** injection

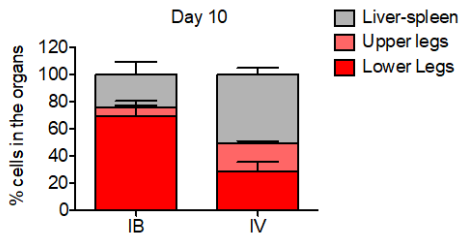


IVIS SpectrumCT®
Perkin Elmer









TIGET-BTHAL: drug products

BTHAL Patient #	Age at GT/gender	PBSC harvest CD34+/kg N° apheresis	Gene therapy dose CD34+/kg	Vector copy number (VCN^)	% Transduction efficiency (TE*)
1	31/M	31 x10^6 (2 aph)	19.4	0.8	60
2	35/F	23 x10^6 (2 aph)	18.4	0.7	63
3	34/M	21 x10^6 (3 aph)	18.7	0.7	68
4	13/M	53 x10^6 (1 aph)	19.5	1.2	53
5	13/M	47 x10^6 (2 aph)	16.3	1.5	77
6	13/M	45 x10^6 (2 aph)	19.5	0.7	62
7	6/M	50 x10^6 (1 aph)	19.7	1.0	59
8	5/F	31 x10^6 (1 aph)	20.0	0.9	55
9	4/F	30 x10^6 (2 aph)	19.8	0.9	38

median 28
Range 21-53

median 19.5
Range 16.3-20.0

median 0.9
Range 0.7-1.5

^ *VCN/cell = average vector copies/cell on d14 CD34⁺ bulk culture*

* *TE = % vector positive CFUs*

Patients' summary, drug products and safety

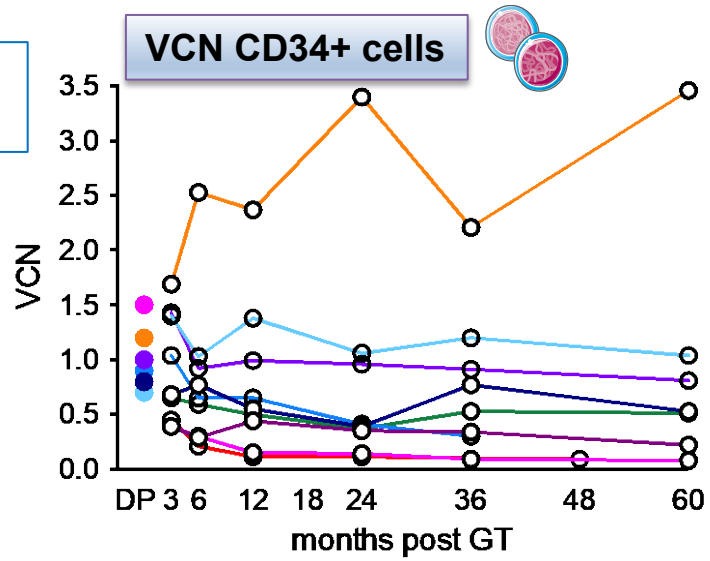
	Pt1	Pt2	Pt3	Pt4	Pt5	Pt6	Pt7	Pt8	Pt9
Age/sex	31/M	35/F	34/M	13/M	13/M	13/M	6/M	5/F	4/F
Mutations	cod39/ IVS I-110 ß0/ß+*	cod39/ IVS I-110 ß0/ß+*	cod39/ IVS I-110 ß0/ß+*	IVS I-110/ IVS I-110 ß+/ß+*	cod39/ cod39 ß0/ß0	IVS I-6/ IVS I-110 ß+/ß+*	cod39/ cod39 ß0/ß0	IVS I-110/ IVS I-110 ß+/ß+*	IVS II-1/ IVS I-110 ß0/ß+*
Pre-GT pRBC (ml/kg/yr)	≥ 200 ml/kg/yr								
FU (yr)	7.2	6.4	6.8	6.4	6.3	6.1	6.0	5.1	5.0

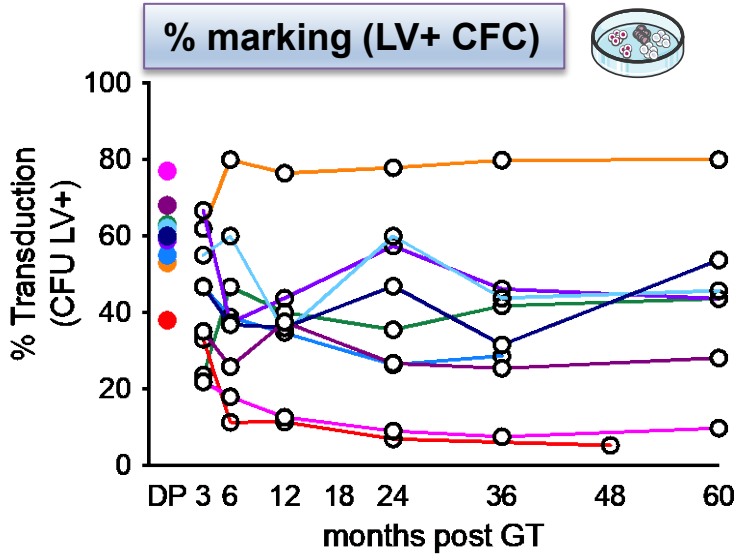
DRUG PRODUCTs	
CD34 cells collected (x 10^6/kg)	median **38** (min-max **21-53**)
CD34 cells dose (x 10^6/kg)	median **19.5** (min-max **16.3-20.0**)
VCN/cell	median **0.9** (min-max **0.7-1.5**)
Transduction efficiency (% LV+ CFU)	median **60** (min-max **38-77**)

- No adverse events related to DP
- Median Neutrophils engraftment: 19 days (15-34)
- Median Platelets engraftment: 15 days (10-24)

Engraftment of marked **BM-CD34⁺** cells

Stable marking in HSPC

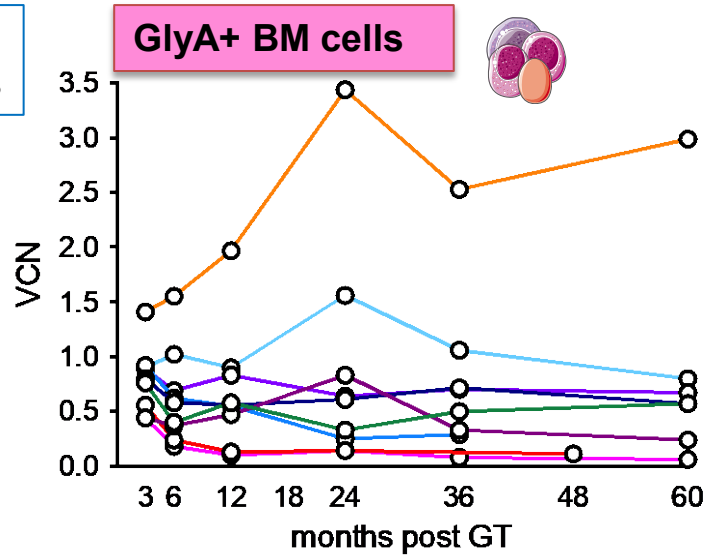




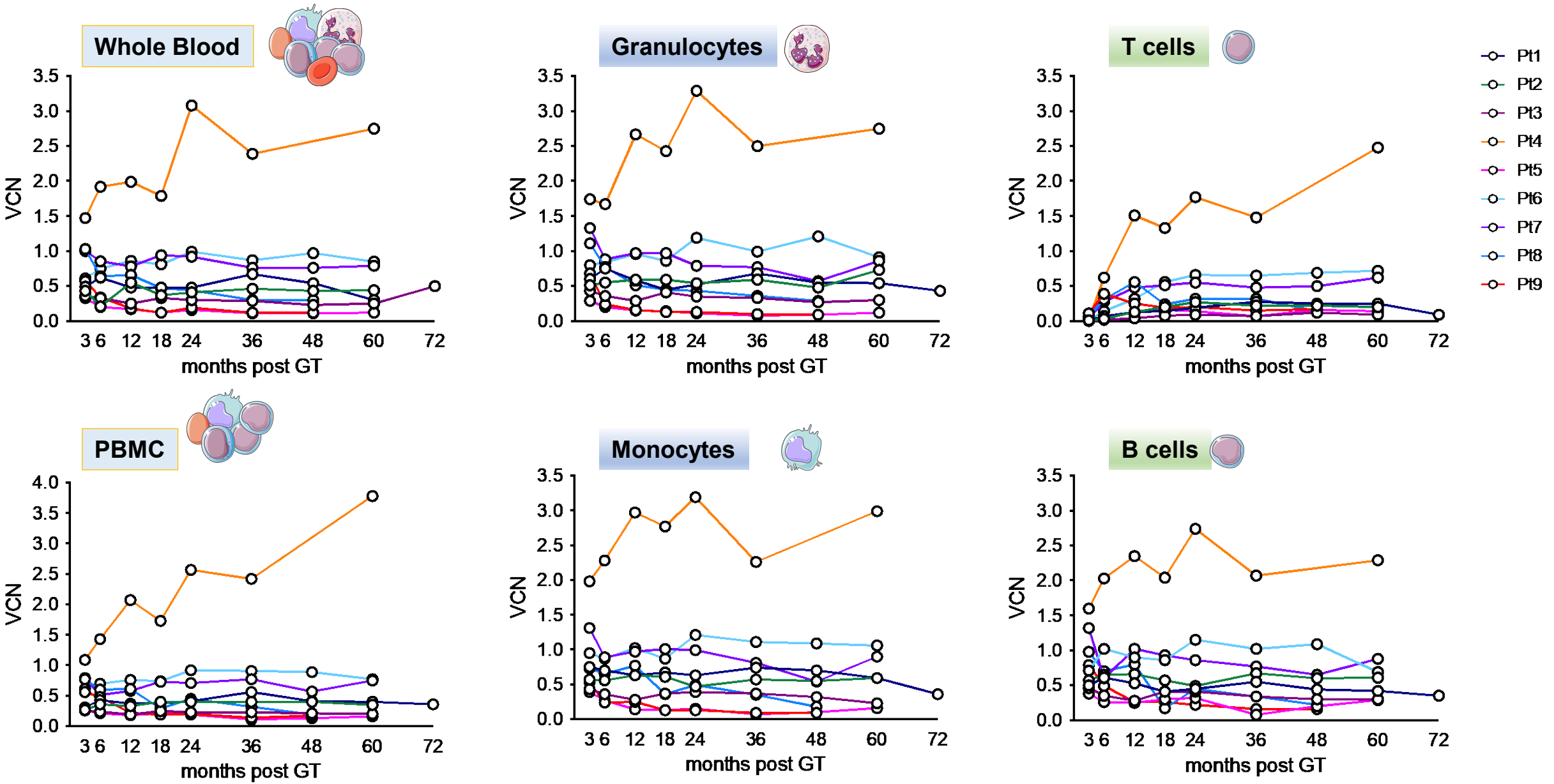
Pt1
Pt2
Pt3
Pt4
Pt5
Pt6
Pt7
Pt8
Pt9

DP= drug product

Stable marking in erythroid cells



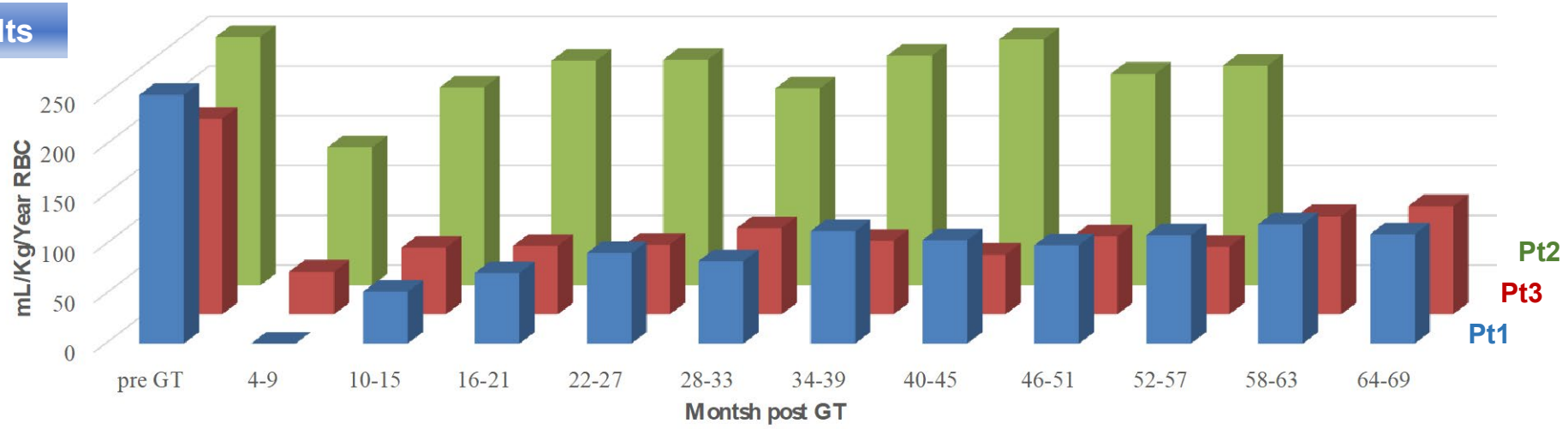
Multilineage marking in **peripheral blood**

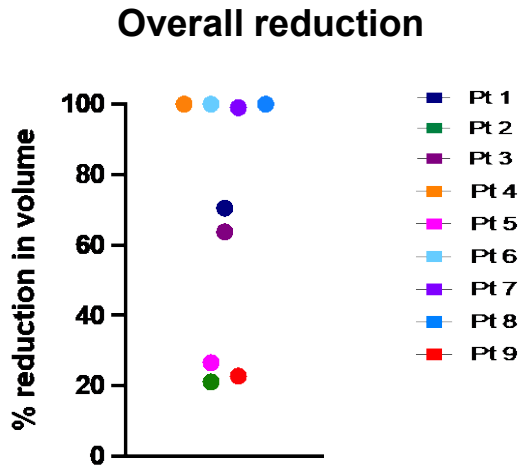


updated from Marktel, Scaramuzza et al., Nat Med, 2019

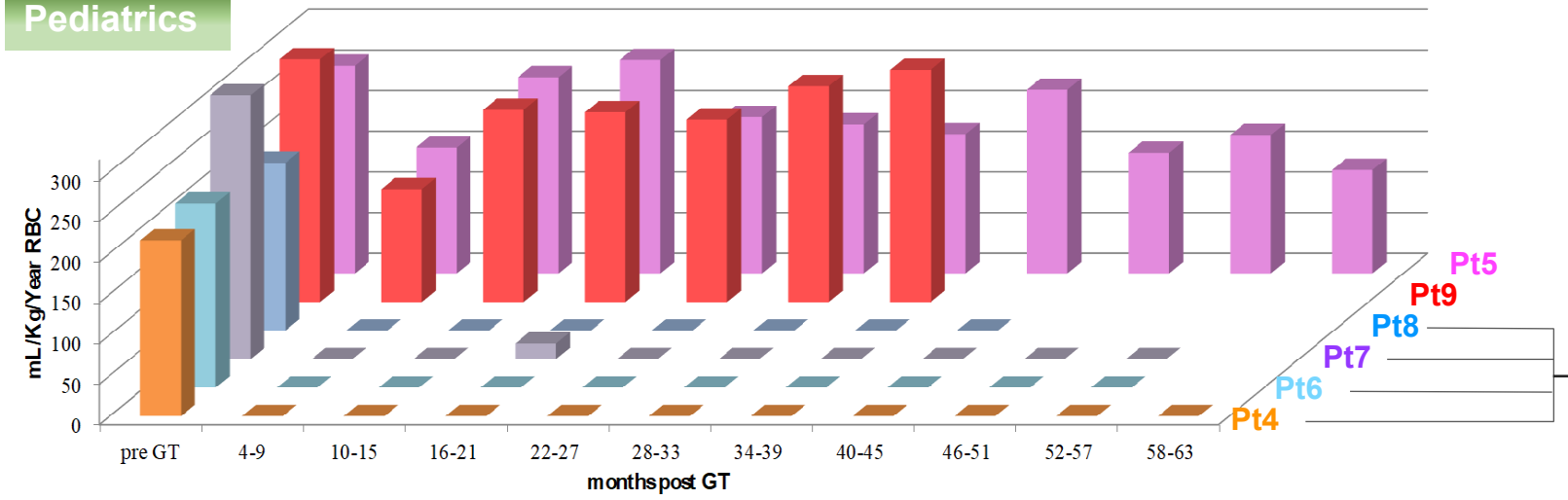
Efficacy: post-GT transfusion requirement

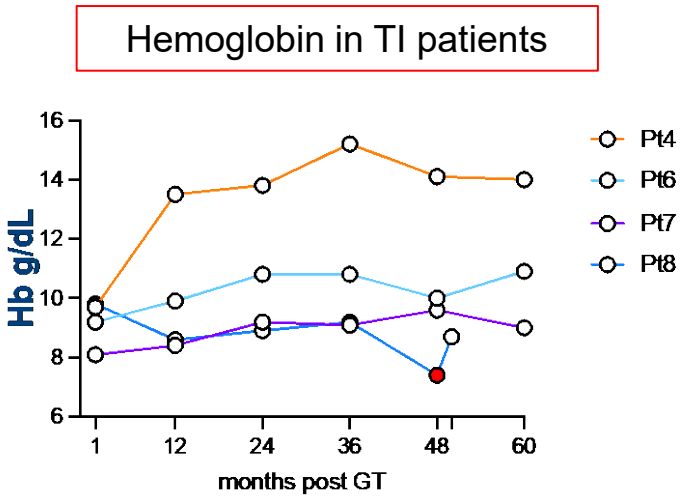
Adults





Pediatrics

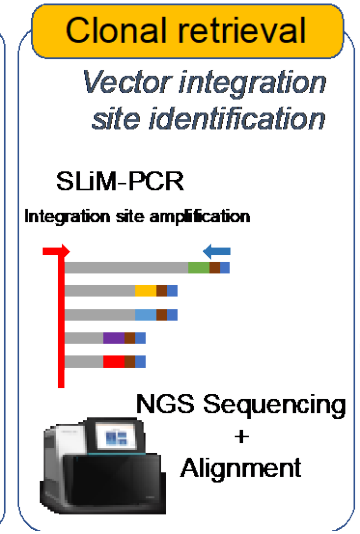


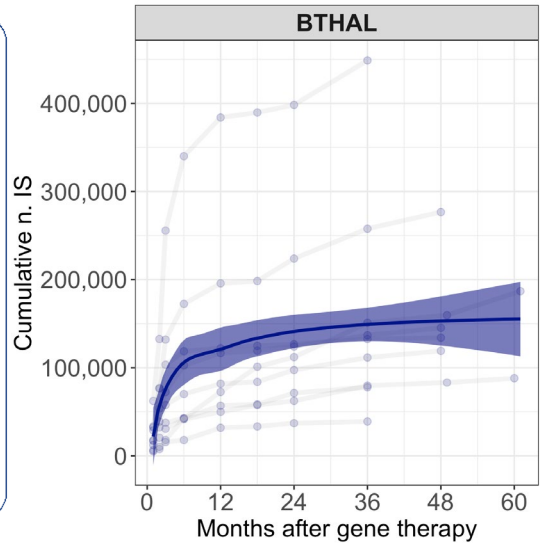


Expressed as mL/kg/y pRBC. According to protocol: analysis on 6 months intervals starting from month 4 from GT

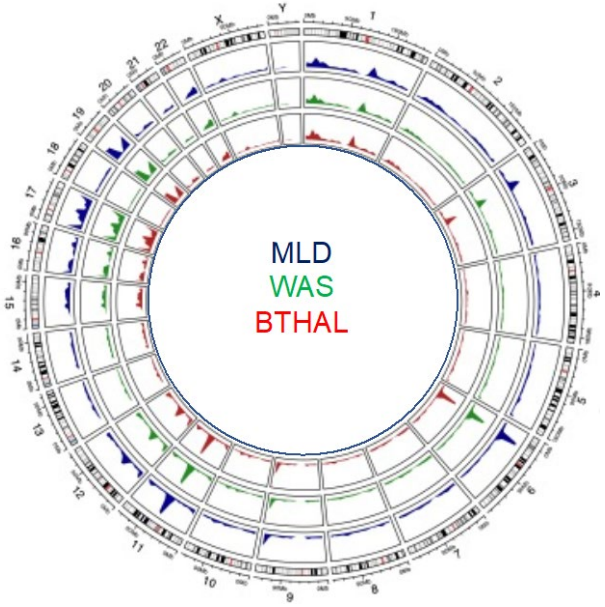
updated from Marktel, Scaramuzza et al., Nat Med, 2019

Safety: vector integration site analysis



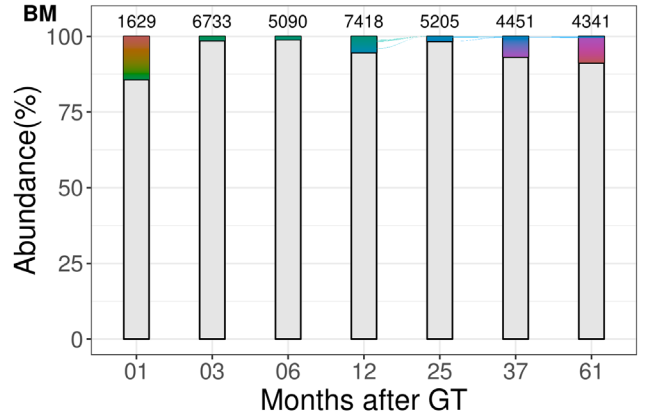


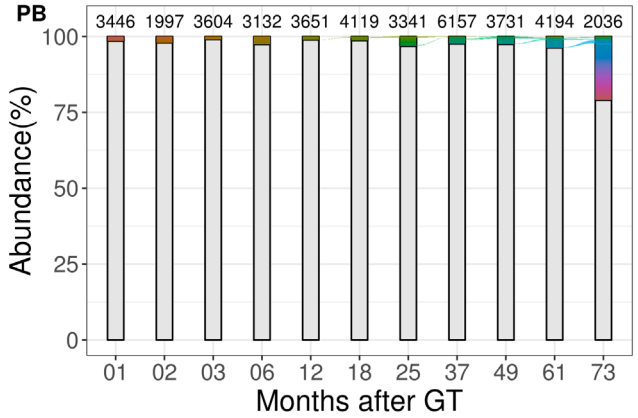
IS genome wide distribution



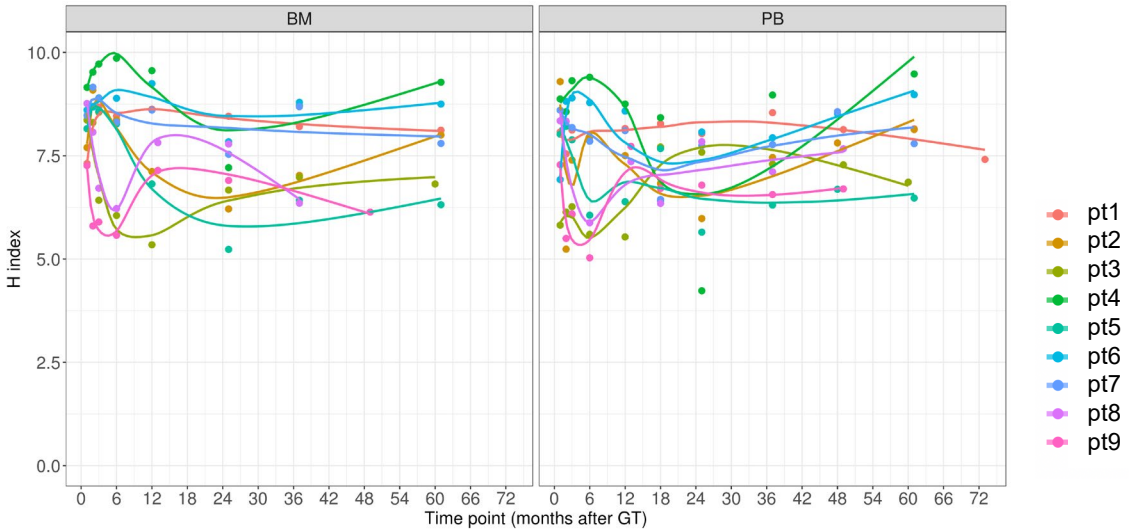
Clonal abundance

clones are colored if >0.01% in one time point





Clonal Population Diversity



- Absence of dominant clones
- No Replication Competent Lentivirus (RCL)
- No evidence of abnormal clonal proliferation

coll. A. Calabria, E. Montini

Tiget Bthal: Summary

Primary safety endpoints achieved:

- ✓ Rapid hematopoietic recovery in all treated patients
- ✓ Good tolerability of the procedure overall with adverse events consistent with autologous HCT, none DP-related
- ✓ Evidence of HSC vector targeting
- ✓ Multilineage and polyclonal engraftment of gene corrected cells, no evidence of clonal dominance at latest time points

Primary efficacy endpoint achieved:

- ✓ Primary efficacy endpoint of transfusion reduction achieved in 8 out of 9 patients at 2yr FU
- ✓ 4/9 patients are transfusion-free
- ✓ Independence from transfusions in younger patients and in those with engraftment of gene corrected $CD34^+$ progenitors above 40% and VCN ≥ 0.8

Secondary efficacy endpoints achieved:

- ✓ Adequate Hb level in transfusion independent patients
- ✓ Multilineage and polyclonal engraftment of gene corrected cells

Ongoing and future studies
…..back to the bench

- Improving transduction efficiency by optimizing protocols preserving biological HSC features

- Unraveling biological key factors for prediction of favourable outcome

HSC and its niche

- What's the impact of the BM niche on the quality of HSC?

- What's the impact of the BM niche on homing, engraftment and hematopoietic output of genetically modified transplanted cells?

1996-2021 25 YEARS SAN RAFFAELE TELETHON INSTITUTE FOR GENE THERAPY

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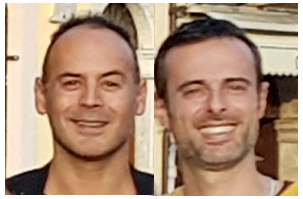

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