SAFETY : CURRENT DATA AND CLUES TO IMPROVE IT

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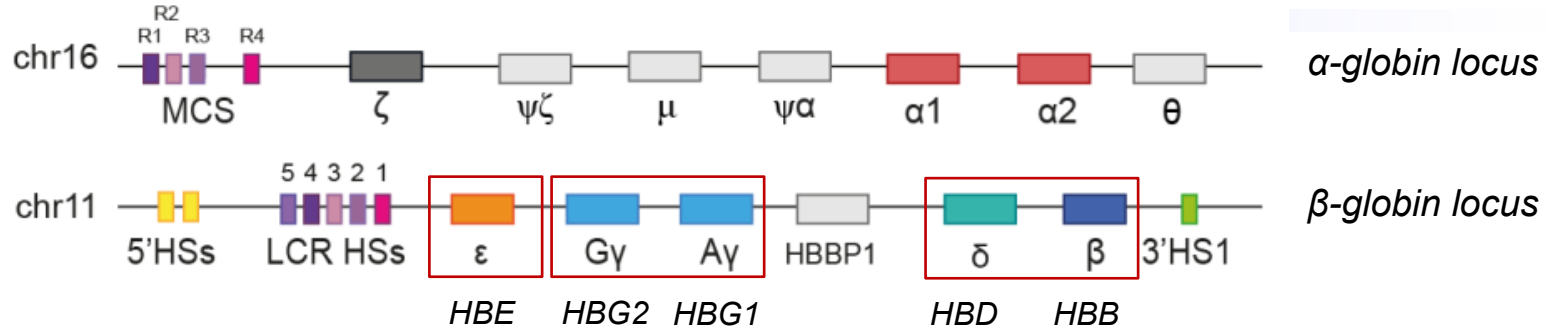
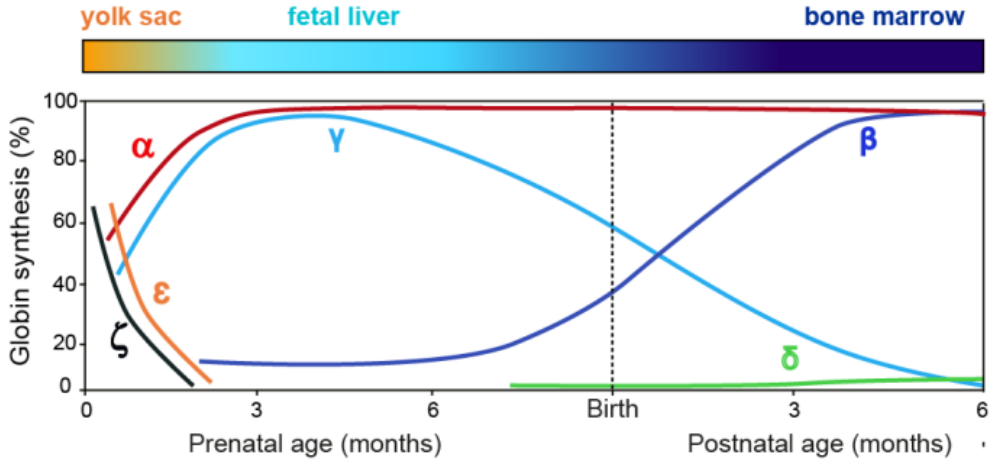
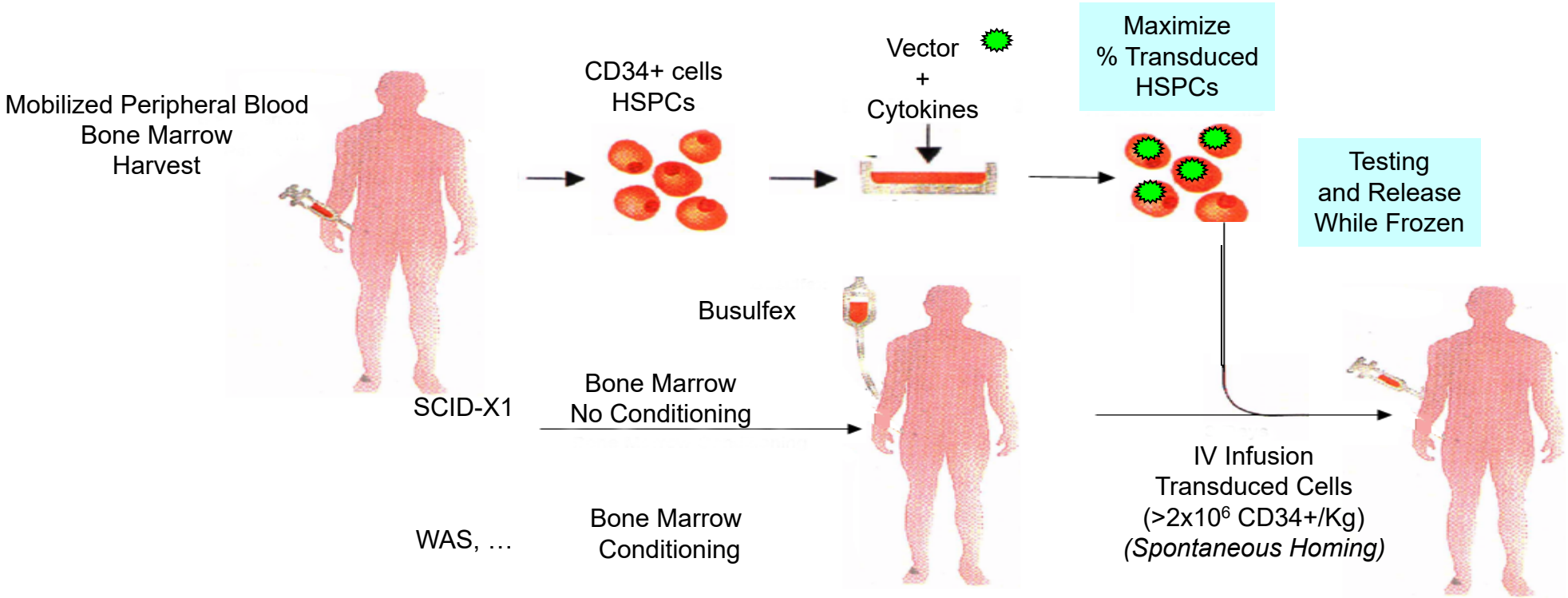
ORGANIZATION AND REGULATION OF GLOBIN GENES



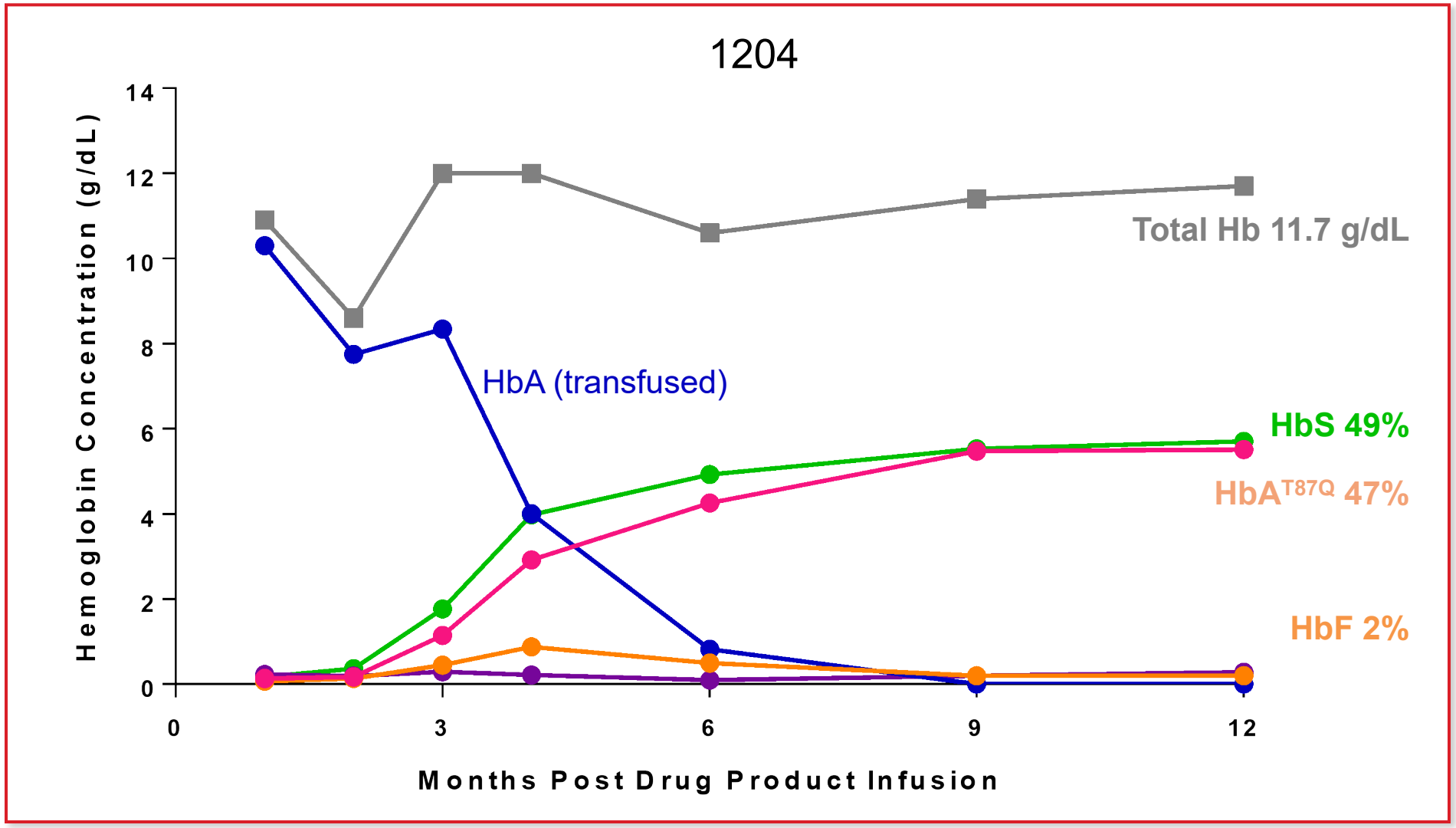


12-16 g/dl Hb

Overview of gene therapy clinical protocol



Severe SCD Subject 1204: Equal ratio of anti-sickling hemoglobin (HbAT87Q + HbF) and HbS by 9 months



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CLINICAL TRIALS FOR B-HEMOGLOBINOPATHIES (LV)

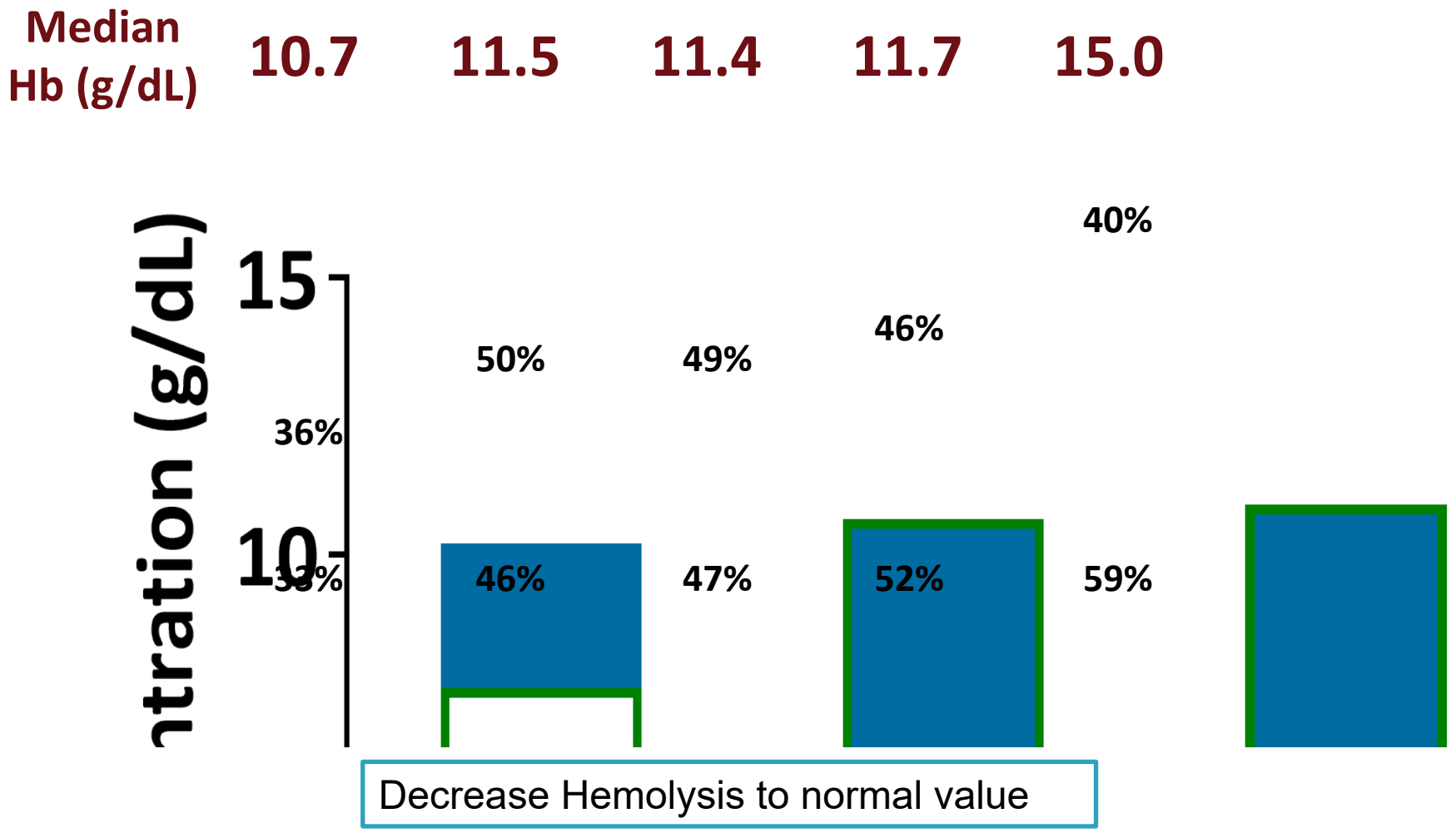
Table 1. Gene therapy clinical trials for TDT and SCD patients

Trial number	Phase	Sponsor	Site	Start date/ recruitment status	Number of patients	Vector and transgene (nuclease and DP name)	Cell source	Conditioning	DP administration	Last update (www.clinicaltrials.gov)
β-Thalassemia										
LG001	1/2	bluebird bio	France	September 2006/ completed	2*	HPV569 ($\beta^{A\text{-}T87Q}$-globin)	G-CSF mPBCs or BM	Myeloablative (busulfan)	IV	NA
NCT01639690	1	Memorial Sloan Kettering Cancer Center	United States	July 2012/active, not recruiting	4	TNS9.3.55 (β^-globin)	G-CSF mPBCs	Nonmyeloablative (busulfan 8 mg/kg)	IV	6 June 2018
NCT02151526 (HGB205)	1/2	bluebird bio	France	July 2013/active, not recruiting	4	BB305 ($\beta^{A\text{-}T87Q}$-globin)	G-CSF + plerixafor mPBCs	Myeloablative (busulfan)	IV	31 January 2019
NCT01745120 (HGB204)	1/2	bluebird bio	United States, Australia, Thailand	August 2013/ completed	18	BB305 ($\beta^{A\text{-}T87Q}$-globin)	G-CSF + plerixafor mPBCs	Myeloablative (busulfan)	IV	8 May 2019
NCT02453477	1/2	IRCCS San Raffaele	Italy	May 2015/active, not recruiting	10	GLOBE (βA-globin)	G-CSF + plerixafor mPBCs	Myeloablative (thiotepa + threosulfan)	IO	4 May 2018
NCT02906202 (HGB207)	3	bluebird bio	United States, France, Germany, Greece, Italy, Thailand, United Kingdom,	July 2016/recruiting	23 (estimated)	BB305 ($\beta^{A\text{-}T87Q}$-globin)	G-CSF + plerixafor mPBCs	Myeloablative (busulfan)	IV	31 January 2019
NCT02906202 (HGB212)	3	bluebird bio	United States, France, Germany, Greece, Italy, Thailand, United Kingdom	June 2017/recruiting	15 (estimated)	BB305 ($\beta^{A\text{-}T87Q}$-globin)	G-CSF + plerixafor mPBC	Myeloablative (busulfan)	IV	31 January 2019
NCT03432364	1/2	Sangamo Therapeutics and Bioverativ Therapeutics	United States	February 2018/ recruiting	6	ZFN (ST-400)	mPBCs	Myeloablative (busulfan)	IV	4 February 2019
NCT03655678	1/2	Vertex Pharmaceuticals and CRISPR Therapeutics	Germany, United Kingdom	September 2018/ recruiting	12 (estimated; may be expanded to 45)	CRISPR/Cas9 (CTX001)	$CD34^+$ human HSPCs (mobilization: NA)	Myeloablative (busulfan)	IV	3 May 2019
SCD										
NCT02151526 (HGB205)	1/2	bluebird bio	France	July 2013/active, not recruiting	3	BB305 ($\beta^{A\text{-}T87Q}$-globin)	BM	Myeloablative (busulfan)	IV	31 January 2019
NCT02186418	1/2	Children's Hospital Medical Center, Cincinnati	United States, Jamaica	July 2014/recruiting	10	sGbG (γ-globin)	BM and plerixafor mPBCs	Reduced intensity conditioning (melphalan 140 mg/m² BSA)	IV	6 May 2019
NCT02247843	1	University of California Children's Hospital, Los Angeles	United States	July 2014/recruiting	6	βAS3-FB (β^{AS3}-globin)	BM	Myeloablative (busulfan)	IV	29 March 2019
NCT02140554 (HGB206)	1	bluebird bio	United States	August 2014/ recruiting	50 (estimated; 3 groups [A, B, C])	BB305 ($\beta^{A\text{-}T87Q}$-globin)	BM (A and B) plerixafor mPBCs (C)	Myeloablative (busulfan)	IV	20 May 2019
NCT03282656	1	David Williams, Boston Children's Hospital	United States	February 2018/ recruiting	7	BCH_BB-LCR shRNA(miR) shRNA-miR	Plerixafor mPBCs	Myeloablative (busulfan)	IV	24 May 2018
NCT03745287	1/2	Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics	United States	November 2018/ recruiting	12 (estimated; may be expanded to 45)	CRISPR/Cas9 (CTX001)	NA	Myeloablative (busulfan)	IV	3 May 2019

BM, bone marrow; BSA, body surface area; CRISPR, clustered regularly interspaced short palindromic repeat; DP, drug product; G-CSF, granulocyte-colony stimulating factor; IO, intraosseously; mPBC, mobilized peripheral blood cell; NA, not av
short hairpin RNA; ZFN, zinc-finger nuclease.

*P1 failed to engraft and received the backup cells.

HGB-206 Group C: Median HbS ≤ 50% at ≥ 6 months post LentiGlobin treatment



Sickle cell disease
Gene addition

Phase I/II + Phase III Gene trials

n	>60 (1 DCD)
OS	98% (1 DCD) + several SAE recently reported
EFS	75% (around 50% HbS)

- No information available on the follow-up of patients with vascular problems / stroke and priapism: stop of progression
- Heterozygote after gene therapy is not a true carrier
- Some concerns on safety issues just reported :all clinical trials on hold

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Optimization of LV-based gene addition therapies

Lentiviral vector

Weber, MTMCD 2018

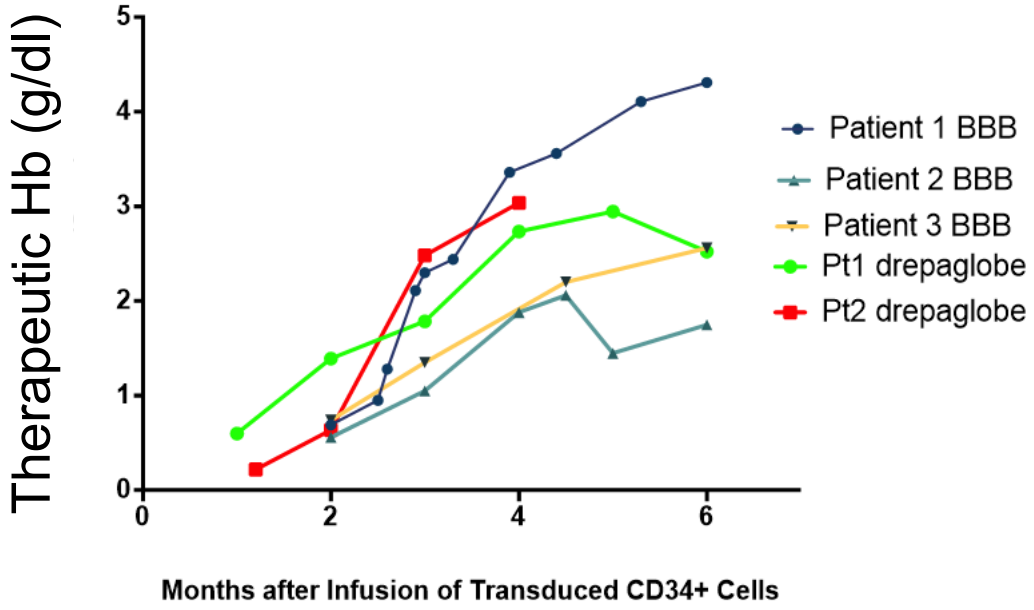
Megane Brusson

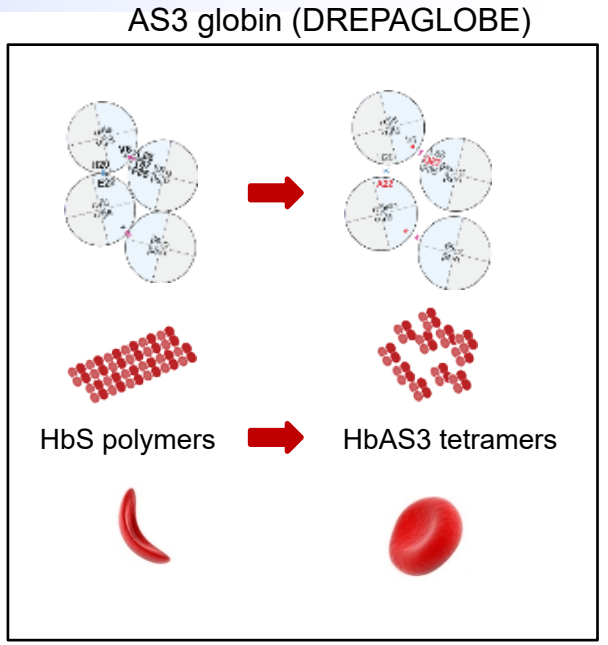
Sophie Ramadier

Vasco Meneghini

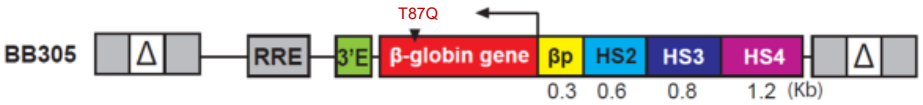
- Increase therapeutic β-like globin expression
- Reduce βS-globin expression (SCD)

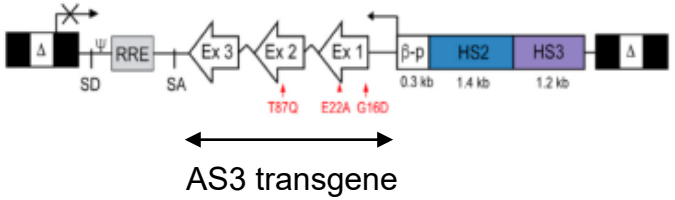
LV-BASED Gene therapy for SCD (BLUEBIRD BIO AND DREPAGLOBE TRIALS)





→ Variable correction of the SCD clinical phenotype



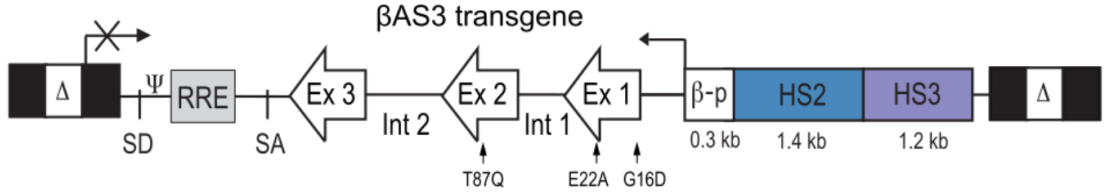


Ribeil, NEJM, 2017); Magrin Nat Med, in press. BBB trial

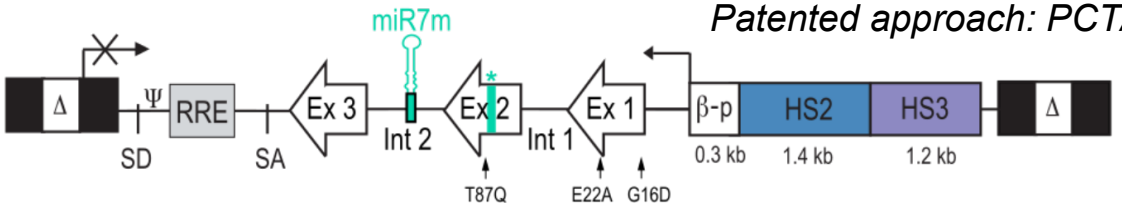
Miccio, PNAS, 2008, Weber, MTCMD, 2018; DREPAGLOBE trial

GLOBE-AS3 WITH MIRNA ANTI-HBS

βAS3-LV (DREPAGLOBE)



βAS3m/miR7m-LV

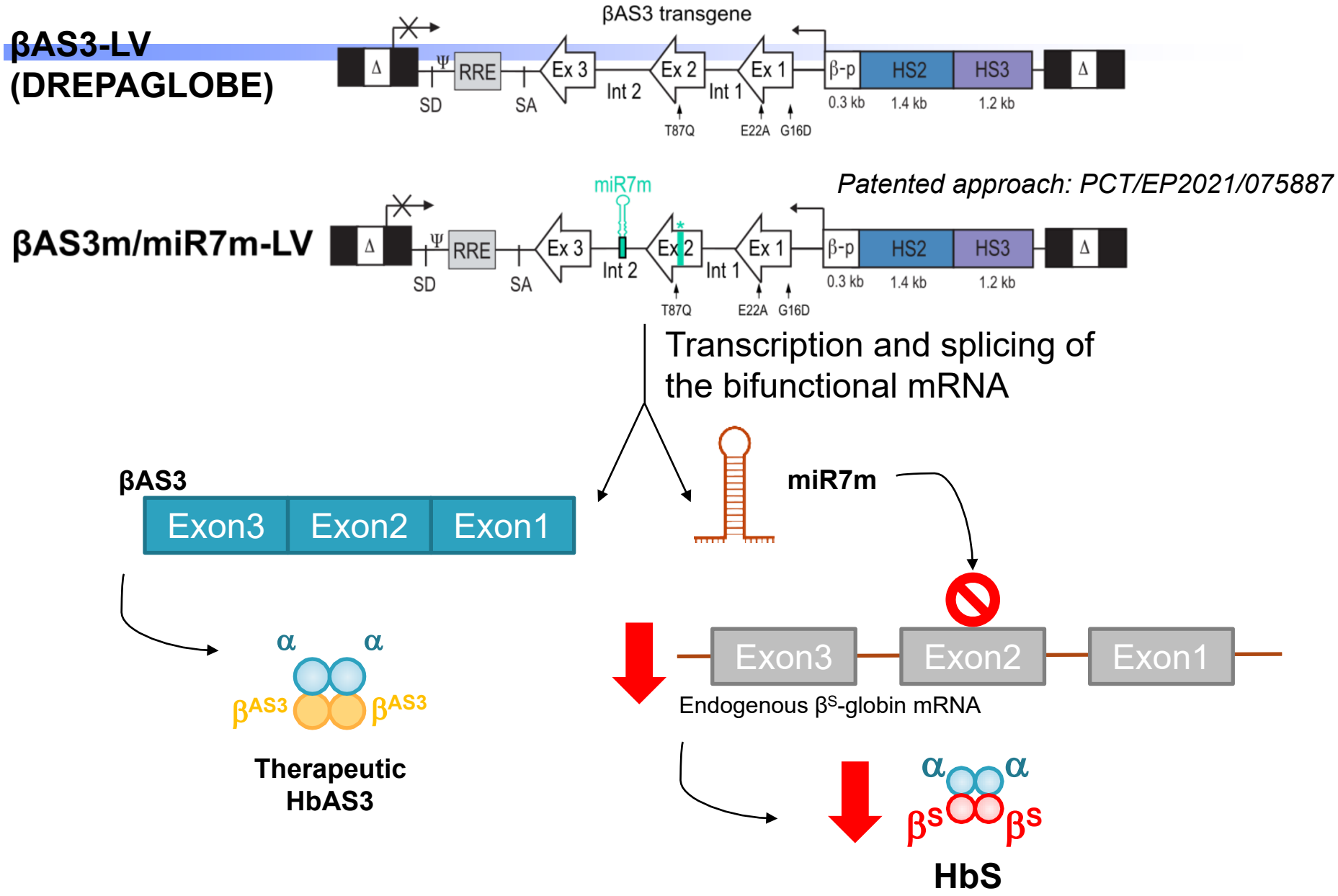


Patented approach: PCT/EP2021/075887

VECTOR: bifunctional lentiviral (LV) vectors to **efficiently transduce HSPCs and to prevent HbS production in their erythroid progeny** by the combination of an anti-sickling β-globin and a miRNA anti-sickle βS-globin.

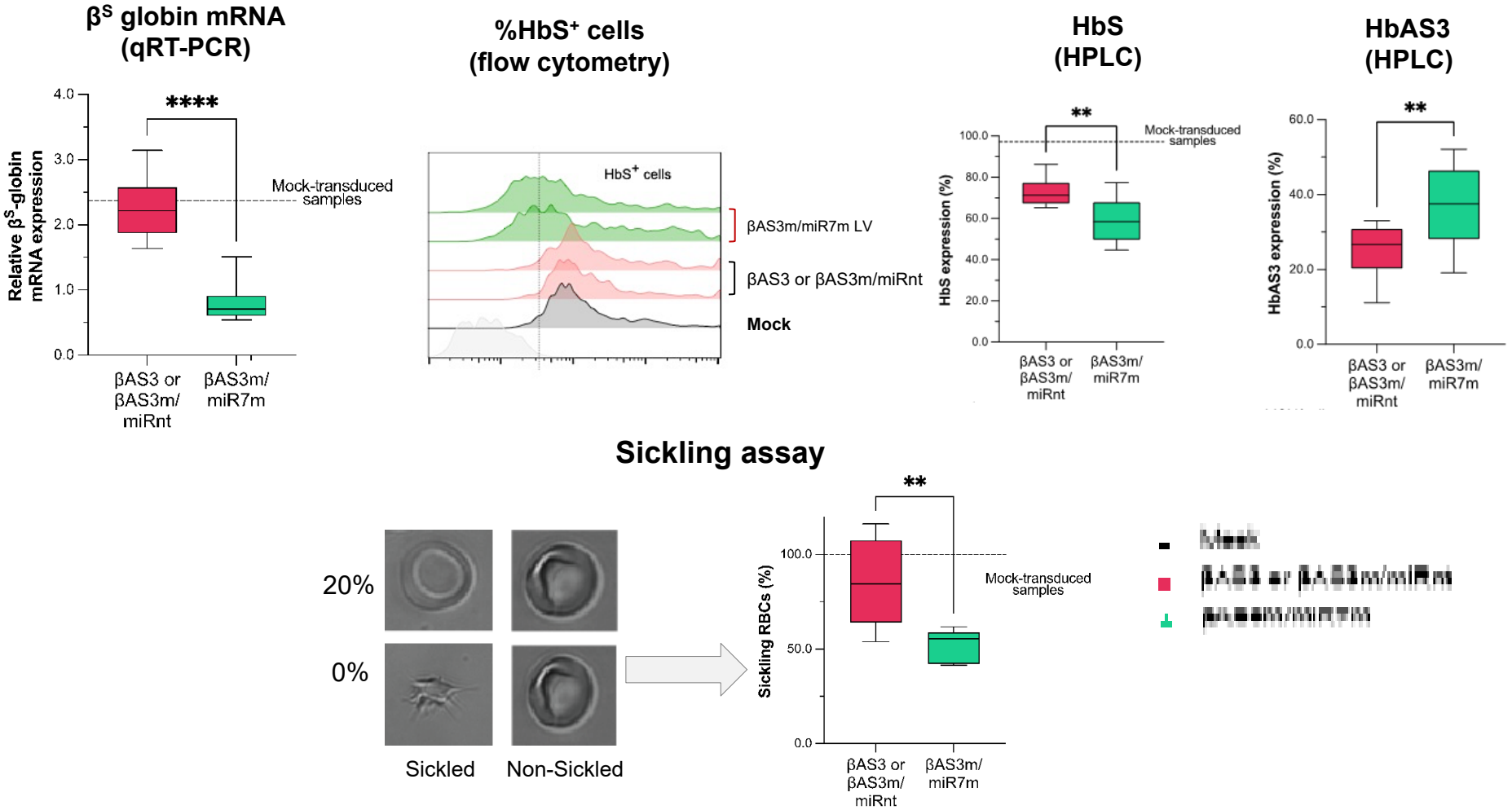
ATMP: Autologous CD34+ cells transduced ex vivo by a bifunctional GLOBE-AS3 lentiviral vector with a micro-RNA (miRNA) targeting specifically the endogenous sickle βS-globin gene.

GLOBE-AS3 WITH MIRNA ANTI-HBS



BIFUNCTIONAL LV MEETS THE EFFICACY REQUIREMENTS

Adult HSPCs from 3 SCD patients (including 2 patients of the DREPAGLOBE trial)

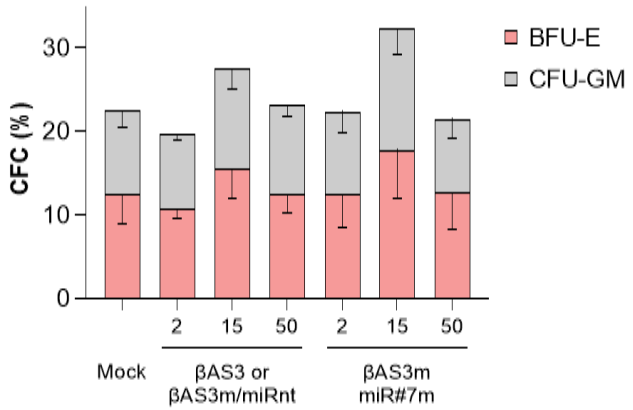


The bifunctional βAS3m/miR7m LV downregulates β^S-globin expression and increases βAS3 incorporation in Hb and ameliorates red blood cell sickling

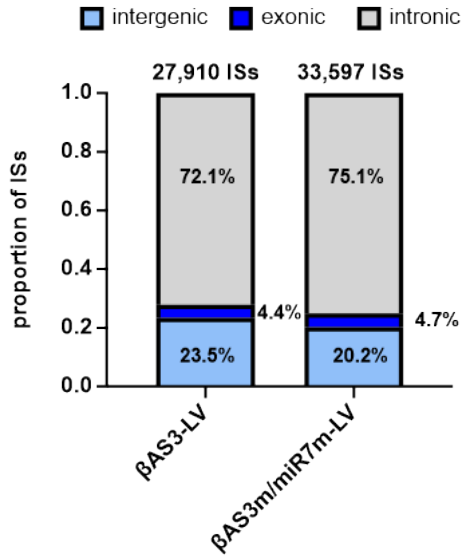
BIFUNCTIONAL LV MEETS THE SAFETY REQUIREMENTS (2)

Adult HSPCs from 3 SCD patients (including 2 patients of the DREPAGLOBE trial)

Progenitor assay

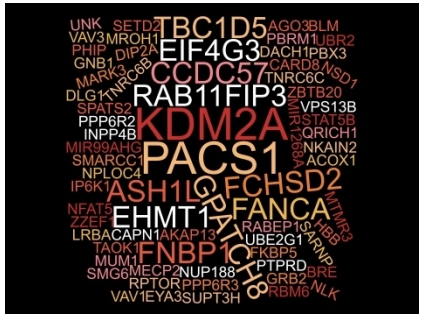


Integration site analysis (1 healthy donor)



Top targeted genes

βAS3-LV



βAS3m/miR7m-LV

The βAS3m/miR7m LV does not affect HSPC clonal growth and differentiation towards erythroid and granulocyte-monocyte lineages

Standard LV integration profile

SAFETY EVENTS UPDATE

	Patient 1303*	Patient 1301	Patient 1351
Reported	2018	Feb 2021	Feb 2021
Age	42	31	20
HGB206 group	A	A	C
Genotype	S/S	S/S	S/S, alpha thal trait
Diagnosis	MDS to AML, death	AML	MDS
Cytogenetics and NGS	Monosomy 7, abnormal 19p, RUNX1 (NP_001745.2:p.Asp198Gly),PTPN11(NP_002825.3:p.Phe71Leu), KRAS NP_203524.1:0.Gly12Ala	Monosomy 7, partial loss of 11p, RUNX1 Exon 5 stop gained p.A149fs, PTPN11 Exon 3 missense: p.A72V	Trisomy 8
Timing	3.5 years after treatment	5.5 years after treatment	6 months after treatment
Vector	Vector not in blasts	Vector in blasts, suspect insertion at VAMP4	No blasts or dysplastic cells reported
Causality	Possibly busulfan	Unlikely related to bb1111	MDS diagnosis changed to transfusion dependent anemia

*prior case of MDS in HGB-206

STATE OF ART: RETROVIRAL VECTORS AND GENOTOXICITY

	ɣ-retrovirus vector	S/N-ɣ	S/N-lentivirus vector
Integration	++	++	++
Cell cycle dependent	+++	+++	+/-
Integration rules	Semi-random promoter oncogene	Semi-random ↘↘ oncogene	Random No preference X regulatory regions
Patients treated	≈ 110	9	> 350
SAE / insertional mutagenisis	20/110	0	X-ALD (n=3) Retroviral promoter (MND)

LENTIVIRUS VECTORS AND GENOTOXICITY

- Strictly dependent on the vector construction

- Mandatory to avoid:
  - Use of large insulators on LTR
  - Use of strong or ubiquitous ɣ-retroviral promoter (cf MND / Bluebird Bio)

- VCN:
  - FDA recommendations
  - ANSM recommendations

} ≠ 1-3 for caution

Clinical and Biological data in favour of accumulation of dangerous hits in SCD -BM cell

Sickle cell disease and hematopoiesis: Mutations, clonal evolution, MDS and AML

Role of the disease ?

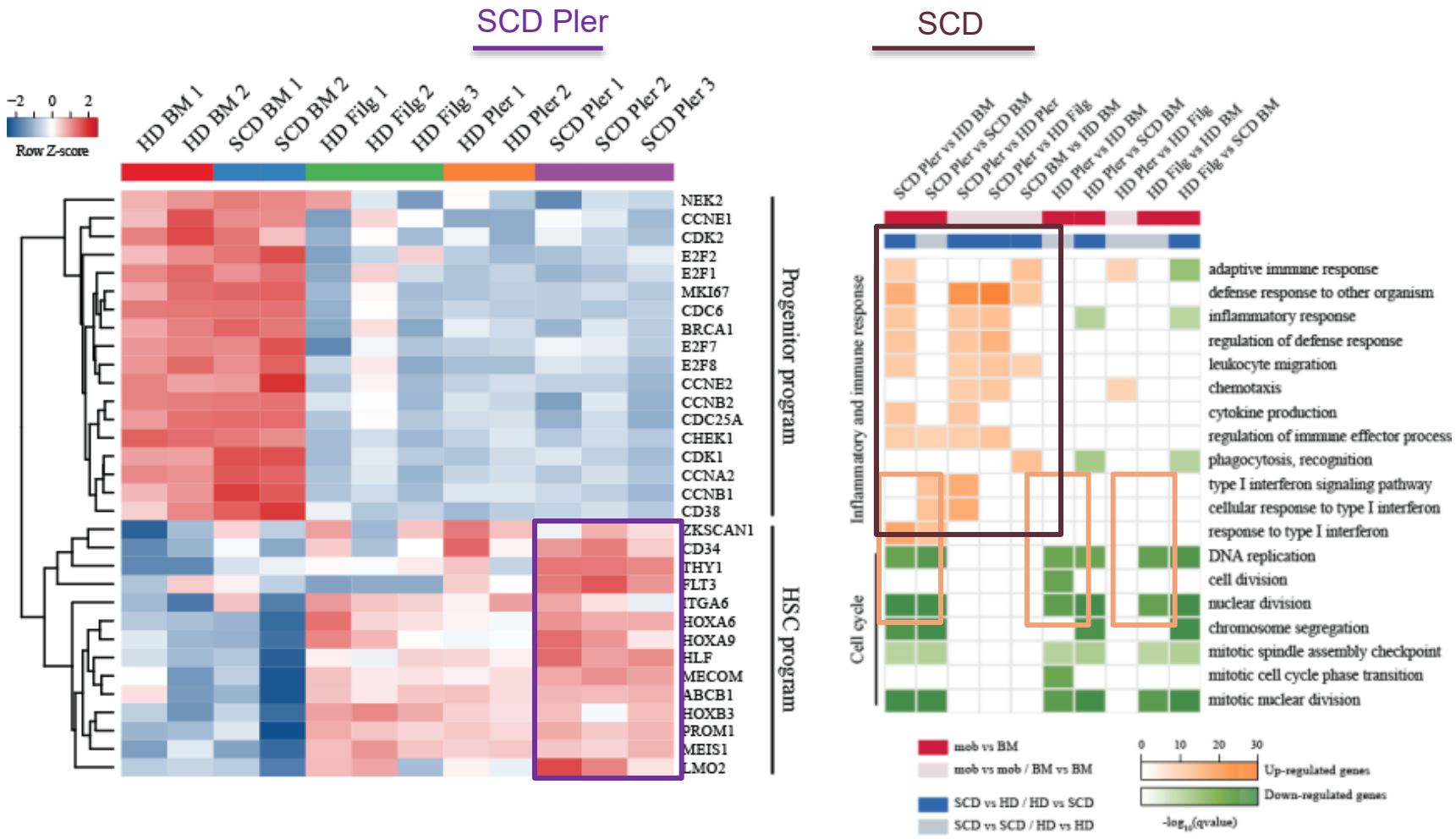
- Chronic Hypoxia and ineffective erythropoiesis
- Endothelium damage
- Chronic acidosis
- Chronic Inflammation
- Chronic Hemolysis /stress erythropoiesis
- Genetic factors*

Role of the treatments?

- Transfusion and iron overload
- CR and HSCT: influence of mixed chimerism or rejection
- Gene Therapy?

*Modified from Bick, BioRxIv 2019

TRANSCRIPTOMIC PROFILE OF PLERIXAFOR-MOBILIZED SCD HSPCS



HD: healthy donor, BM : bone marrow, SCD: sickle cell disease, Pler: Plerixafor, Filg: Filgrastim

CONSEQUENCES ON BMC OF INFLAMMATION AND HYPOXIA

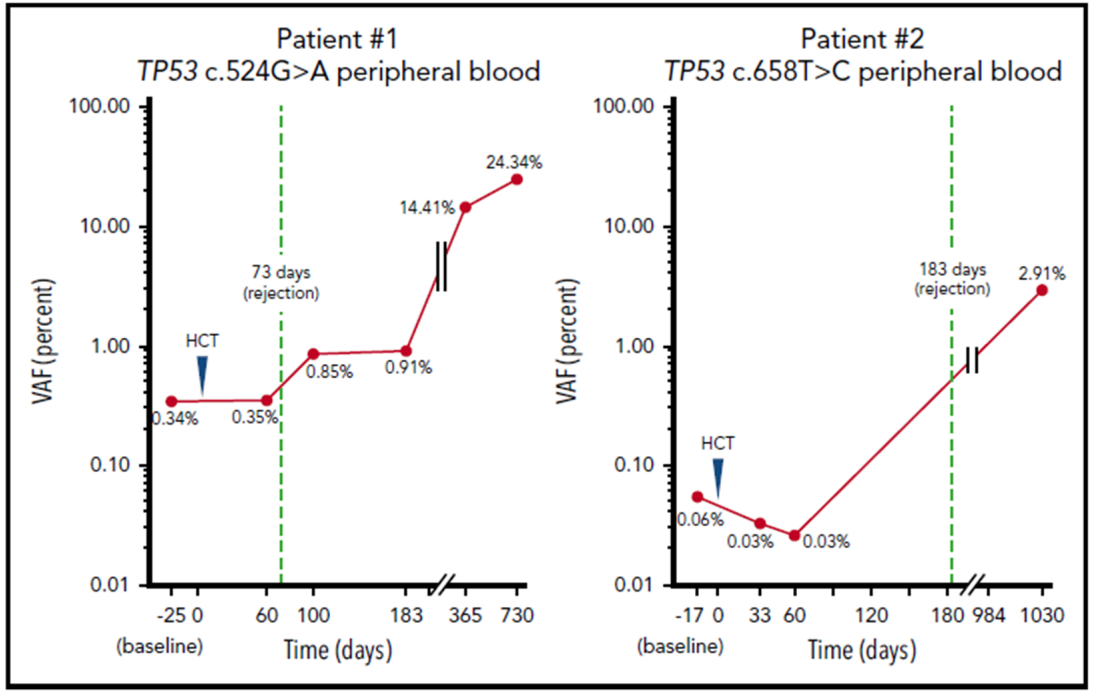
1. Clonal hematopoiesis
2. Decreased number of HSC: poor stem cell reservoir > exhaustion > failure to engraft
3. Increase number of precursor cells committed to erythroid and myeloid lineages
4. Trained immunity: long term functional reprogramming of innate immune cells

Clinical consequences of CHIP in patients undergoing HSCT (auto + allo)

- Affect stem cell collection and engraftment following auto HSCT
- Presence of CHIP is associated with adverse outcomes: 10 years of cumulative evidence of therapy-related neoplasm 14% vs 4.3%; OS 61% vs 19%
- CHIP and Allo HSCT: - Donor: pre-existing or during HSC
  - - Host: mixed chimerism
- VAF > 0.2% High risk of relapse (VAF 5.9% in 16% of donors)

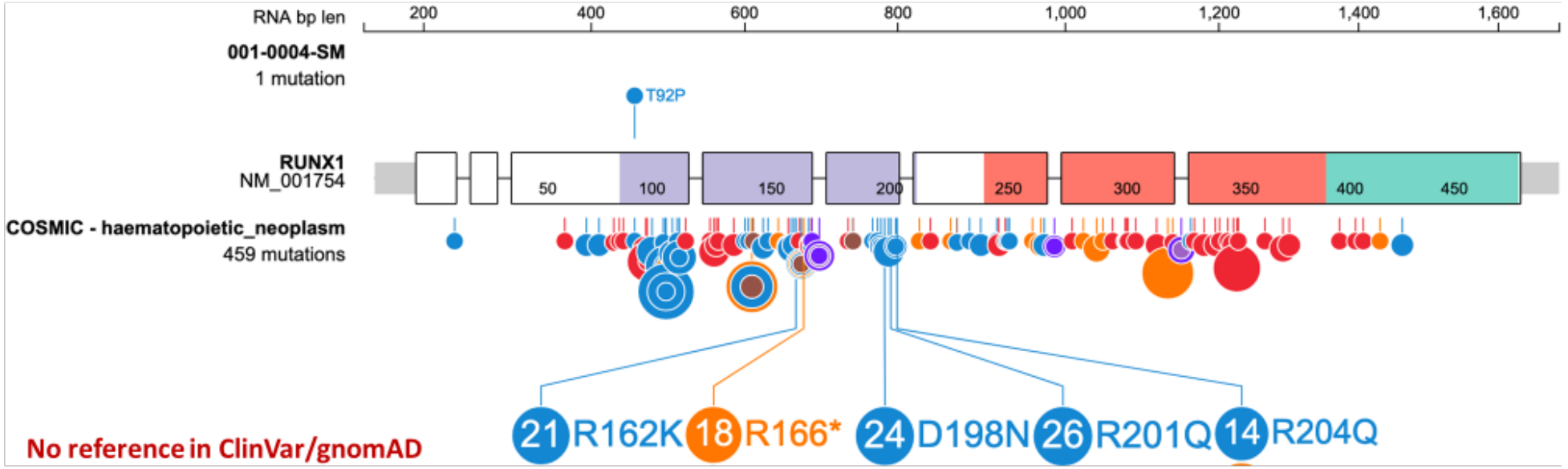
Baseline *TP53* mutations in adults with SCD developing myeloid malignancy following hematopoietic cell transplantation

Jack Y. Ghannam,[1,*] Xin Xu,[2,*] Irina Maric,[3] Laura Dillon,[1] Yuesheng Li,[4] Matthew M. Hsieh,[2] Christopher S. Hourigan,[1,†] and Courtney D. Fitzhugh[2,†]



VAF Detection during Screening before GT Enrollment (18 yrs – Bone Marrow Cells)

Mutation	VAF Before Gene therapy	VAF After Gene therapy	VAF During Follow up
RUNX1 - c.274A>C – **p.T92P**	**17%**	-	-



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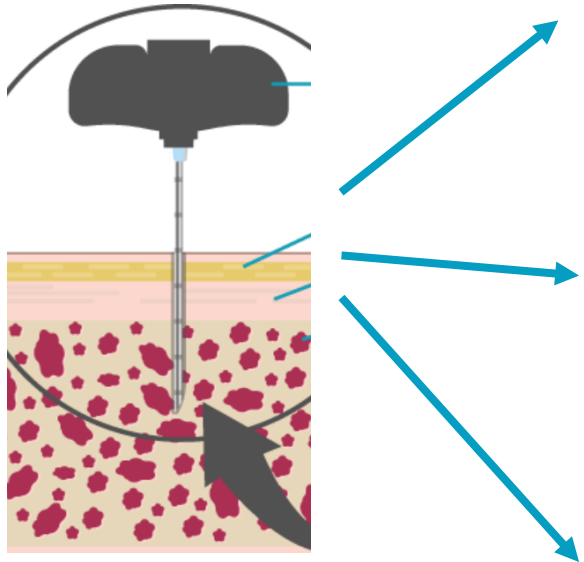
ASSESSMENT OF CLONAL HEMATOPOIESIS, TELOMERE LENGTH AND INFLAMMATION IN SICKLE CELL DISEASE

- SAE (AML, MDS) after in gene therapy in SCD patients → need of safety screening

100 severe SCD patients eligible to gene therapy (12 – 50 yrs)



Clonal hematopoiesis
NGS panel, 38 most frequently mutated genes in CH
BioNano technology

Inflammation
single cell-RNA Seq

Telomere lenght
Flow FISH

Predictive model of risk for hematological malignancies

Aknowledgements

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