Gene therapy for hemoglobinopathies

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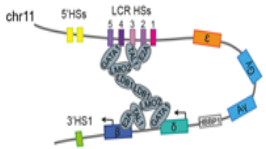
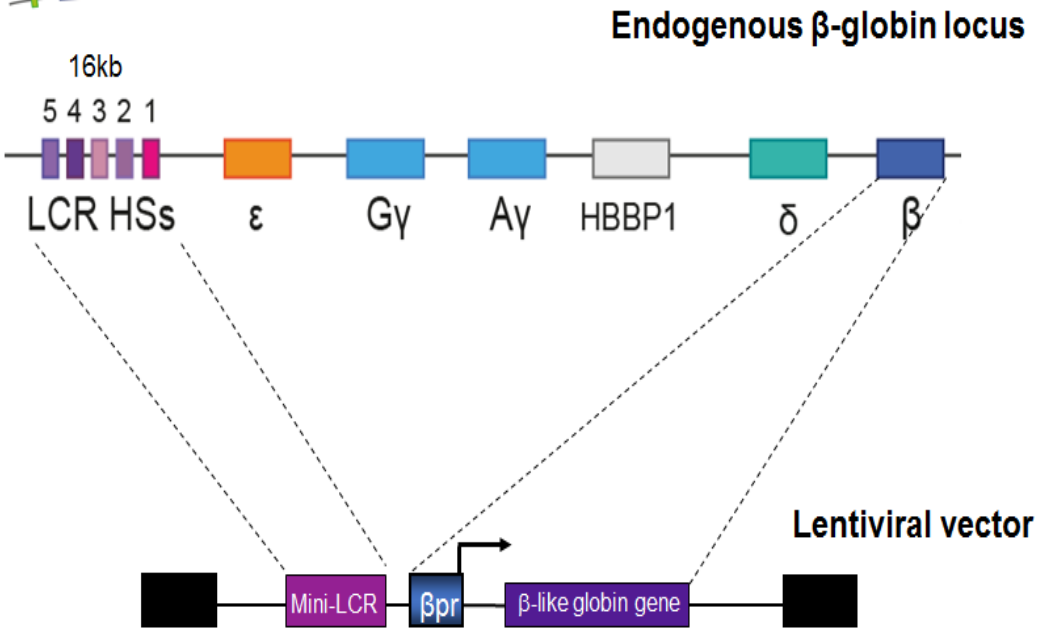
LV-BASED GENE ADDITION THERAPIES





Clinical trials for β-hemoglobinopathies (LV)

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

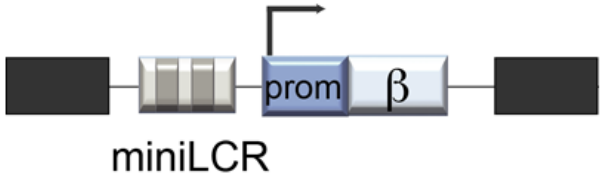
Trial number	Phase	Sponsor	Site	Start date/ recruitment status	Number of patients	Vector and transgene (nuclease and DP name)	Cell source	Conditioning	DP administration	Last update (www.clinicaltrials.gov)
β-Thalassemia										
LG001	1/2	bluebird bio	France	September 2006/ completed	2*	HPV569 (β^{A-T87Q}-globin)	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^{A}-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 (β^{A-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 (β^{A-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 (β^{A-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 (β^{A-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 (β^{A-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 (β^{A-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) shRNAmiR	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.

LV-BASED GENE ADDITION THERAPIES

Lentiviral vector



β-thalassemia

Partial/Full correction of β+ thal

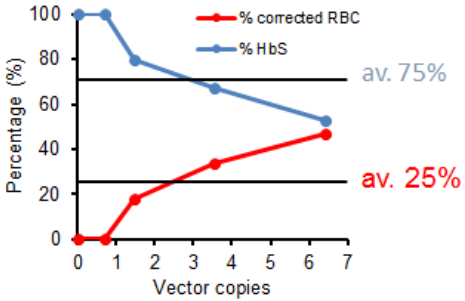
Partial correction of β0 thal

→ Increase β-like globin expression

Cavazzana, Nature, 2010; Marktel, Nature Medicine, 2019; Thompson, NEJM, 2018

SCD

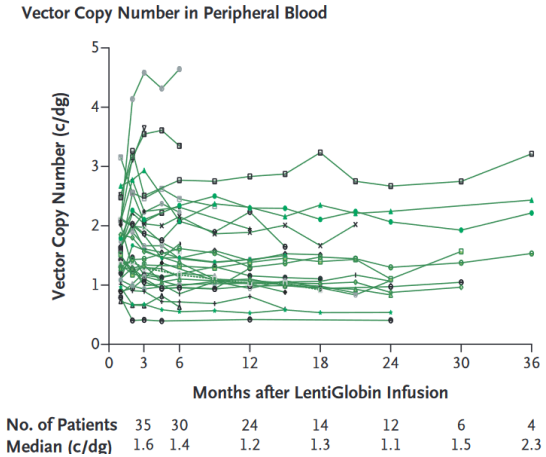
Partial correction *(Weber, MTCMD, 2018; DREPAGLOBE trial)*

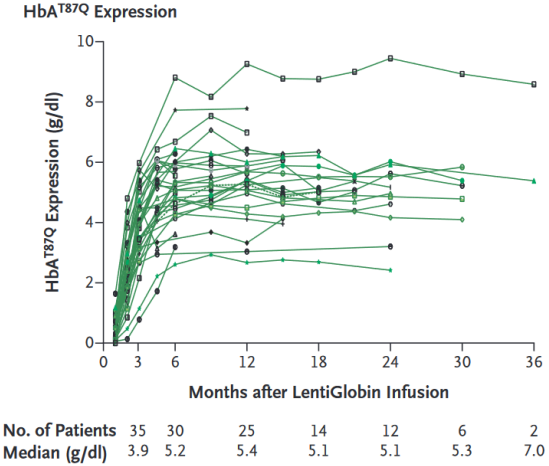


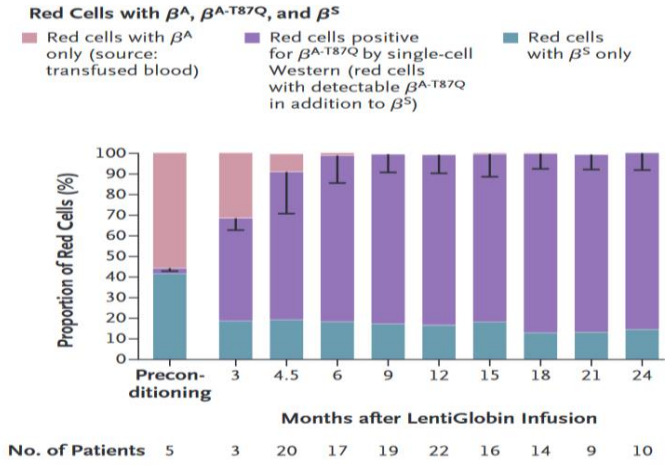
→ Increase β-like globin and reduce βS-globin

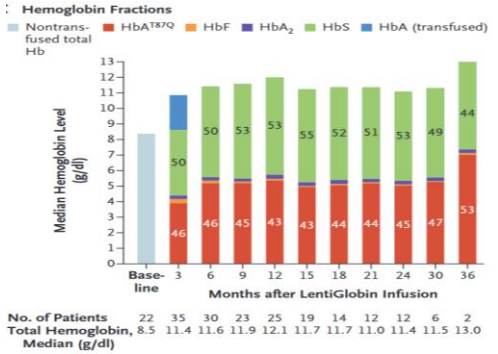
Ribeil, NEJM, 2017; Magrin, Nat Med., under rev.

Biological and Clinical Efficacy of Lentiglobin for Sickle Cell Disease





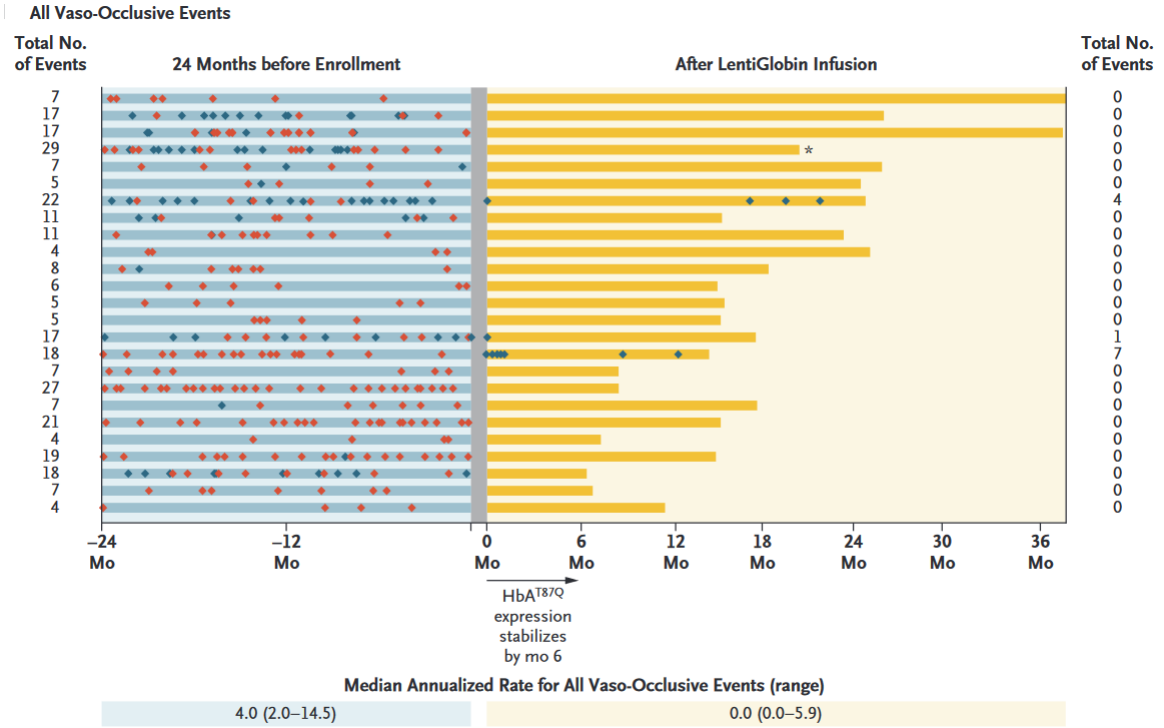




Median FU 17 months

J. Kanter et al. 2022 NEJM

Changes in the Rate of Vaso-occlusive Events before and after LentiGlobin infusion



J. Kanter et al. 2022 NEJM

Sickle cell disease
Gene addition

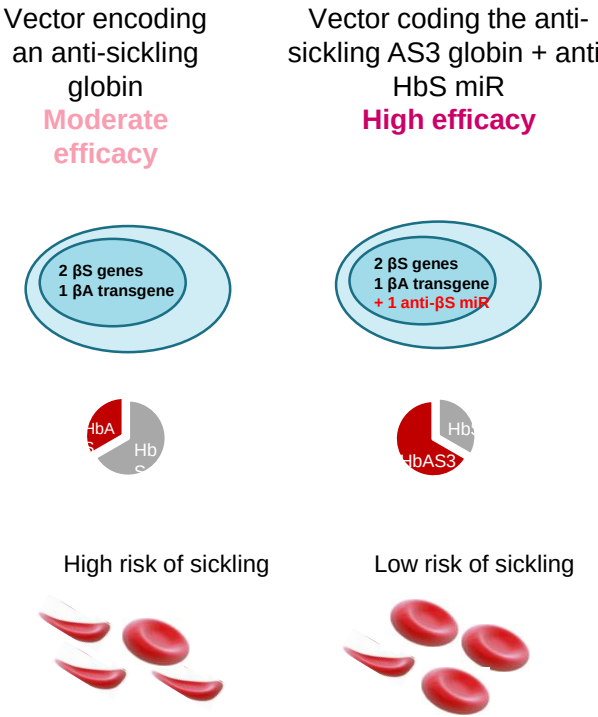
Phase I/II + Phase III Gene trials

n	32 (1 DCD)
OS	98% (1 DCD) +2 SAE recently reported
EFS	75% (around 50% HbS)

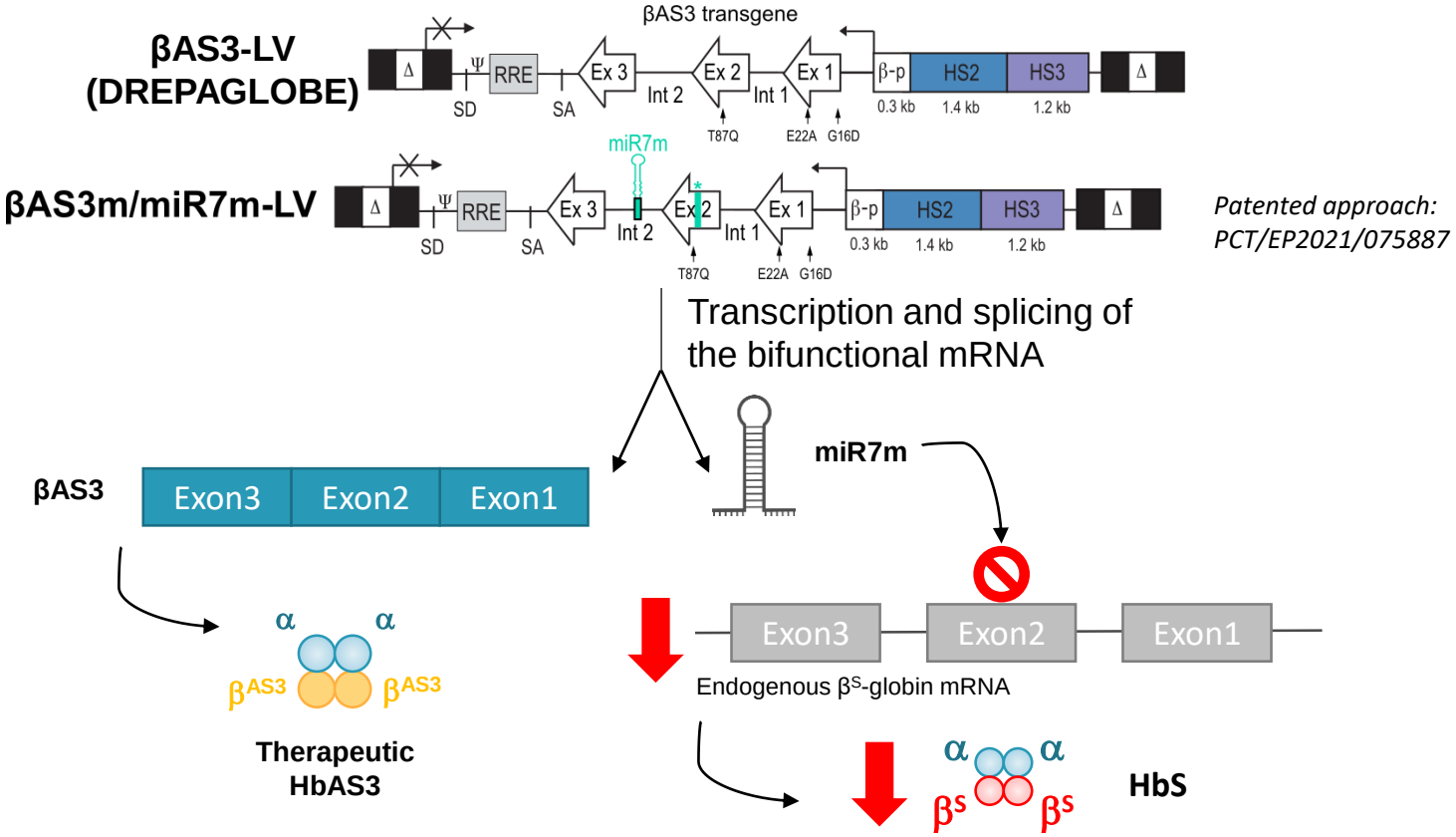
- No information available on the follow-up of patients with vascular problems / stroke and priapism: stop of progression
- Heterozygote after gene therapy is not a true carrier
- Some concerns on safety issues due the diseased bone marrow

Silencing HbS synthesis is crucial for the efficacy of gene therapy

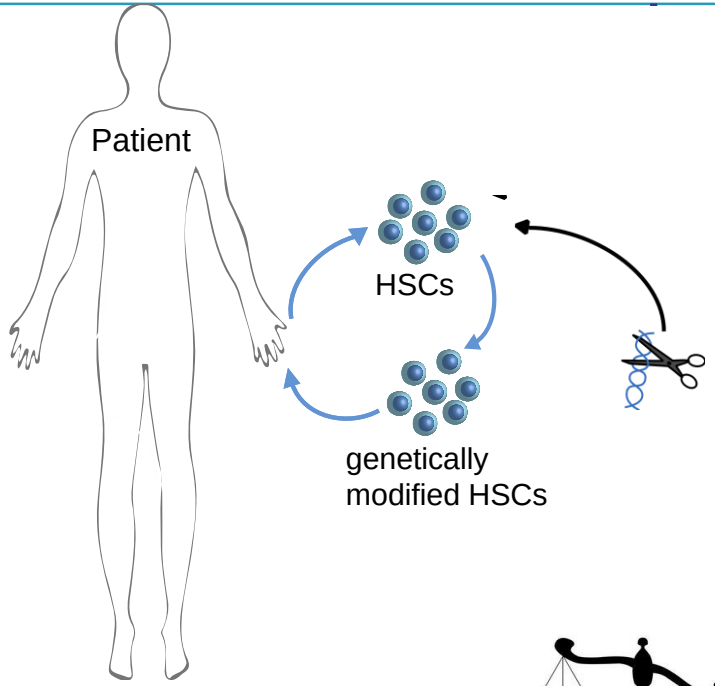
- The natural history of SCD indicates that the risk of sickling is reduced when the amount of HbS per cell is <40%
- The experience of allogeneic HSC transplantation indicates that SCD is corrected when the proportion of S-cells in the circulation is <30-40%
- The target of gene therapy is therefore to reduce the proportion of HbS to <40% of total Hb in >60-70% of the circulating erythrocytes

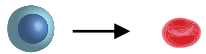


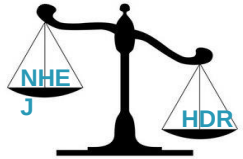
GLOBE-AS3 WITH miRNA ANTI-HbS



Nuclease mediated strategy for γ-globin reactivation



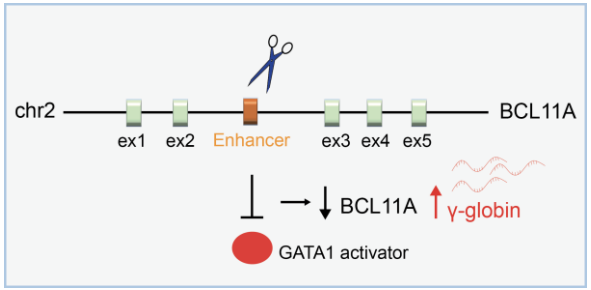




Quiescent HSCs

γ-globin reactivation

KO of the γ-globin repressor BCL11A



Pros: efficient in HSCs
no need for corrective dDNA

Cons: DSB-induced toxicity

Baseline Demographics and Clinical Characteristics of the 31 Patients With SCD Infused With Exa-cel

CLIMB SCD-121

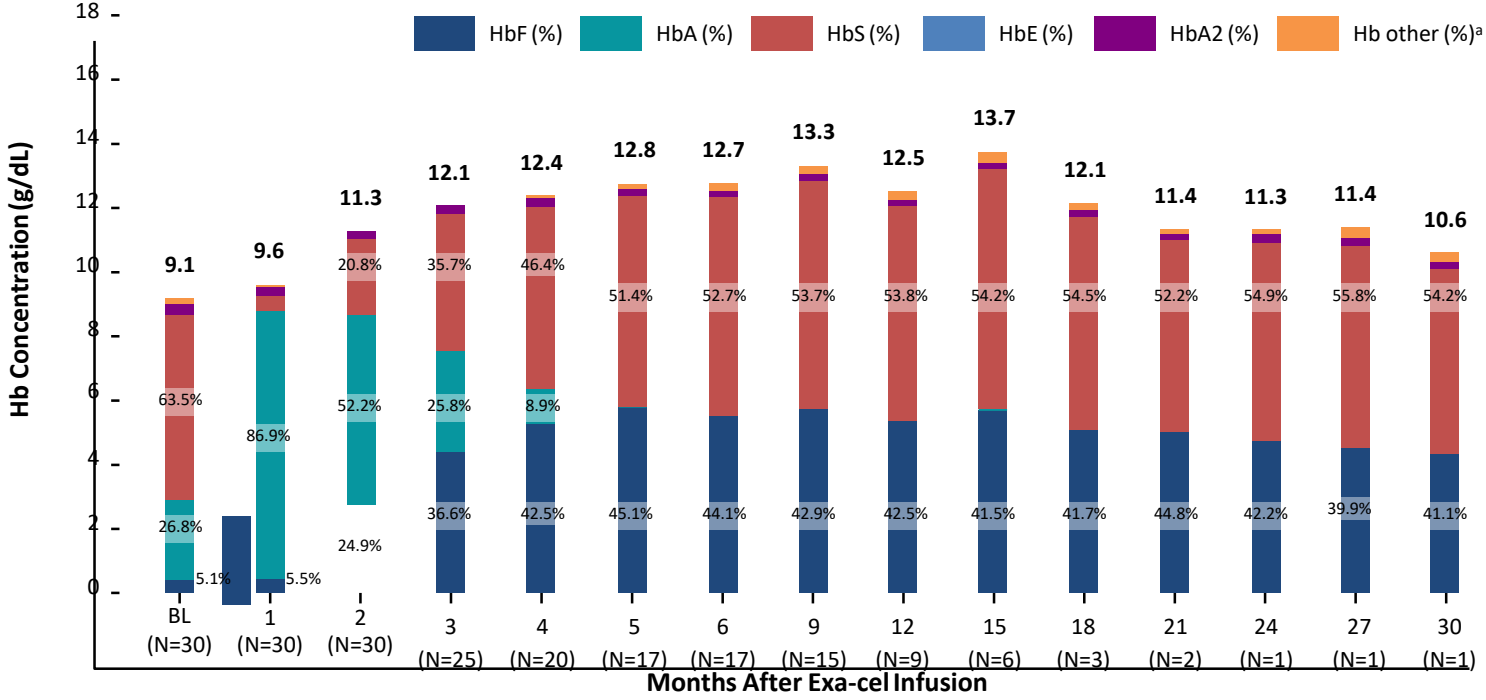
	Exa-cel (SCD) n = 31
Sex, n (%)	
Male	16 (51.6)
Female	15 (48.4)
Genotype, n (%)	
β^S/β^S	29 (93.5)
β^S/β^0	2 (6.5)
Age at baseline, years, mean (min, max)	22.5 (12, 34)
Historical VOC episodes per year,[a] mean (min, max)	3.9 (2.0, 9.5)

Data cut-off February 2022

SCD, sickle cell disease; **VOC,** vaso-occlusive crisis.

[a] Annualized rate during the 2 years before signing of the informed consent form or the latest rescreening.

Patients With SCD Had Clinically Meaningful Increases in HbF (>20% that Occurred Early and Were Sustained Over Time



Data cut-off February 2022

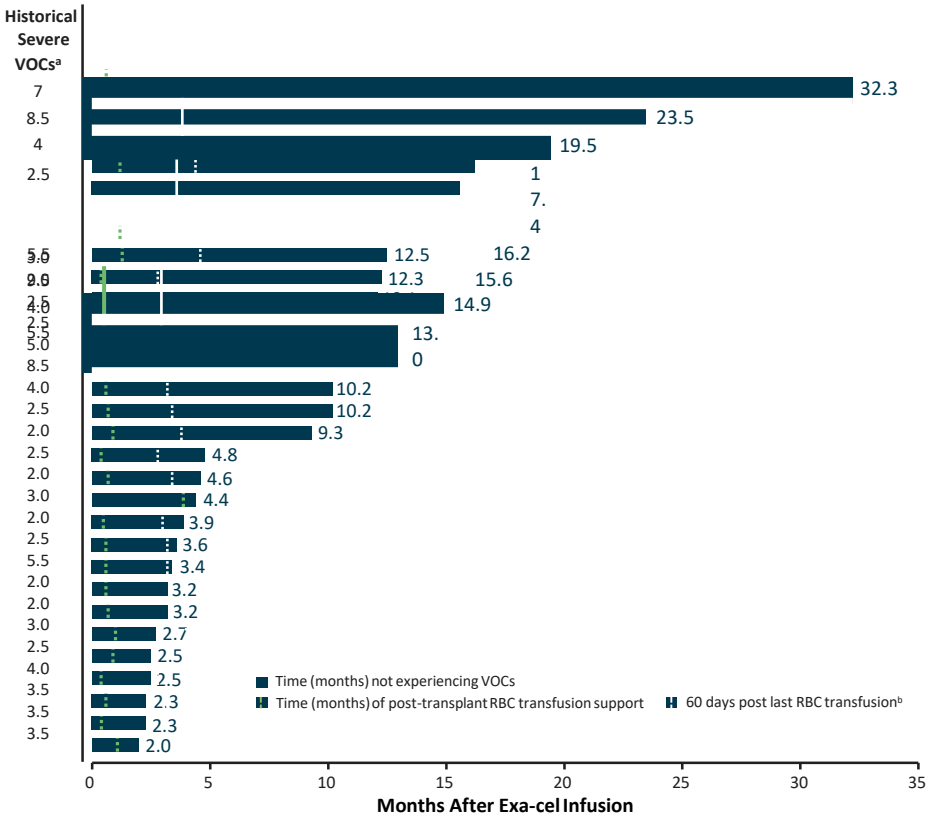
BL, baseline; **Hb,** hemoglobin; **HbA,** adult hemoglobin; **HbA2,** hemoglobin alpha 2; **HbE,** hemoglobin E; **HbF,** fetal hemoglobin; **HbS,** sickle hemoglobin; **SCD,** sickle cell disease.

Bars show mean Hb (g/dL). Labels indicate mean proportion of HbS and HbF as a percentage of total Hb. Mean total Hb concentrations are shown directly above bars.

[a] Hb adducts and other variants.

All Patients With SCD Treated With Exa-cel Were VOC-Free

- Time (months) since **exa-cel infusion** is indicated by the dark bar
- 31 of 31 patients were **VOC-free** after exa-cel infusion (duration from 2.0 to 32.3 months)



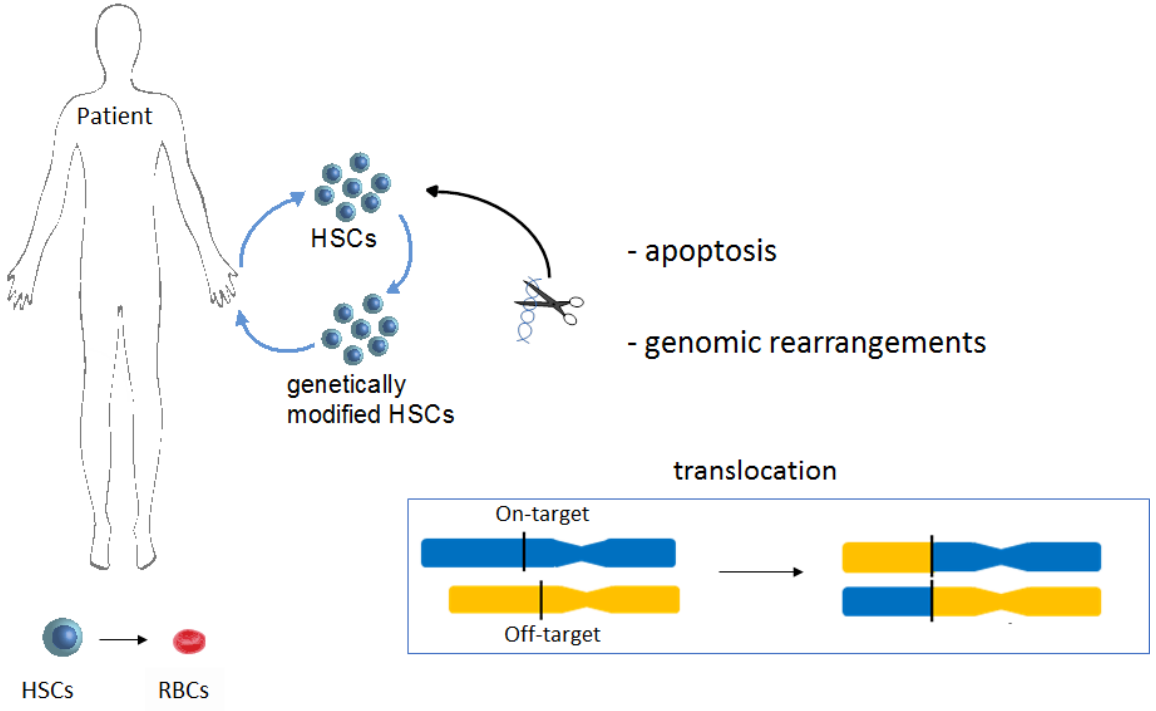
Data cut-off February 2022

Each row in the figure on the right represents an individual patient.

[a]Pre-study severe VOCs annualized over 2 years; [b]Patients are evaluated for elimination of VOCs starting 60 days after their last transfusion.

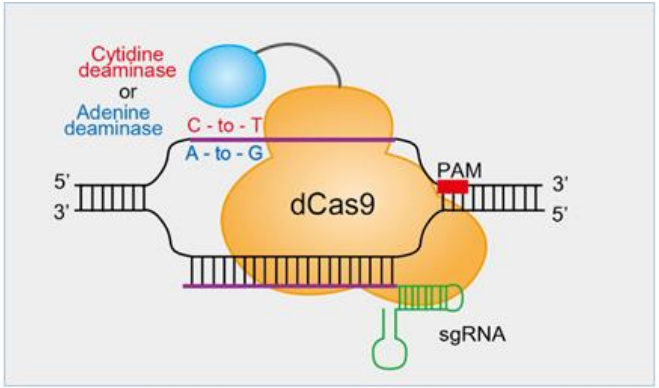
RBC, red blood cell; **SCD,** sickle cell disease; **VOC,** vaso-occlusive crisis.

DSB-induced toxicity: the main drawback of nuclease-based approaches



Base editing

David Liu,
Broad Institute



CBE C>T G>A

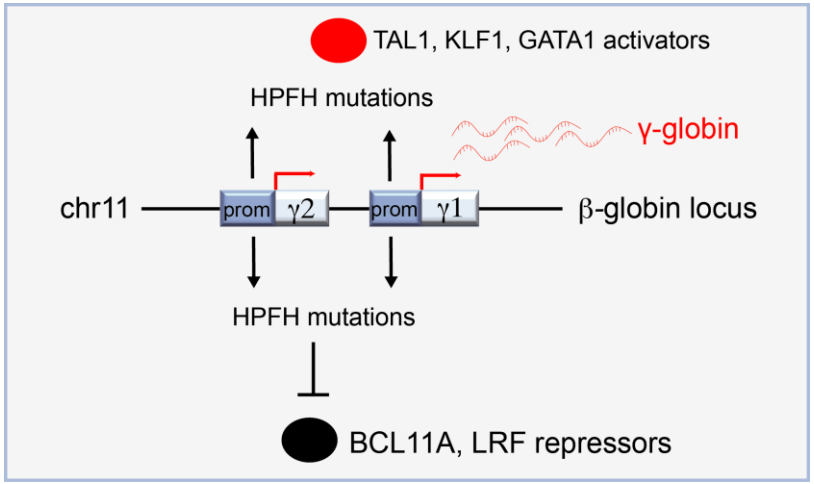
ABE A>G T>C

Pros:

- *no DNA damage response/genotoxicity*
- *no donor DNA template*
- *efficient in quiescent cells (e.g. HSCs)*

Hereditary persistence of fetal hemoglobin (HPFH)

HbF



HbF accounting for 10 to 40% of total Hb

Genome editing vs LV gene addition therapy of hematopoietic disorders

- Lentivirus vector approach is safe and efficient approach in the correction of inherited disorders, but further effort is necessary to improve the access of the patients and substantially decrease the cost
- HDR-based gene correction, as opposed to gene addition, may not only restore the function but also the physiological expression of the gene. However, HDR has a low efficiency in hematopoietic stem cells and presents important side effects . Further improvements are needed in HSC
- Base editing is very efficient in hematopoietic stem cells, with lower detection of off-target or immune response
- Costs: non-viral delivery costs might be lower than LV

Aknowledgements

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