CRISPR-Cas9 Gene Editing for Treatment of Sickle Cell Disease

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Conflict of Interest

- Vertex Pharmaceutical Steering committee membership

Celebrating Scientific Milestones

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

MARTIN JINEK, KRZYSZTOF CHYLINSKI, INES FONFARA, MICHAEL HAUER, JENNIFER A. DOUDNA , AND , EMMANUELLE CHARPENTIER

Science June 28,2012

Nobel Prize in Chemistry Awarded to 2 Scientists for Work on Genome Editing

Emmanuelle Charpentier and Jennifer A. Doudna developed the Crispr tool, which can change the DNA of animals, plants and microorganisms with high precision.

"One of gene technology's sharpest tools: the CRISPR-Cas9 genetic scissors"

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Gene Editing

- Gene editing tools allows scientists to make very precise changes in the DNA.
- These tools allow genetic material to be disrupted, deleted, corrected or inserted at precise locations at specific locations in the genome.
- Several ongoing clinical trials using gene editing to treat sickle cell disease
  - Gene disruption to induce fetal hemoglobin production (CLIMB-121, BEAM-101, EDIT-301)
  - Gene correction of sickle cell mutation (BEAM-102, GPH-101)

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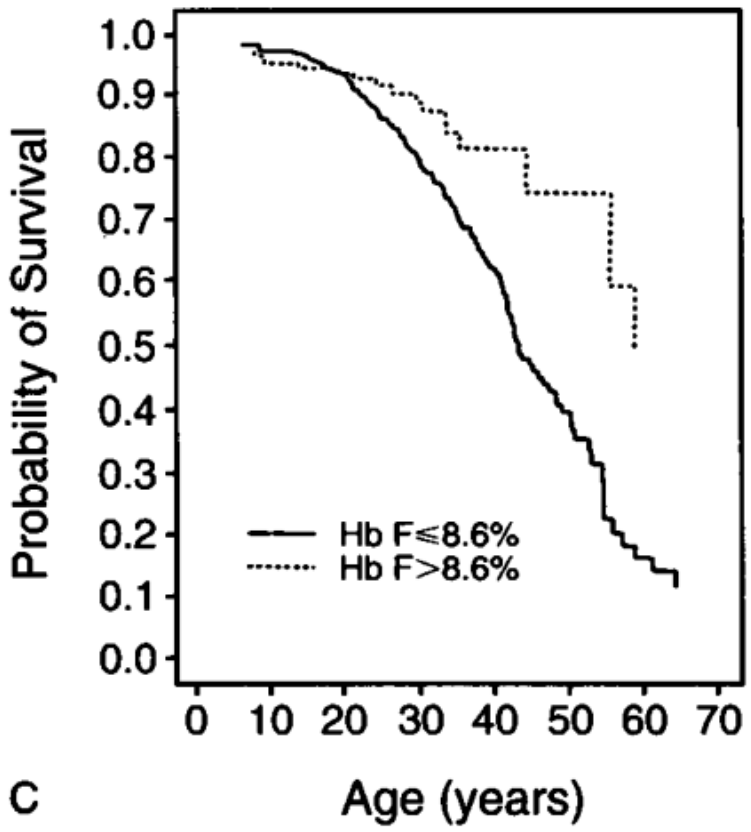
Protective Effect of Fetal Hemoglobin



ORIGINAL ARTICLE

Mortality In Sickle Cell Disease -- Life Expectancy and Risk Factors for Early Death

Orah S. Platt, Donald J. Brambilla, Wendell F. Rosse, Paul F. Milner, Oswaldo Castro, Martin H. Steinberg, and Panpit P. Klug*et al.

June 9, 1994

The NEW ENGLAND JOURNAL of MEDICINE

In 3764 patients in the pre-hydroxyurea era, HbF > 8.6% was associated with improved survival

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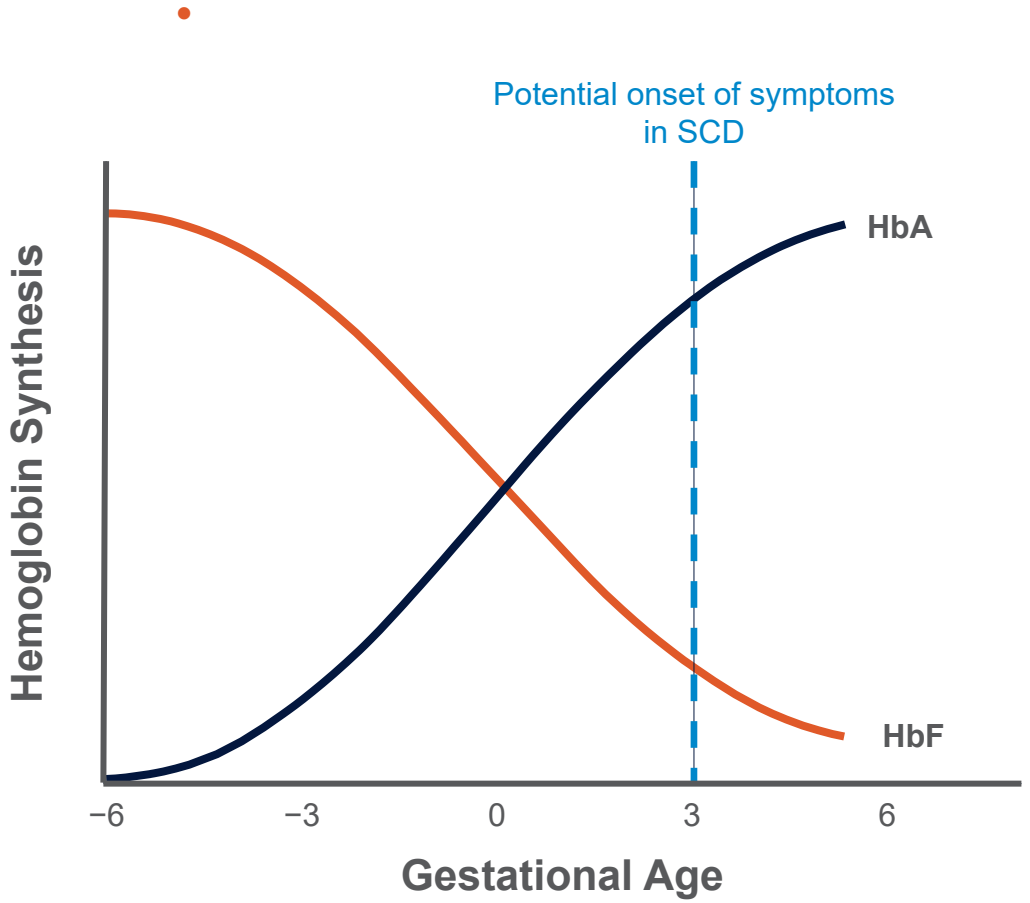
Platt OS, et al. *N Engl J Med*. 1994;330(23):1639-1644.

Symptoms arise as hemoglobin switches from fetal to adult

- The main type of hemoglobin during fetal development and shortly after birth is fetal hemoglobin (HbF)
- A few months after birth, HbF is replaced with adult hemoglobin (HbA)
- In patients with sickle cell disease, the onset of symptoms occurs as HbF switches to HbA, which is affected by disease-causing mutations



HbA: adult hemoglobin; HbF: fetal hemoglobin; SCD: sickle cell disease; 1. Canver MC, et al. *Blood*. 2016;127:2536-2545; 2. Sankaran VG, et al. *Cold Spring Harb Perspect Med*. 2013;3:a011643.

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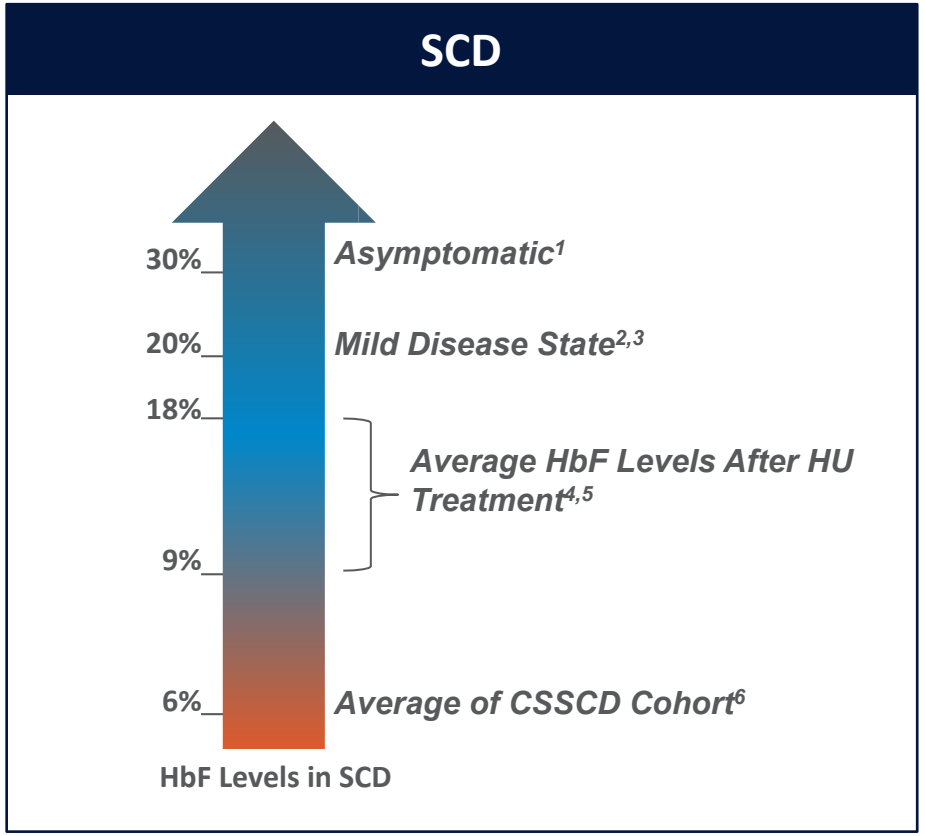
Persistence of HbF can Alleviates Symptoms in Patients with SCD

A subset of patients with SCD continue to express HbF into adulthood, a condition known as Hereditary Persistence of HbF (HPFH) these patients experience reduced or no symptoms with no detrimental effects



1. Ngo DA, et al. *Br J Haematol*. 2012;156:259-264. 2. Alsultan A, et al. *Am J Hematol*. 2012;87:824-826. 3. Fitzhugh CD, et al. *PLoS One*. 2015;10:e0141706. 4. Platt OS, et al. *N Engl J Med*. 1991;325:11-16. 5. Murray N, et al. *Br J Haematol*. 1988;69:89-92. 6. Conley CL, et al. *Blood*. 1963;21:261-281. 7. Bank A. *Blood*. 2006;107:435-443.

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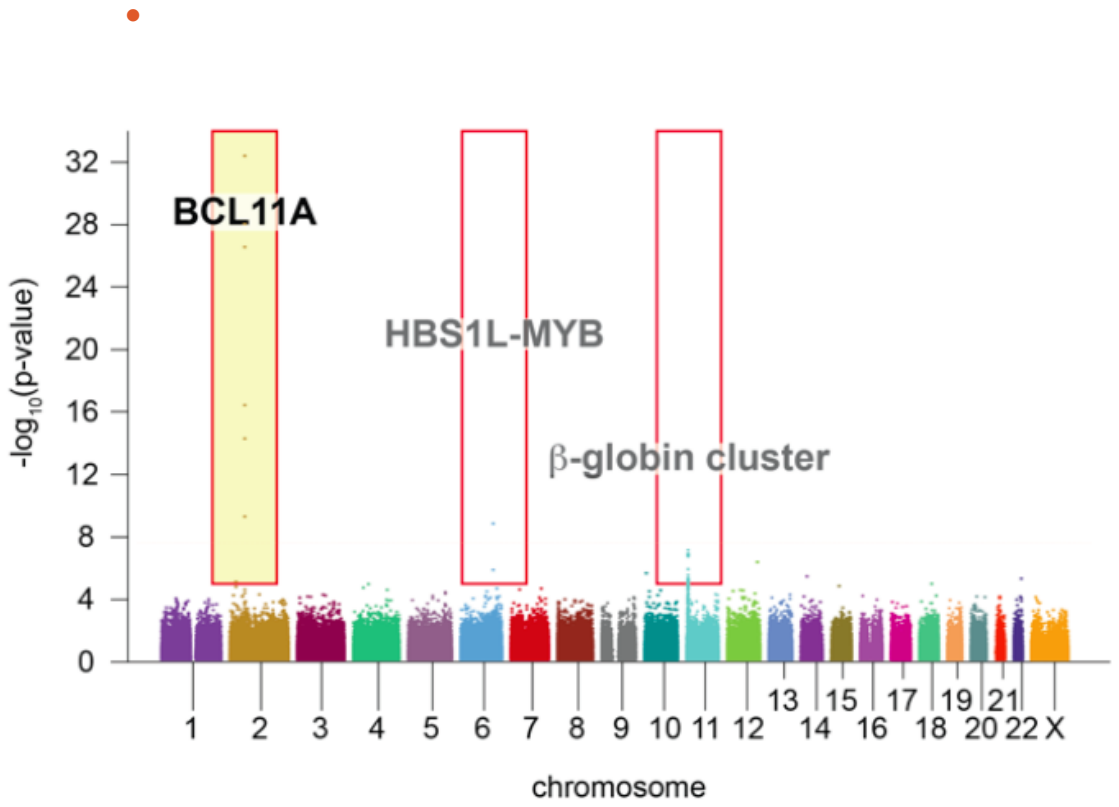
Genetic studies discovered that genomic variation leading to decreased expression of BCL11A result in increased HbF expression

- BCL11A represses HbF production; decreasing expression of BCL11A increases HbF production
- The strong correlation between HbF expression and a decrease in symptoms of SCD highlight the treatment opportunity targeting *BCL11A* to increase production of HbF



Adapted from Bauer DE, et al. *Blood*. 2012;120:2945-2953.

HbF: fetal hemoglobin; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia.

1. Bhatnagar P, et al. *J Hum Genet*. 2011;56:316-323; 2. Bauer DE, et al. *Blood*. 2012;120:2945-2953.

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Exa-cel Is a Cell Therapy That Uses Non-Viral, *Ex Vivo* CRISPR/Cas9-Mediated Editing of *BCL11A* to Increase HbF Levels

- Naturally occurring genetic polymorphisms in *BCL11A* are associated with elevated HbF and decreased severity of SCD[2-4]
- *BCL11A* suppresses expression of γ-globin and thus HbF
- Editing of *BCL11A* reactivates γ-globin expression and formation of HbF (α2γ2) in mouse models
- Exa-cel is produced using non-viral, *ex vivo* editing of the erythroid-specific enhancer region of *BCL11A* in CD34$^+$ HSPCs and reduces erythroid-specific expression of *BCL11A*

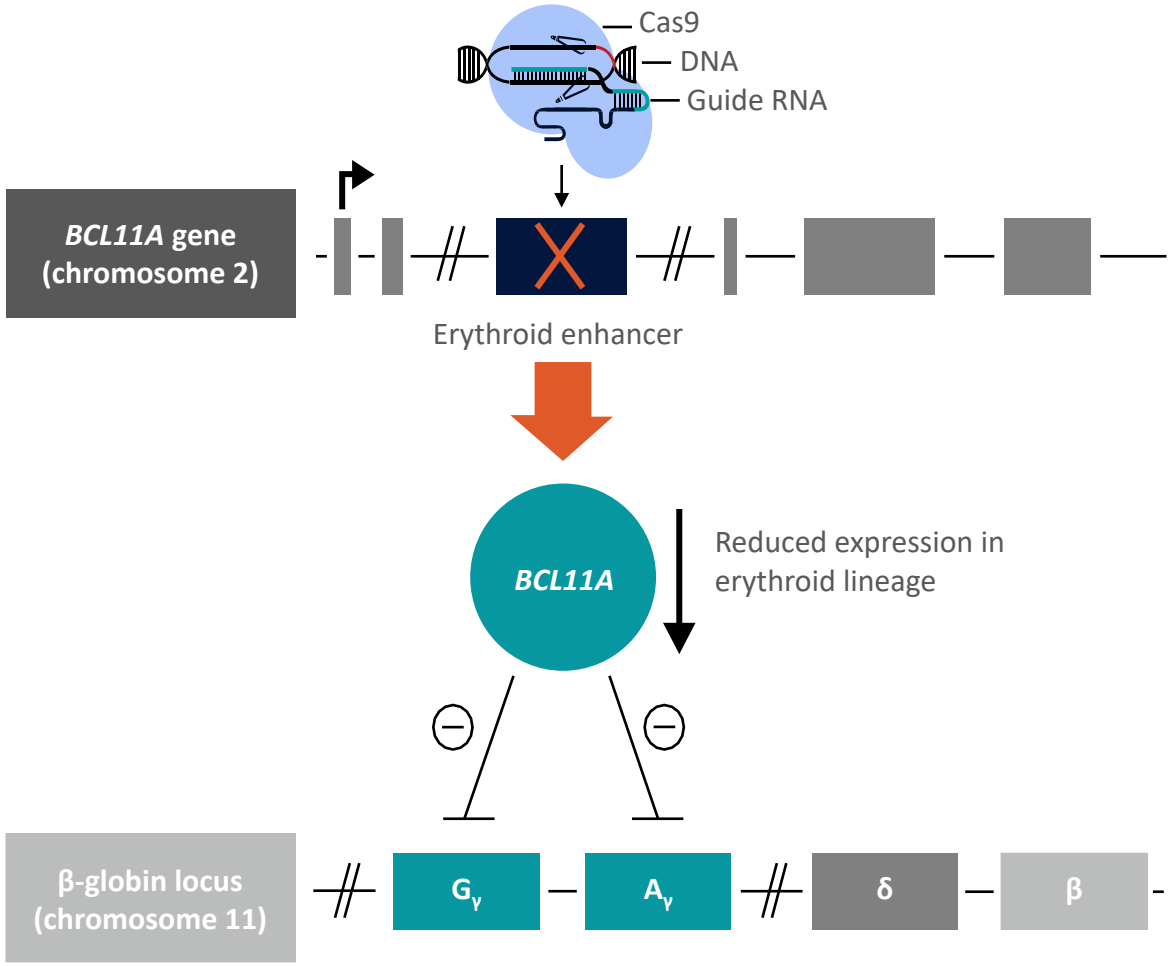

Figure modified from Canver, et al.[1]

BCL11A, B-cell lymphoma/leukemia 11A; CRISPR, clustered regularly interspaced short palindromic repeats; DNA, deoxyribonucleic acid; HbF, fetal hemoglobin; HSPC, hematopoietic stem and progenitor cell; RNA, ribonucleic acid;
SCD, sickle cell disease; TDT, transfusion dependent β-thalassemia.

1. Canver MC, et al. *Blood*. 2016;127:2536-2545; 2. Murray N, et al. *Br J Haematol*. 1988;69:89-92; 3. Conley CL, et al. *Blood*. 1963;21:261-281; 4. Bank A. *Blood*. 2006;107:435-443.

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Phase 1/2/3 Pivotal Trials of Exa-cel in Patients Severe SCD Are Ongoing

	CLIMB SCD-121
Design	International, multicenter, open-label, single-arm pivotal study of exa-cel (NCT03745287)
Key Inclusion Criteria	Twelve to 35 years of age with severe SCD and a history of ≥2 VOCs per year in the previous 2 years
Primary Endpoint	*Primary efficacy endpoint:* Proportion of patients who have not experienced any severe VOC for at least 12 consecutive months after exa-cel infusion
Clinical Assessments	Engraftment, total Hb, HbF, *BCL11A* edited alleles, transfusions, VOCs, and AEs

AE, adverse event; *BCL11A*, B-cell lymphoma/leukemia 11A; Hb, hemoglobin; HbF, fetal hemoglobin; pRBC, packed red blood cell; RBC, red blood cell; SCD, sickle cell disease; TDT, transfusion dependent β-thalassemia; VOC, vaso-occlusive crisis.

Locatelli F, et al. Presented at the 27th Annual European Hematology Association; 12 June 2022

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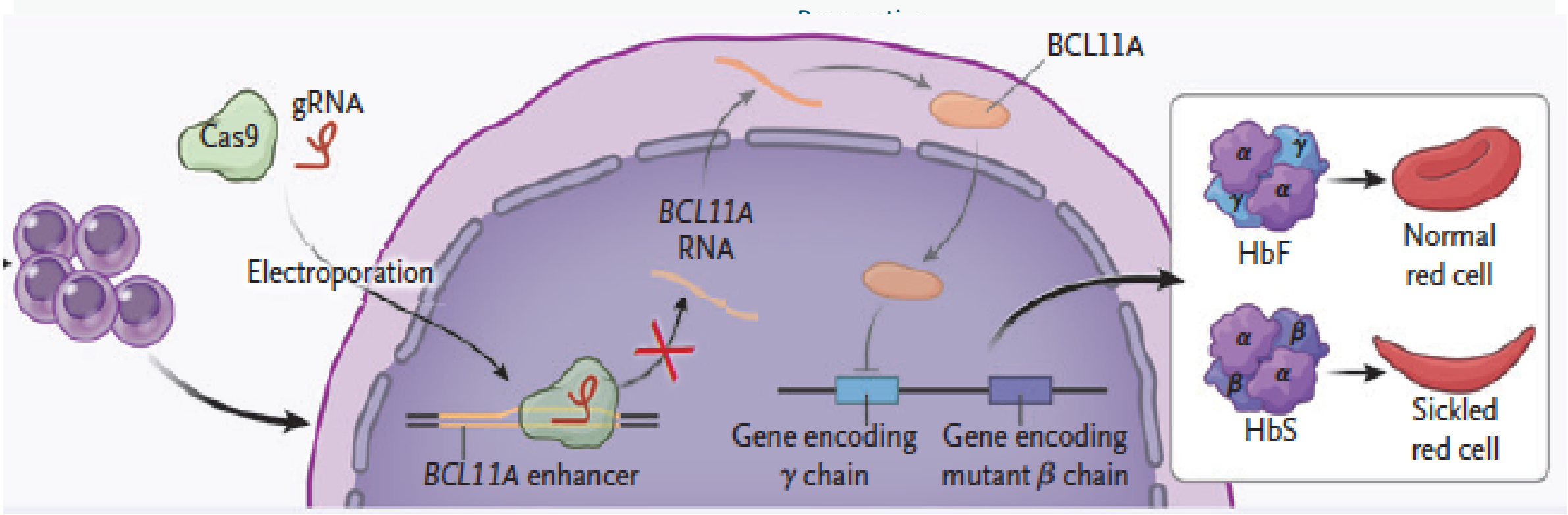
In the clinic: Exa-cel – an autologous ex vivo CRISPR-Cas9 gene-edited therapy



Steinberg NEJM 2022

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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)

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

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All Patients Engrafted Neutrophils and Platelets After Exa-cel Infusion (N=31)

	Median (Range)
Drug product cell dose,[a] median (range) CD34+ cells × 10^6/kg	4.0 (2.9-14.4)
Neutrophil engraftment,[b] median (range) Study Day[c]	27.0 (15-38)
Platelet engraftment,[d] median (range) Study Day[c]	32.0 (23-74)
Duration of follow-up, median (range) Months[c]	10.2 (2.0-32.3)

SCD, sickle cell disease; TDT, transfusion dependent β-thalassemia.

[a]Across multiple drug product lots per patient; [b]Defined as the first day of 3 consecutive measurements of absolute neutrophil count ≥500 cells/μL on 3 different days; [c]Defined as day after exa-cel infusion; [d]Defined as the first day of 3 consecutive measurements of unsupported (no platelet transfusion in last 7 days) platelet count ≥20,000/μL on 3 different days after exa-cel infusion (TDT) and as the first day of 3 consecutive measurements of unsupported (no platelet transfusions for last 7 days) platelet count ≥50,000/μL on 3 different days after exa-cel infusion (SCD).

Exa-cel Safety Profile Is Consistent With That of Busulfan Myeloablation and Autologous HSCT

CLIMB SCD-121

Post-Exa-cel AE Overview	SCD (n = 31)
Patient-time exposure, Patient-months	288.6
Patients with any AEs, n (%)	31 (100.0)
Patients with AEs related to exa-cel, n (%)[a]	9 (29.0)
Patients with AEs related to busulfan, n (%)[a]	31 (100.0)
Patients with AEs Grade 3/4, n (%)	31 (100.0)
Patients with SAEs, n (%)	10 (32.3)
Patients with SAEs related to exa-cel, n (%)[a]	0
Patients with AEs leading to death, n (%)	0

No patients with SCD had an SAE considered related or possibly related to exa-cel

AE, adverse event; HSCT, hematopoietic stem cell transplantation; SAE, serious adverse event; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia.

[a]Includes related and possibly related AEs.

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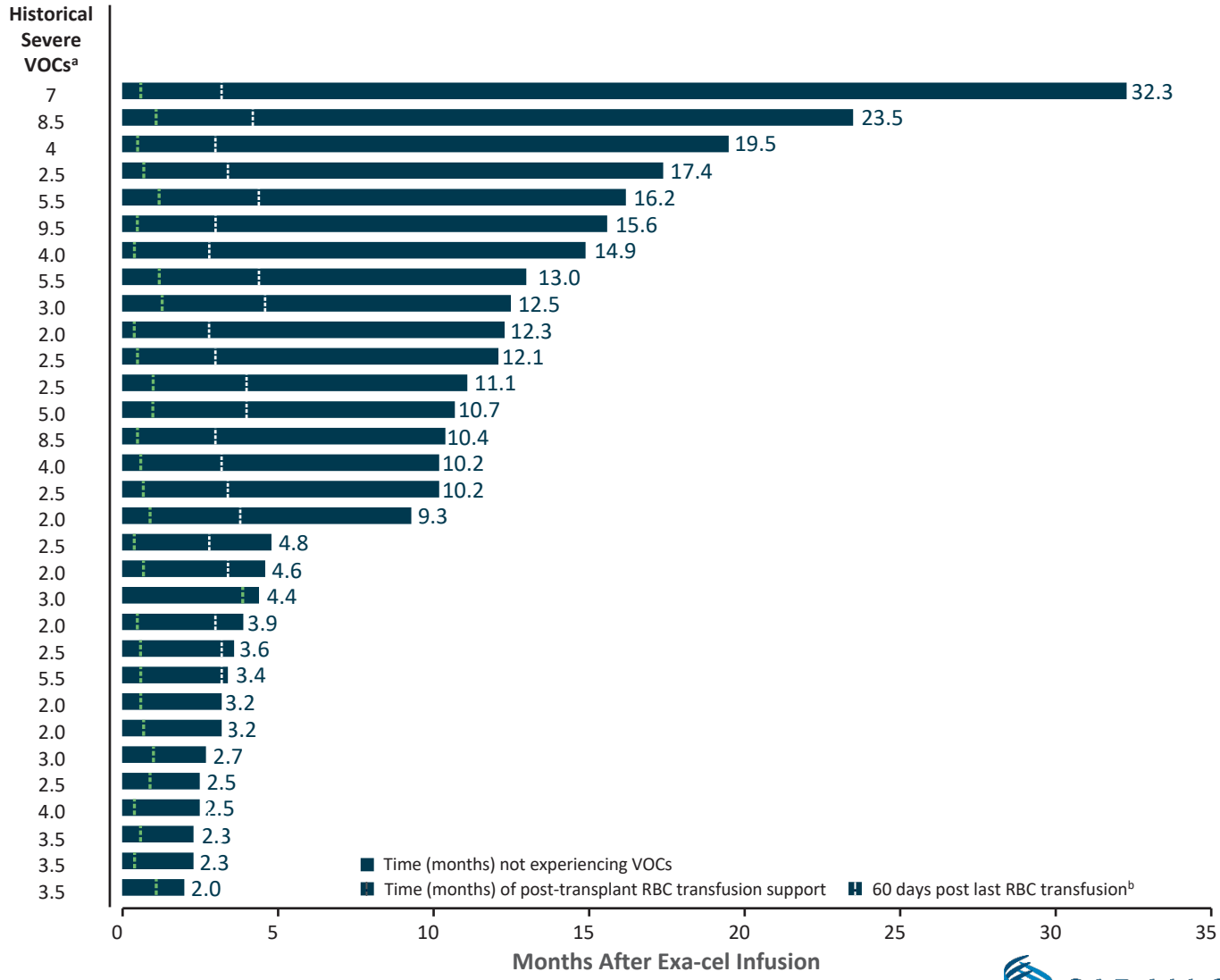
All Patients With SCD Treated With Exa-cel are VOC-Free

- 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)



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

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

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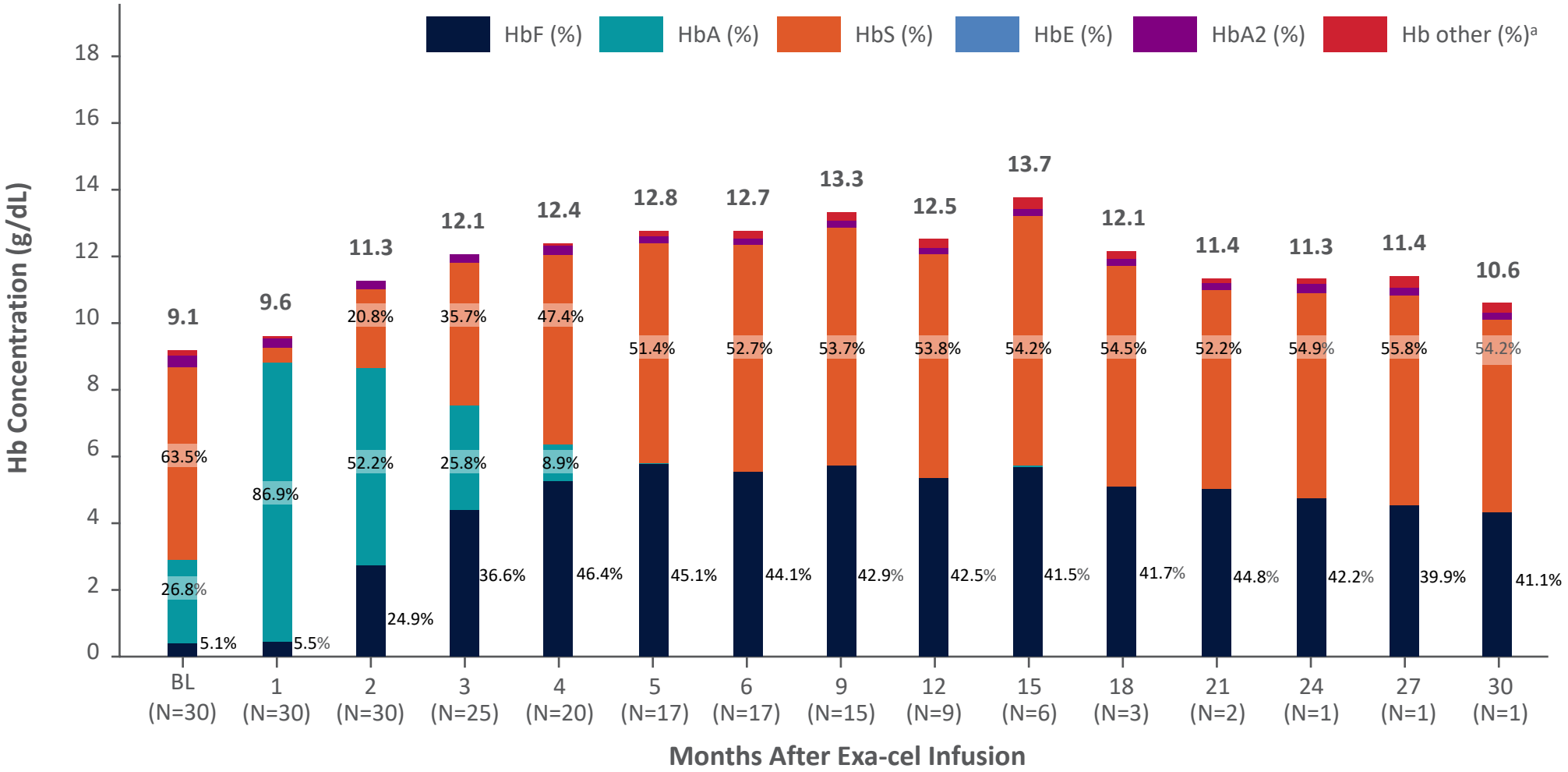
Patients With SCD Had Clinically Meaningful Increases in HbF (>20%) That Occurred Early and Were Sustained Over Time



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.

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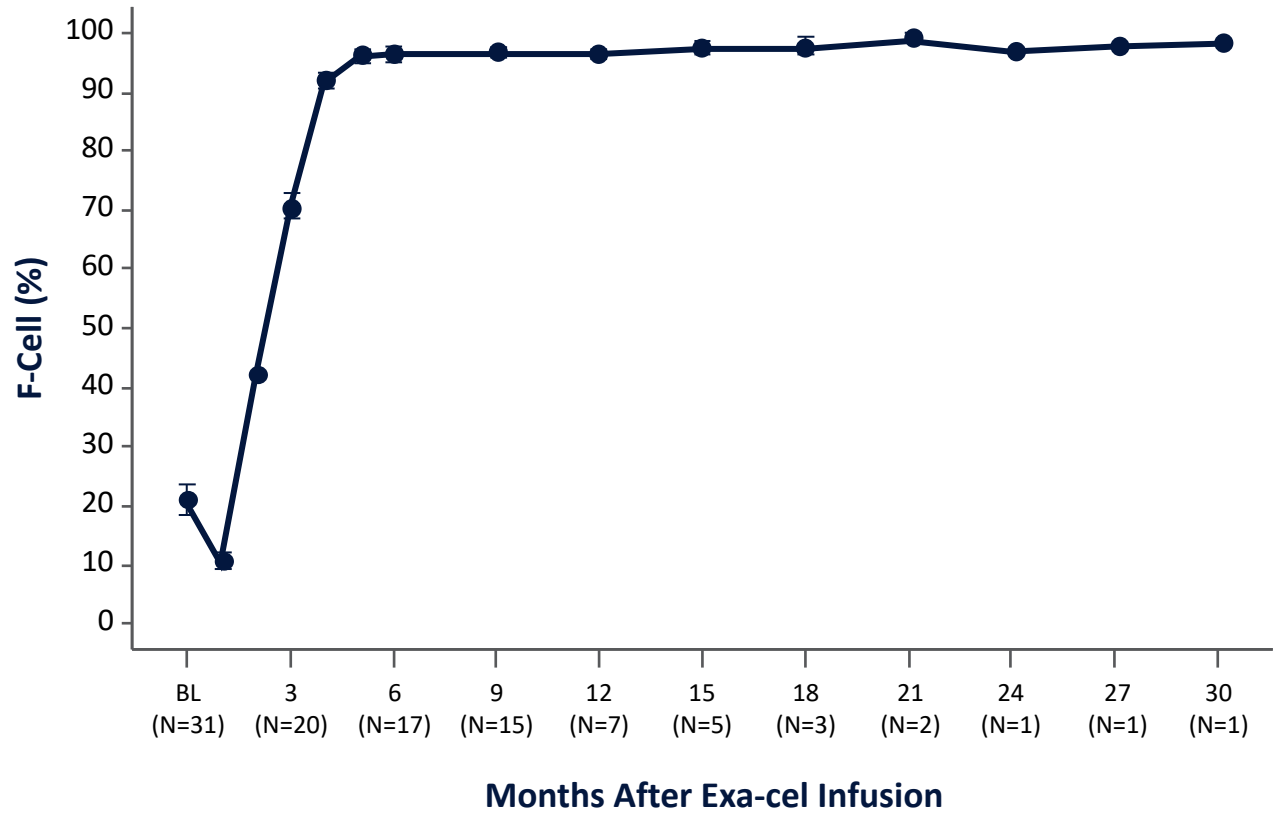
Pancellular Distribution of HbF Is Maintained Over Time



Figures show mean (SE)
BL, baseline; F-cells, HbF-containing cells; HbF, fetal hemoglobin; SCD, sickle cell disease.

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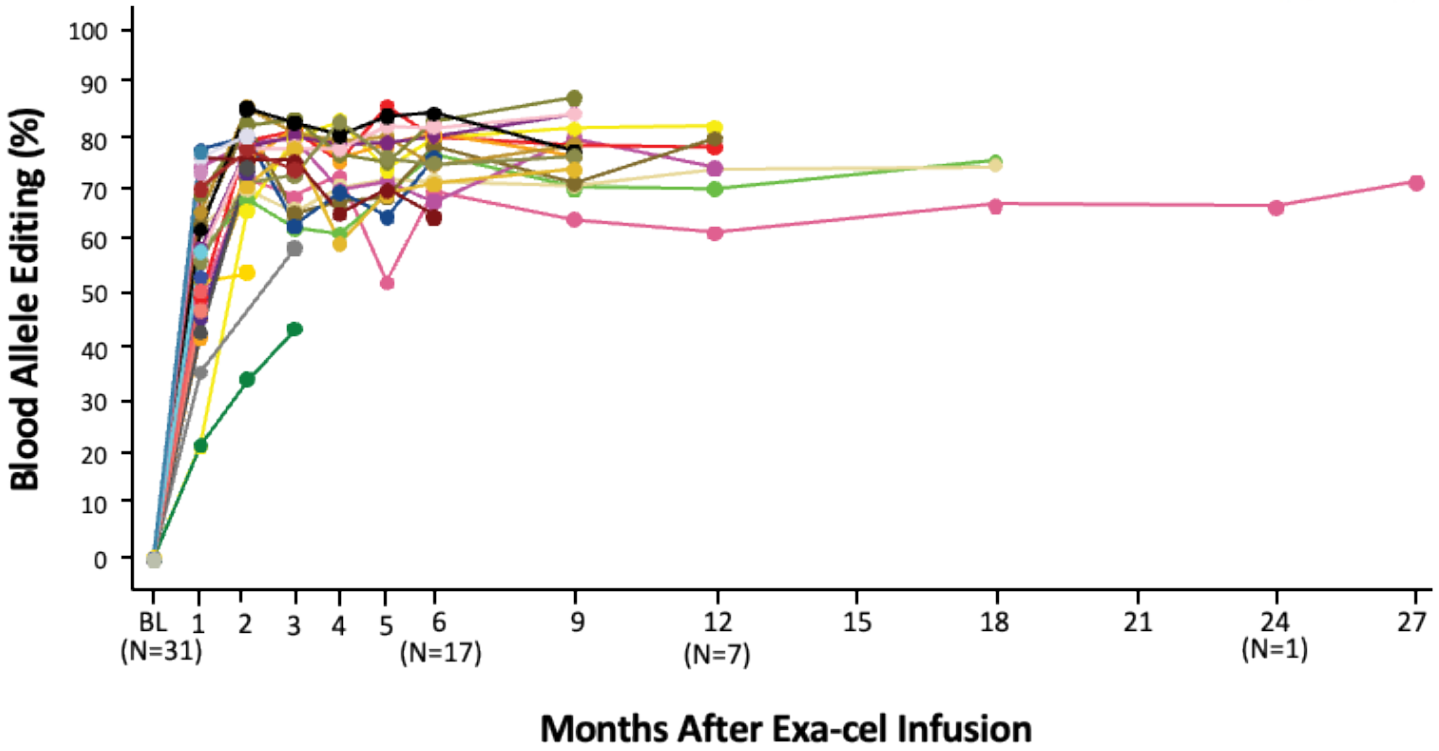
Durable *BCL11A* Editing Achieved in Peripheral Blood (Nucleated Cells)



Bone marrow allele editing figures include patients who have at least 12 months of follow-up whereas the blood allele editing figures include all patients.
BCL11A, B-cell lymphoma/leukemia 11A; BL, baseline; SCD, sickle cell disease.

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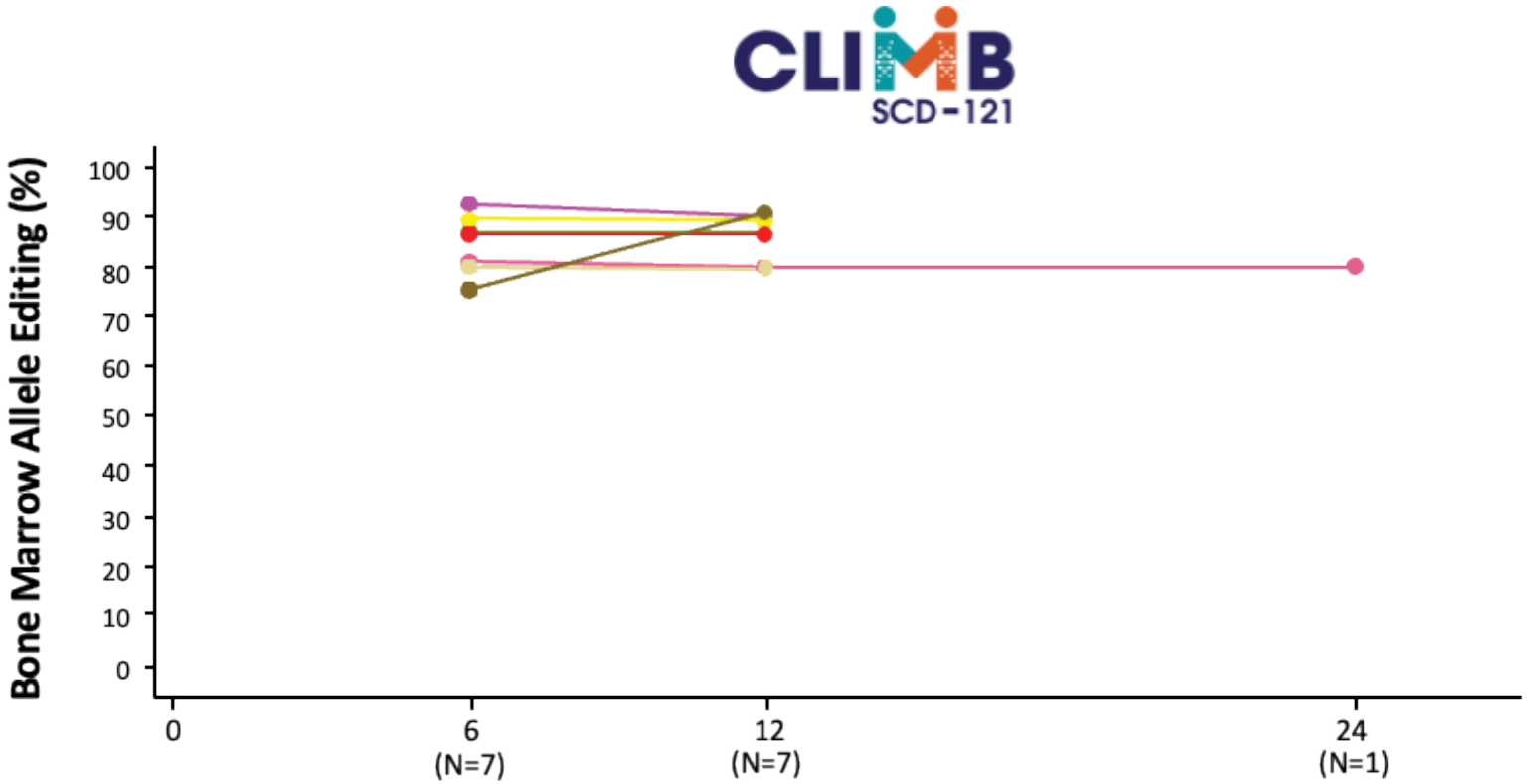
Durable *BCL11A* Editing Achieved in Bone Marrow (CD34^{+} Cells)



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Conclusions

- Data from 31 patients with severe SCD shows a **single dose of exa-cel leads to early increases in HbF and total Hb** that are durable up to 32 month
- All 31 patients with severe SCD are **free of VOCs**
- Patients with ≥1 year of follow up have stable proportions of *BCL11A* edited alleles in bone marrow and peripheral blood, **indicating successful and durable editing** of long-term HSCs
- **Safety profile of exa-cel is consistent** with that of busulfan myeloablative conditioning and autologous hematopoietic stem cell transplantation
- Exa-cel has the potential to be the first CRISPR/Cas9-based therapy to provide a **functional cure for patients with severe SCD**

Hb: hemoglobin; HbF: fetal hemoglobin; HSC: hematopoietic stem cell; RBC: red blood cell; SCD: sickle cell disease; TDT, transfusion-dependent β-thalassemia; VOCs: vaso-occlusive crises.

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Gene therapy approaches to treatment of hemoglobinopathies

Advantages:

- No need for a donor
- No risk of rejection or graft versus host disease
- No viral vector is used

Limitations:

- Short follow up
- Need for high dose chemotherapy
- Absence of data in patients with SCD and CNS disease
- Too early to evaluate if this approach will alter end organ damage overtime

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Celebrating Scientific Milestones

1st Patients To Get CRISPR Gene-Editing Treatment Continue To Thrive

December 15, 2020 · 5:02 AM ET
Heard on Morning Edition

ROB STEIN

npr

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Thank you to study participants and their families, as well as sites, investigators, nurses, and the entire exa-cel team

The authors acknowledge the site investigators for CLIMB THAL-111 and CLIMB SCD-121 trials

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