Understanding the Complexities of Patient Selection, Enrollment, and the Consent Process: Gene Therapy for Sickle Cell Disease

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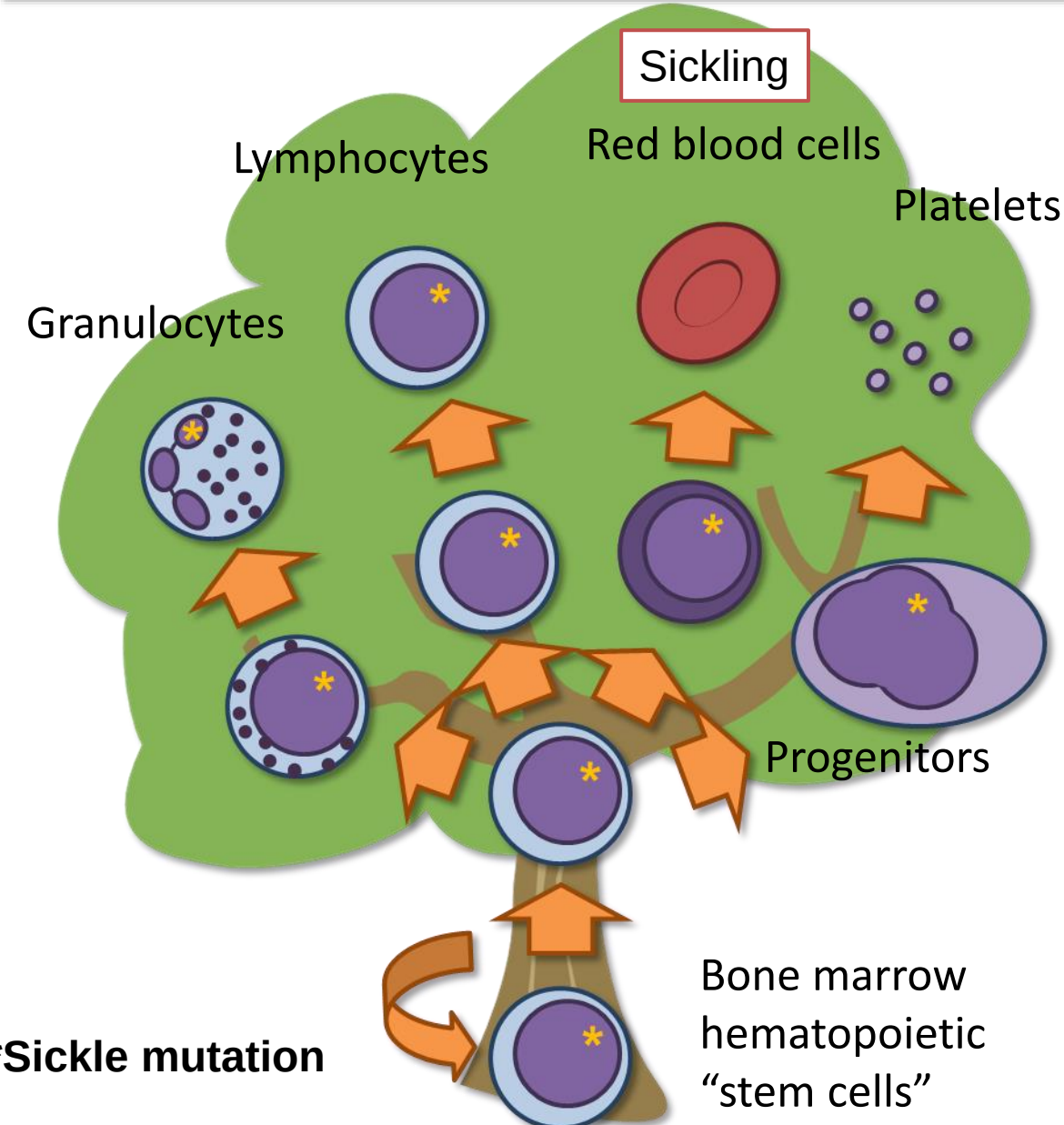
Bone marrow transplants replace the seeds of the blood



***Sickle mutation**

Bone marrow stem cells produce all types of blood cells for the life of a patient.

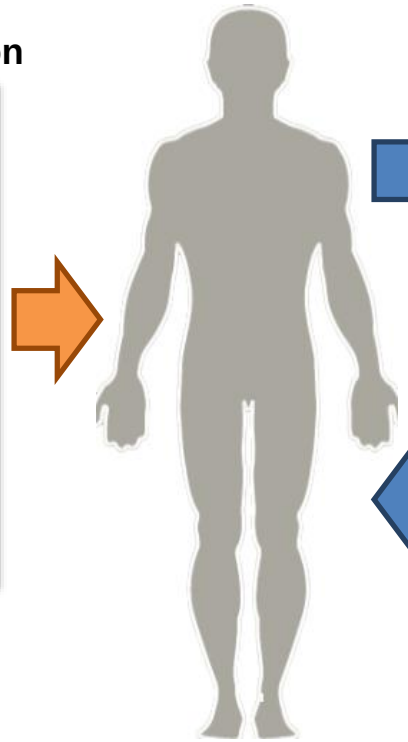
We have sought to develop curative strategies based upon replacing or repairing bone marrow stem cells.

Bone marrow stem cell transplant strategies for SCD

1. Allogeneic transplantation

Bone marrow transplant from someone who does not have SCD

Donor is usually an HLA-matched sibling, but could include cord blood, matched unrelated, or half-matched family member



Sickle cell disease patients

2. Autologous gene therapy

Bone marrow transplant from patient's own bone marrow

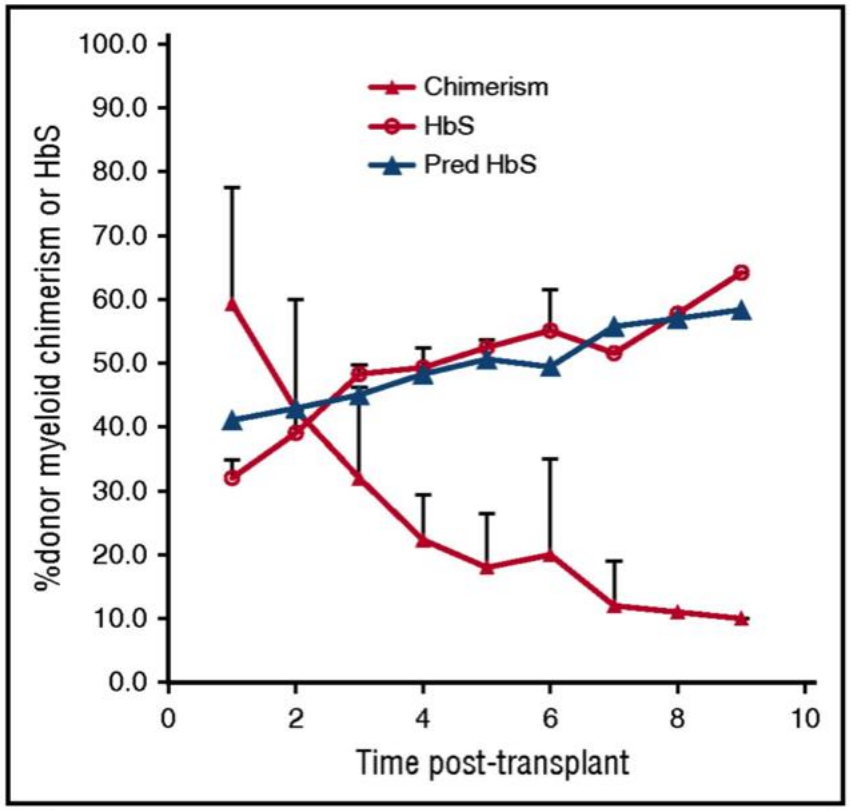
β-globin gene transfer with an engineered virus to transfer or gene editing with an engineered endonuclease

Comparison between NIH transplant results and mathematical modeling demonstrates that only 20% donor level needed and is dependent only on red blood cell life span differences

$$f_M = \frac{f_P t^D}{f_P t^D + (1 - f_P) t^H}$$

In our model the fraction of mature donor erythrocytes in the periphery (f_M) is a function of

- Progenitor chimerism, f_P
- Donor and recipient erythrocyte half-lives, t^D and t^H, respectively.

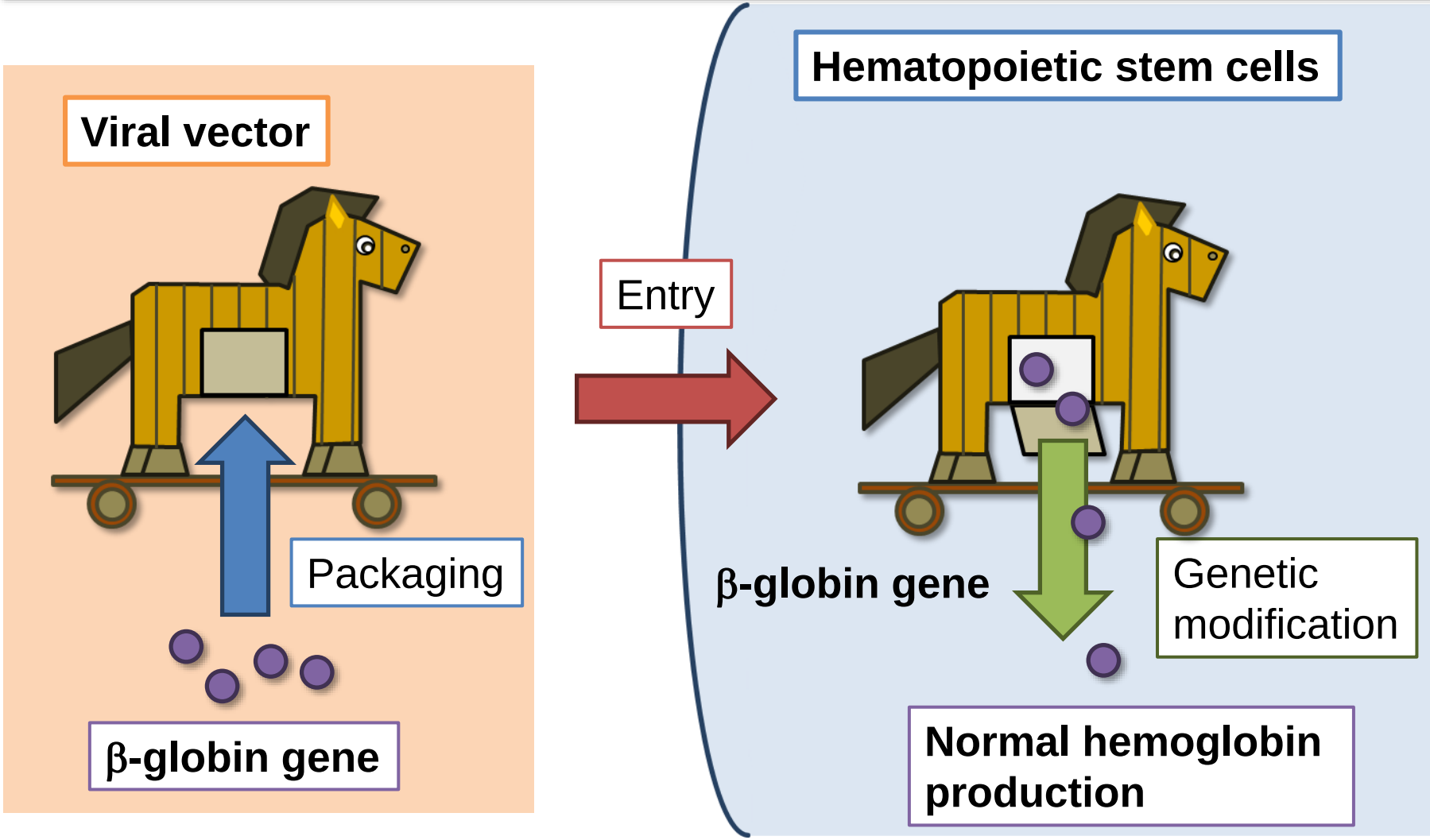


Can we achieve this modest 20% correction level with gene addition using the patients' own bone marrow HSCs?

Fitzhugh, Cordes et al, Blood, 2017 Oct 26;130(17):1946-1948

NIH National Institutes of Health

1. How do we introduce the experimental vector?



Patients with sickle cell disease when confronted with our experimental trial that employs HIV as the delivery vector may think back on Tuskegee experiments

2. How do we determine that the first trial patients get a potentially therapeutic dose?

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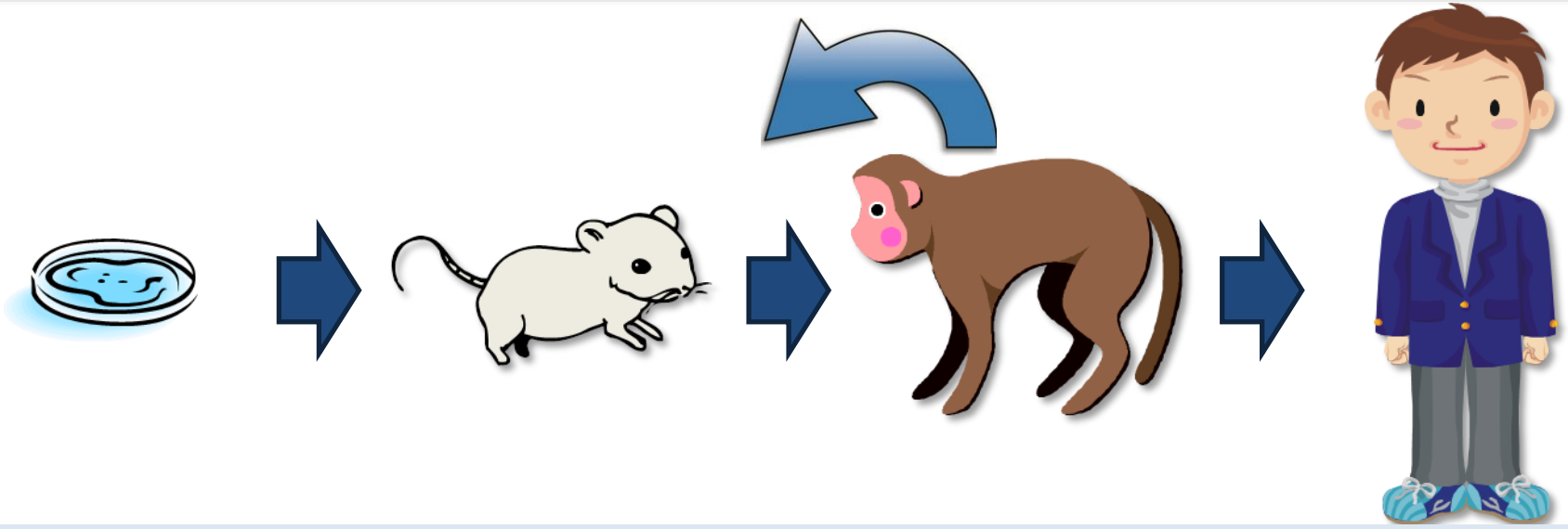


Cell culture	Small animal	Large animal	Clinical trial
Cell lines iPS cells	Mice Disease model mice Humanized mice	Non-human primates	Phase I Phase II Phase III Phase IV

Efficiency

Cell lines $>$ Mouse HSCs $>>$ Rhesus HSCs $\approx$ Human HSCs

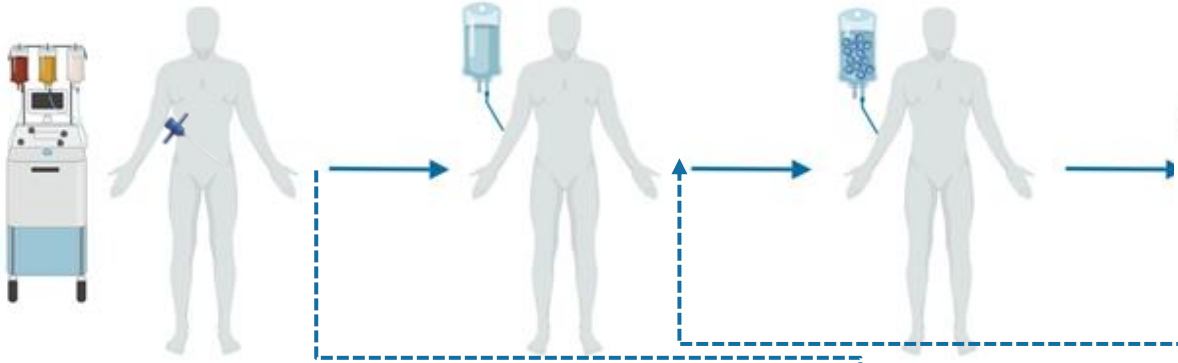
HGB-206: Further evolution of the protocol allowed refinements during the course of the study

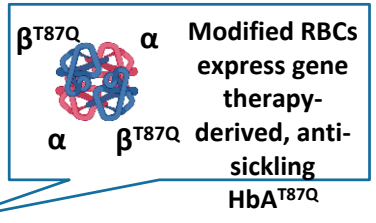
HSC collection
Bone marrow harvest or mobilization with plerixafor & apheresis

Busulfan myeloablative conditioning

DP infusion

Transduced HSCs engraft and contribute to reconstitution of functional RBCs





2-yr follow-up

Long-Term Follow-Up Study

	Group A	Group B	Group C
Pre-collection transfusion regimen	Optional	Required	Required
HSC source	Bone marrow	Bone marrow	Mobilized PB
Manufacturing process	Original	Orig ➔ Refined	Refined

LentiGlobin DP centralized manufacturing

Select CD34+ cells | *Transduce with BB305 lentiviral vector* | *Cryopreserve, test, release DP*

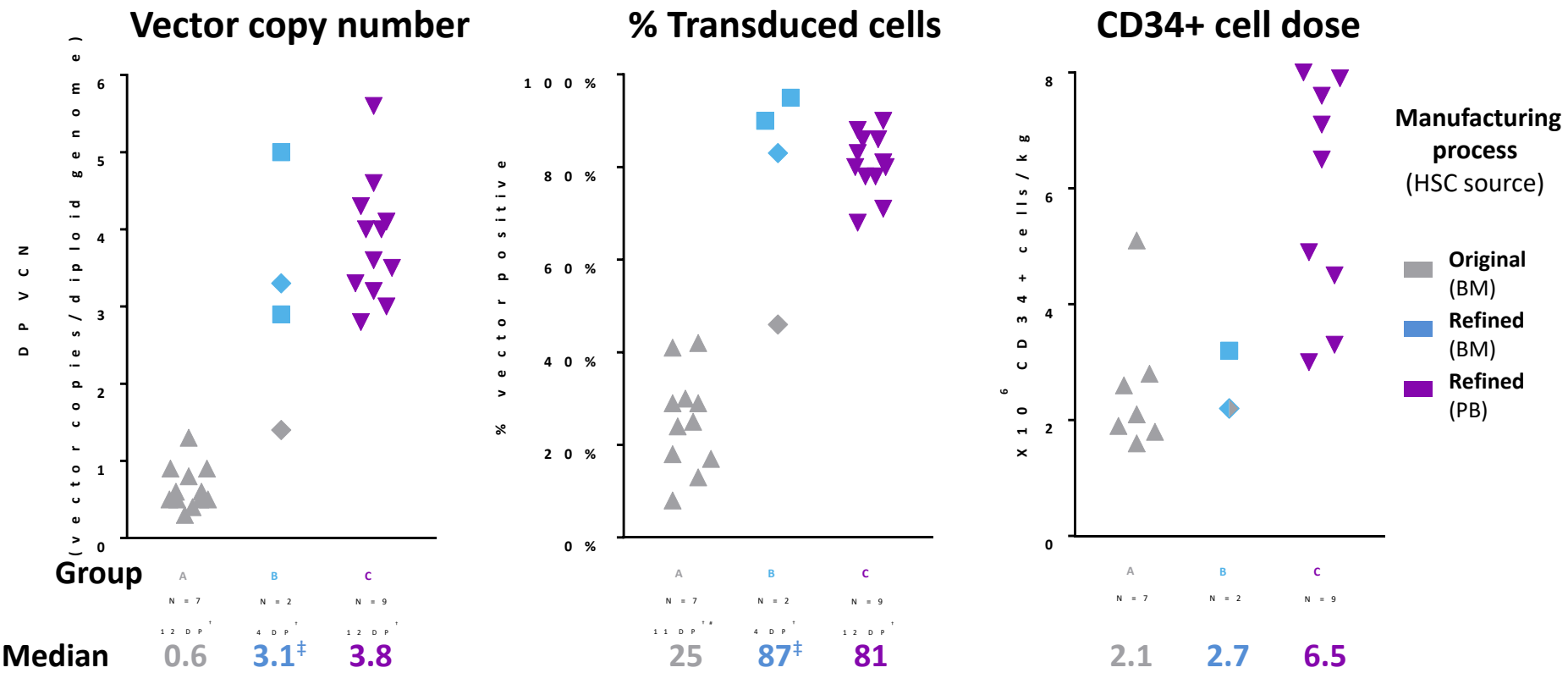
Key Enrollment Criteria

- 18+ years of age
- History of symptomatic SCD
- Adequate organ function
- No previous HSCT or gene therapy

Study Objectives

- Primary objective: Safety
- Key Secondary Objectives:
  - Frequency of VOCs and ACS
  - Total Hb and Hb fractions
  - Vector copies in peripheral blood

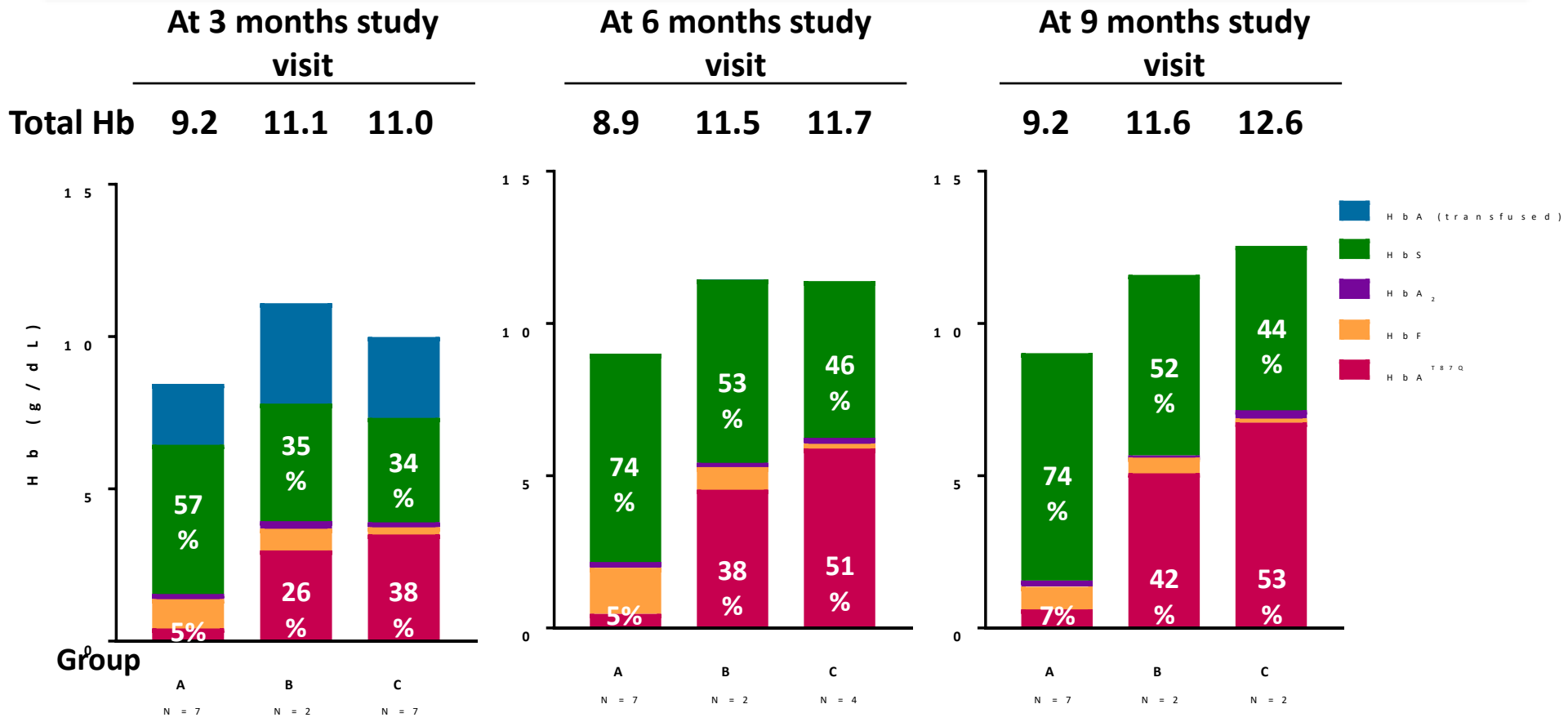
HGB-206: Refinements to manufacturing and cell harvesting improved product characteristics



†Number of DP exceeds number of patients since some patients were harvested or mobilized more than once; # % Transduced cells not available for 1 DP at time of analyses; ‡1 Group B DP lot was made using original manufacturing process, while the other 3 DP lots were made using refined manufacturing process

BM, bone marrow; DP, drug product; HSC, hematopoietic stem cell; PB, peripheral blood; VCN, vector copy number

HGB-206: Gene therapy-derived hemoglobin mirrors the carrier state at ≥ 3 months in Group C



% represent median Hb fractions as % of total; Hb, hemoglobin

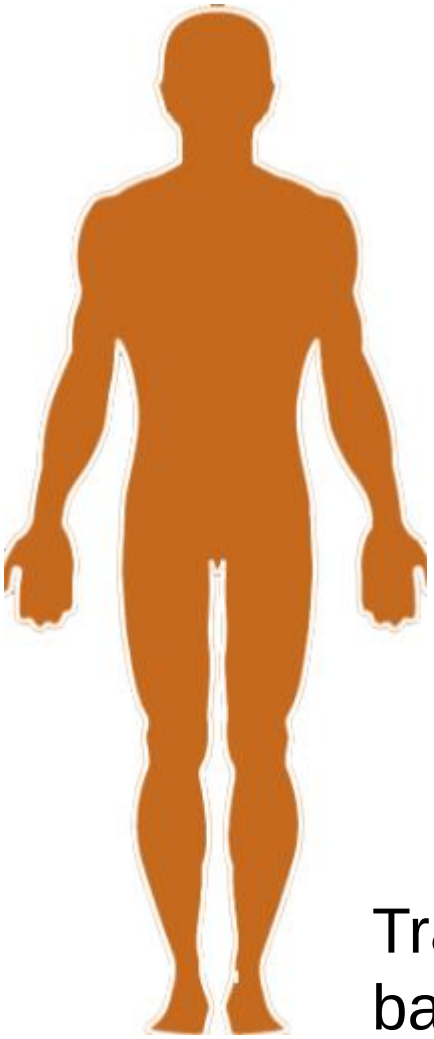
Further issues that arose along the course of the first gene therapy trial for sickle cell disease

3. How do we enroll pre-symptomatic patients?

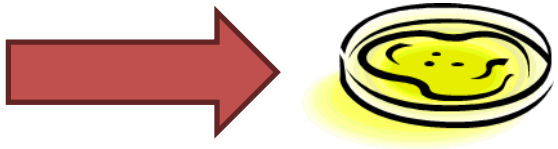
4. How do we follow up with participants on the results of clinical trials?

5. What are the scientific and clinical reasons a patient might be precluded from gene therapy trials after participating in a trial for a gene therapy investigational agent?

Autologous bone marrow stem cell-targeted gene editing



Harvest



Patients' own bone marrow stem cells

Reactivate fetal hemoglobin by cutting repressor genes or correct the mutation by cutting and repairing

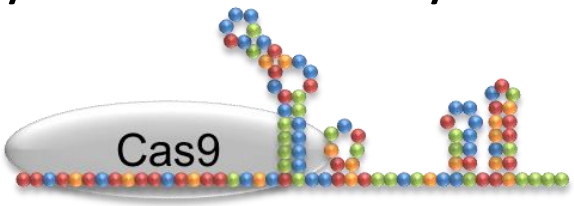
Transplantation back to patients

- Patients serve as their own donor
- Available for all patients
- No need for immunosuppression
- No risk of GVHD

SCD patients

CRISPR/Cas9 system for genome editing

- Arose from basic science studies of bacteria
- Achieves targeted disruption of genomes with enzyme + guide RNA
  - Initial approaches to create double strand breaks
  - Can serve a “find and replace” function when delivered with template DNA
- Has revolutionized basic molecular biology due to accuracy and ease of use
- Paves the way for new therapeutics



CRISPR/Cas9 system for genome editing, just a click away….

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DIY Bacterial Genome Engineering CRISPR Kit

by The ODIN

3 customer reviews | 9 answered questions

Available from these sellers.

- All-In-One kit, you don't need anything else but water and a microwave
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- Learn CRISPR Technology through Hands-On experimentation

Specifications for this item

Part Number	CRISPR1
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EAN	0636391770750
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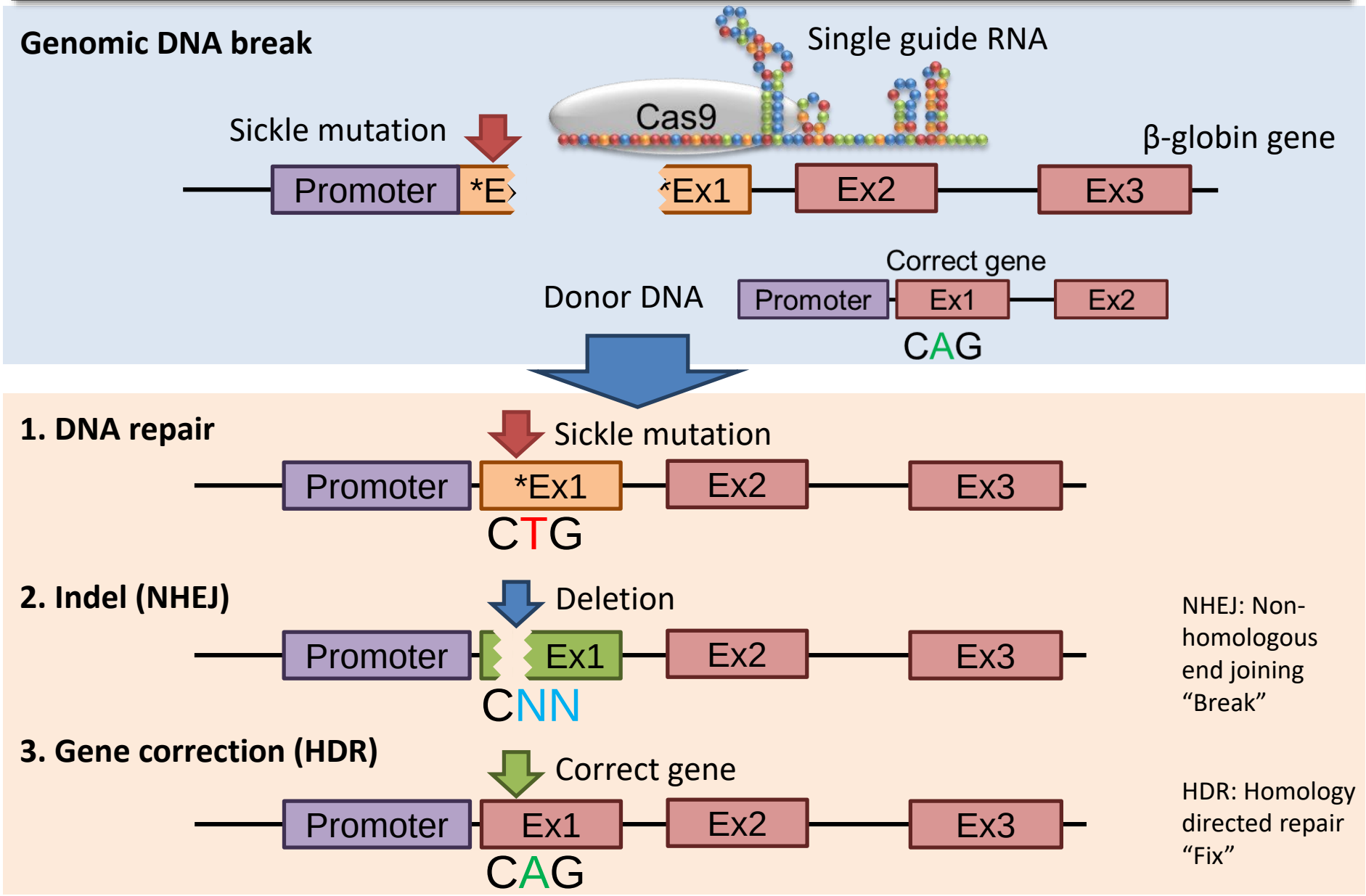
CRISPR/Cas9 system for genome editing



NHEJ: Non-homologous end joining "Break"

HDR: Homology directed repair "Fix"

Gene correction with CRISPR/Cas9 in SCD bone marrow stem cells

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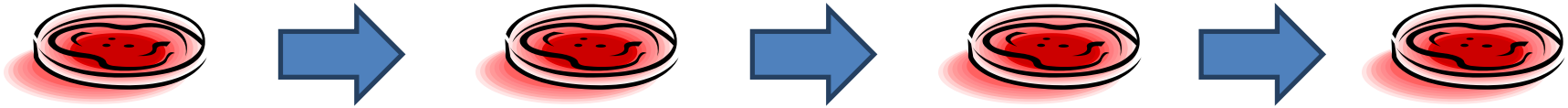
Guide RNA targeting the β-globin gene
Cas9 mRNA or Cas9 protein
Donor ssDNA : 80, 120, or 200 µg/ml



SCD CD34+ cells

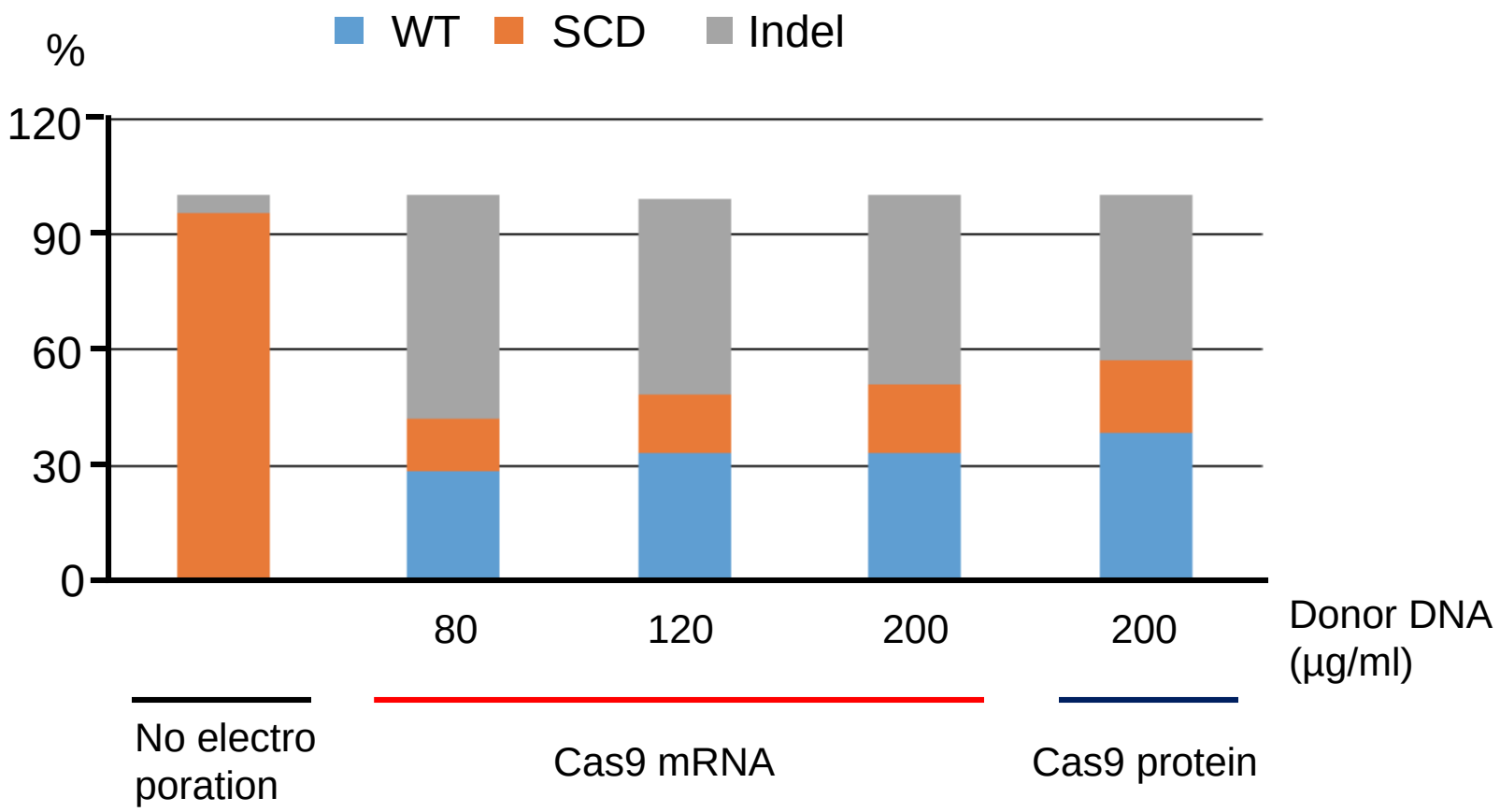
Electroporation

Grow red blood cells in flasks

Electrophoresis
RP-HPLC
SNP PCR
Targeted sequence

Colony assay → Targeted sequence

~30% of gene correction evaluated by DNA sequencing



Robust hemoglobin formation following red blood cell production

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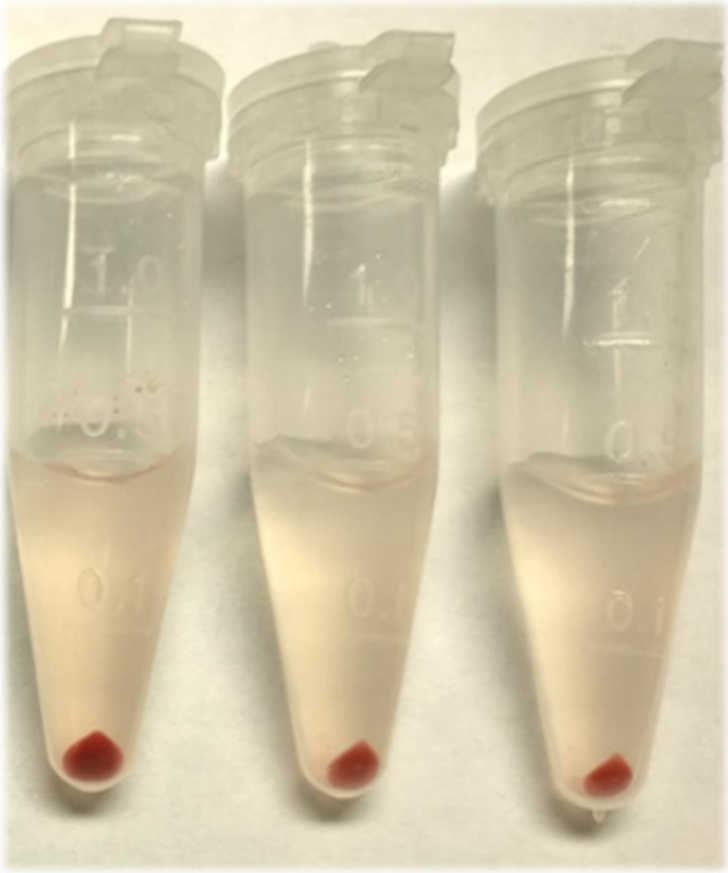
SCD CD34+ cell gene correction

No electro poration	Gene correction 120 µg/ml DNA	Gene correction 200 µg/ml DNA



High-efficiency gene correction from βs-globin to β-globin

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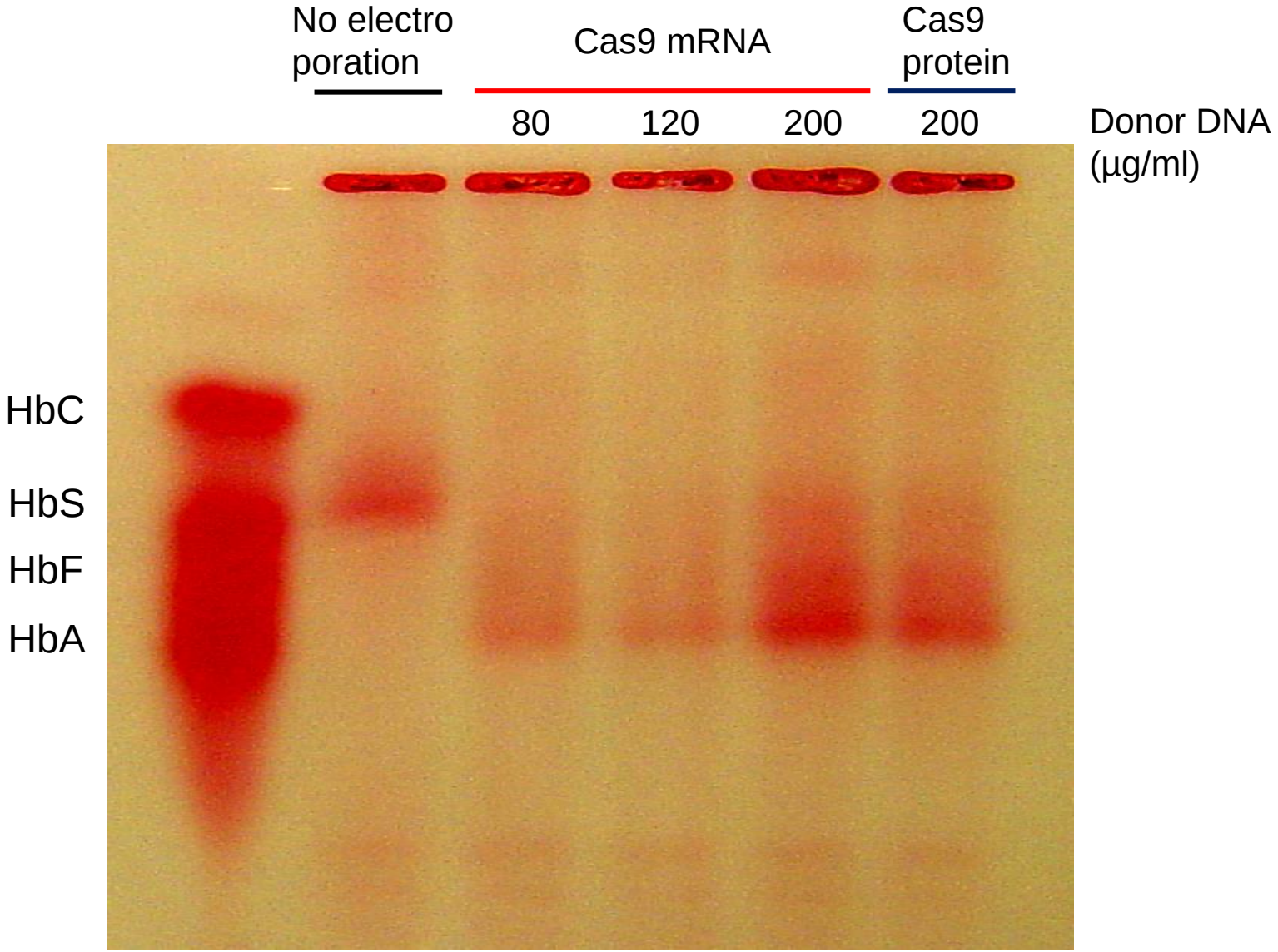
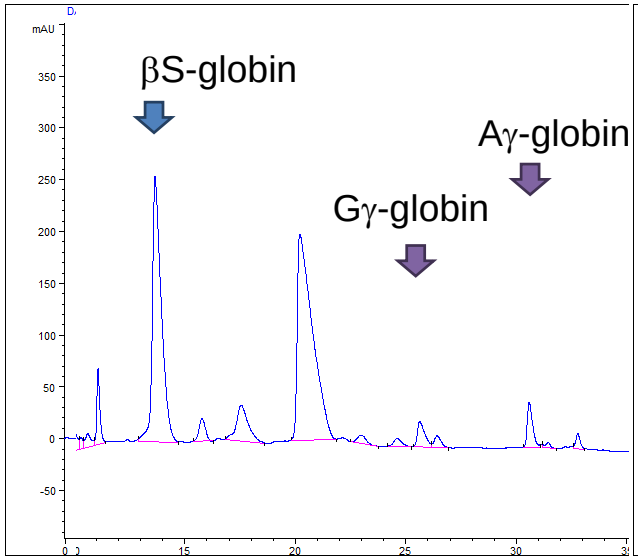
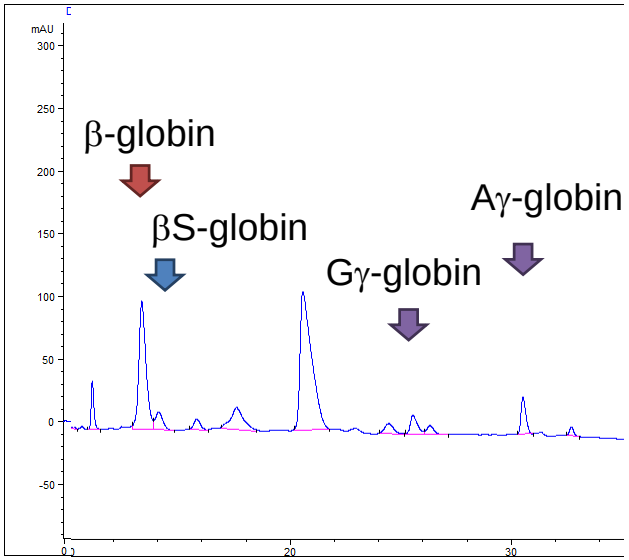
Electrophoresis after erythroid differentiation of CD34+ cells with electroporation



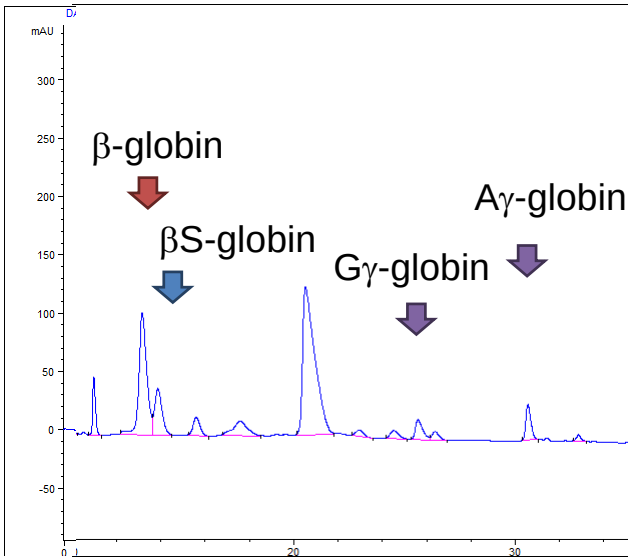
Detection of normal globin peaks in red blood cells by HPLC



No electroporation



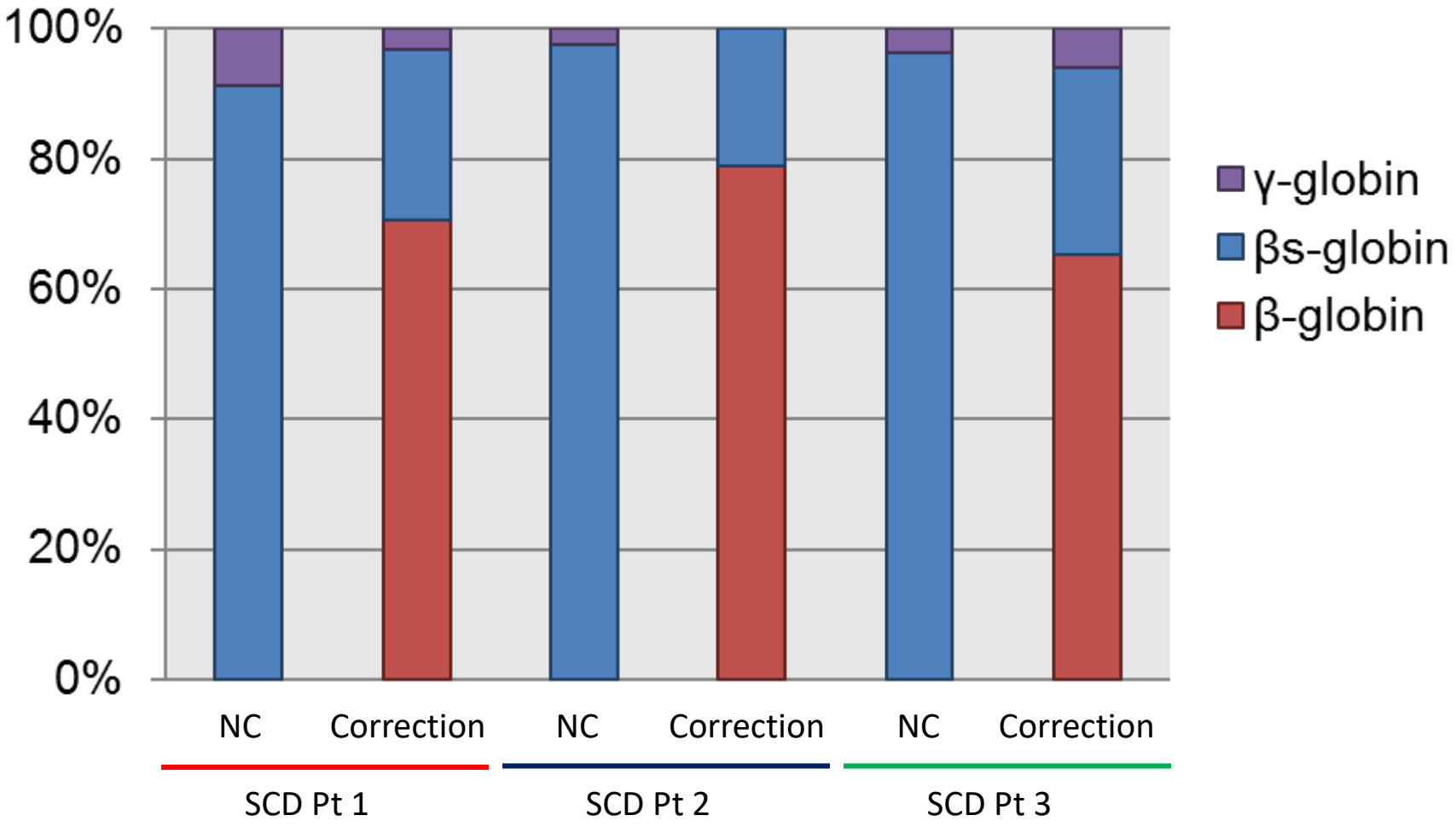
Gene correction
120 µg/ml DNA



Gene correction
200 µg/ml DNA

SCD CD34+ cell gene correction

~70% β-globin production in gene-corrected red blood cells *in vitro*



Control: no electroporation

How will gene editing technologies be received by the stakeholders?

A CRISPR focus on attitudes and beliefs toward somatic genome editing from stakeholders within the sickle cell disease community

Anitra Persaud, BA[1], Stacy Desine, BA[1], Katherine Blizinsky, PhD[1,2,3] and Vence L. Bonham, JD[1]

Purpose: Genome editing holds both tremendous therapeutic promise and significant potential risk. Sickle cell disease (SCD), the most commonly inherited blood disorder, is a frontline candidate for the clinical applications of this tool. However, there is limited knowledge of patient community values and concerns regarding this new technology. This study aims to investigate the perspectives of three key decision-makers (patients, parents, and physicians) toward participation in future CRISPR-mediated somatic genome editing clinical trials.

Methods: We utilized a mixed-methods approach, involving an educational video tool, two-part survey, and 15 moderated, audio-recorded focus groups, which were conducted in seven U.S. cities.

Results: Study participants expressed hope that genome editing technology would rechart the course for SCD, but concerns related to involvement burden, uncertainty of clinical outcomes, and equity in access were identified. Major themes emerged from the focus groups: facilitators of, and barriers to, participation in future somatic genome editing clinical trials; information pertinent to the decision-making process; persons from whom participants would seek counsel before making a decision; and recommendations for the research community on meaningful engagement as clinical trials are designed and approved.

Conclusion: The advent of genome editing has renewed hope for the SCD community, but caution tempers this optimism.

Genetics in Medicine (2019) 21:1726–1734; https://doi.org/10.1038/s41436-018-0409-6

Keywords: sickle cell disease; somatic genome editing; CRISPR; clinical trials; ELSI

INTRODUCTION

One of the first targets of CRISPR-mediated somatic genome editing will likely be sickle cell disease (SCD, OMIM 603903).[1–6] SCD affects millions of people, particularly those in regions where malaria is highly prevalent, such as sub-Saharan Africa, India, and the Mediterranean.[7,8]

SCD is caused by a single pathogenic variation (A→T) in the sixth codon of the β-globin gene. Affected individuals inherit two abnormal copies of the gene, resulting in the production of malformed hemoglobin. This diminishes the oxygen carrying capacity of erythrocytes, resulting in medical complications, including pain crises, strokes, pulmonary hypertension, leg ulcers, priapism, and acute chest syndrome.[7–9]

Despite being identified over a century ago and posing a significant global health burden, those living with SCD have limited treatments available to them.[9,10] Hematopoietic stem cell transplantation (HSCT) remains the only nonexperimental cure for SCD.[11,12] However, while the event-free survival rate of HSCT exceeds 90%, few patients can access this curative therapy due in part to stringent eligibility criteria.[11,12] Further, while the life expectancy of the general adult SCD population has increased over the past 40 years, premature death continues.[8,9]

Because SCD is a well-studied molecular disorder impacting the blood system, it comprises an ideal candidate for gene editing therapies, with different approaches under current investigation. One mechanism involves promoting fetal hemoglobin (HbF) levels, which can reduce the disease's severity by inhibiting HbS polymerization.[5,7,13] However, HbF expression is typically suppressed after birth.[13] Genome editing can be used to deactivate the B-cell lymphoma/leukemia 11A (*BCL11A*) transcription factor promoter, allowing HbF to persist.[5,13] Other researchers have displayed proof of principle success in removing hematopoietic stem and progenitor cells (HSPC) from the bone marrow, correcting the pathogenic variation itself with CRISPR, and repopulating the bone marrow with the edited cells.[2,4,14]

Given these preliminary results, clinical trials are soon expected. On 13 September 2018, the National Heart, Lung,

[1]Social and Behavioral Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; [2]Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, USA; [3]All of Us Research Program, National Institutes of HealthBethesda, MD, USA. Correspondence: Vence L. Bonham (bonhamv@nih.gov)

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Educational video, 2-part survey, 15 moderated focus groups in 7 U.S. cities

- **Motivators** included hope in technology, altruism, shortcomings of current treatment, increased awareness of the importance of clinical trials
- **Deterrents** included uncertainty about consequences, permanence of change, trial burden, mistrust, reproductive risk, cost, lack of access
- **Mediators** included religiosity, capacity to manage disease and life
- **Information desired** included specific details, expected interpatient variability, optimal timing, track record of treatment

Somatic versus germline gene editing, a caution.

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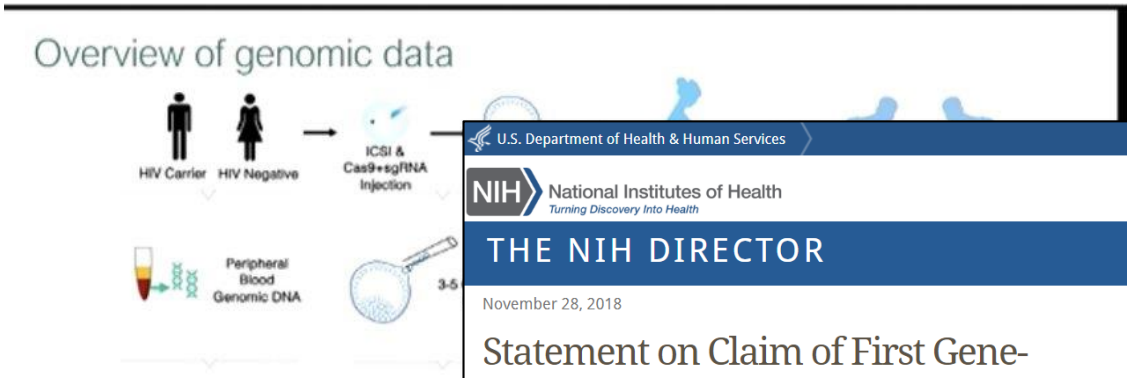

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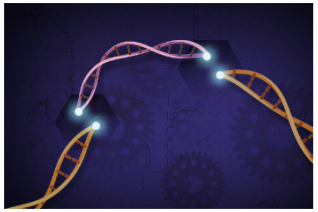
THE NIH DIRECTOR

November 28, 2018

Statement on Claim of First Gene-Edited Babies by Chinese Researcher

NIH is deeply concerned about the work just presented at the Second International Summit on Human Genome Editing in Hong Kong by Dr. He Jiankui, who described his effort using CRISPR-Cas9 on human embryos to disable the CCR5 gene. He claims that the two embryos were subsequently implanted, and infant twins have been born. This work represents a deeply disturbing willingness by Dr. He and his team to flout international ethical norms. The project was largely carried out in secret, the medical necessity for inactivation of CCR5 in these infants is utterly unconvincing, the informed consent process appears highly questionable, and the



CRISPR-Cas9 is a customizable tool that lets scientists cut and insert small pieces of DNA at precise areas along a DNA strand. This lets scientists study our genes in a specific, targeted way. *Image Credit: Ernesto del Aguila III, NHGRI.*

"Lest there be any doubt, and as we have stated previously, NIH does not support the use of gene-editing technologies in human embryos."

Summary

1. Sickle cell disease is a single-gene disorder.

2. Clinical trials have established bone marrow transplant as a one time cure for SCD.
   - Bone marrow transplantation can cure >90% of SCD patients.

3. Gene therapy trials are open at NIH.
   - Early results demonstrate efficacy with gene addition

4. Gene editing trials are now being developed.

5. Access to and participation in clinical trials should improve the outlook for patients with SCD.