The future of gene therapy: from science fiction to reality for LMICs

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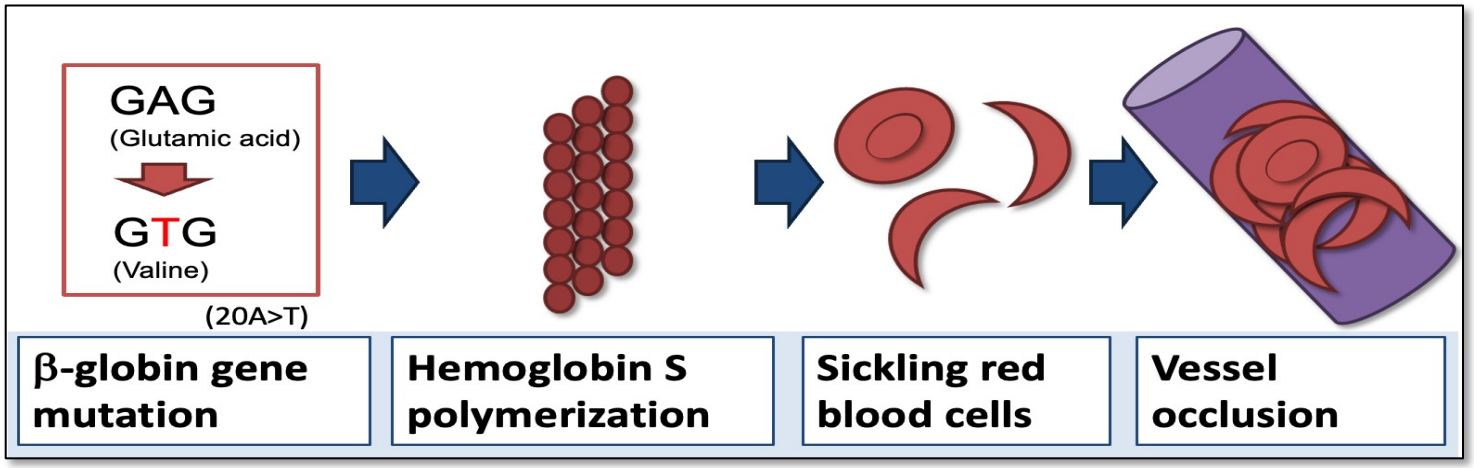
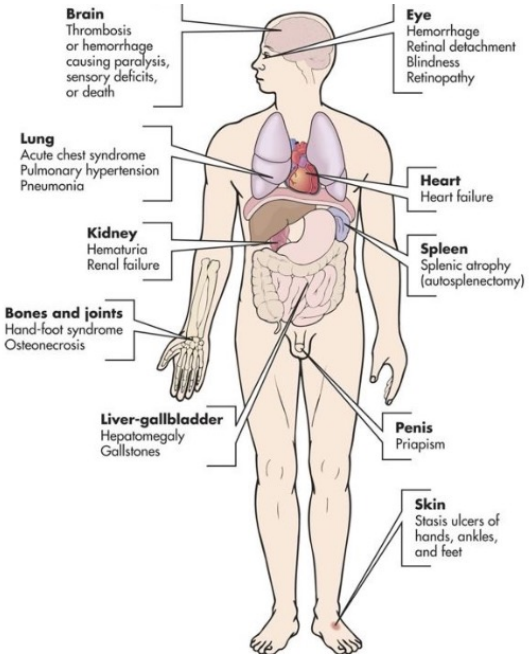
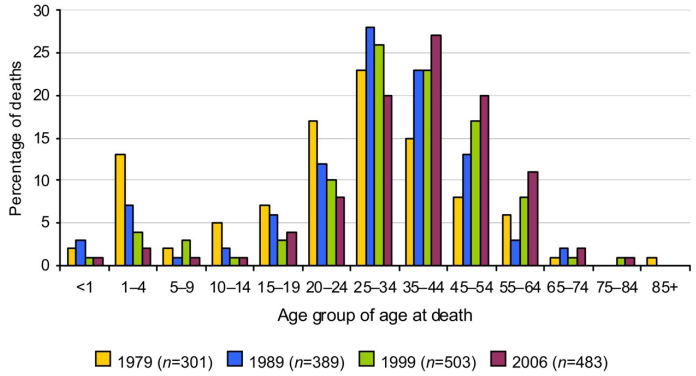
Chief, Cellular and Molecular Therapeutics Branch,
NHLBI, National Institutes of Health

Sickle cell disease: a single gene disorder in need of a cure



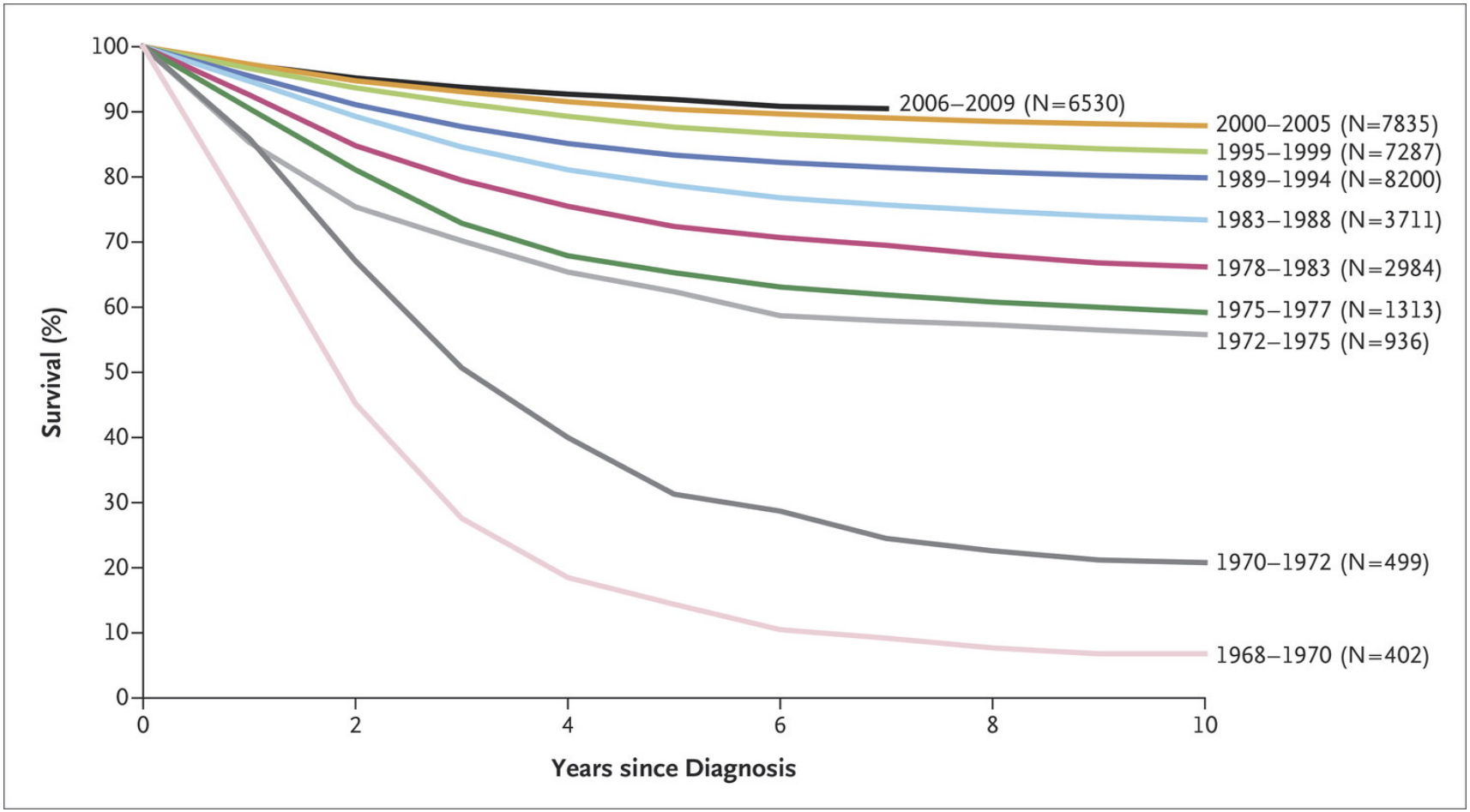




Mean age at death was 39 in 2006, only 35% alive at age >45 years

Hassell / Am J Prev Med 2010;38(4S):S512–S521

Clinical trials have improved the cure rate for acute leukemia in children from Less than 10% to over 95%, by trying



The urgency for treatment of childhood ALL encourages participation in clinical trials

Hunger SP, Mullighan CG. N Engl J Med 2015;373:1541-1552.

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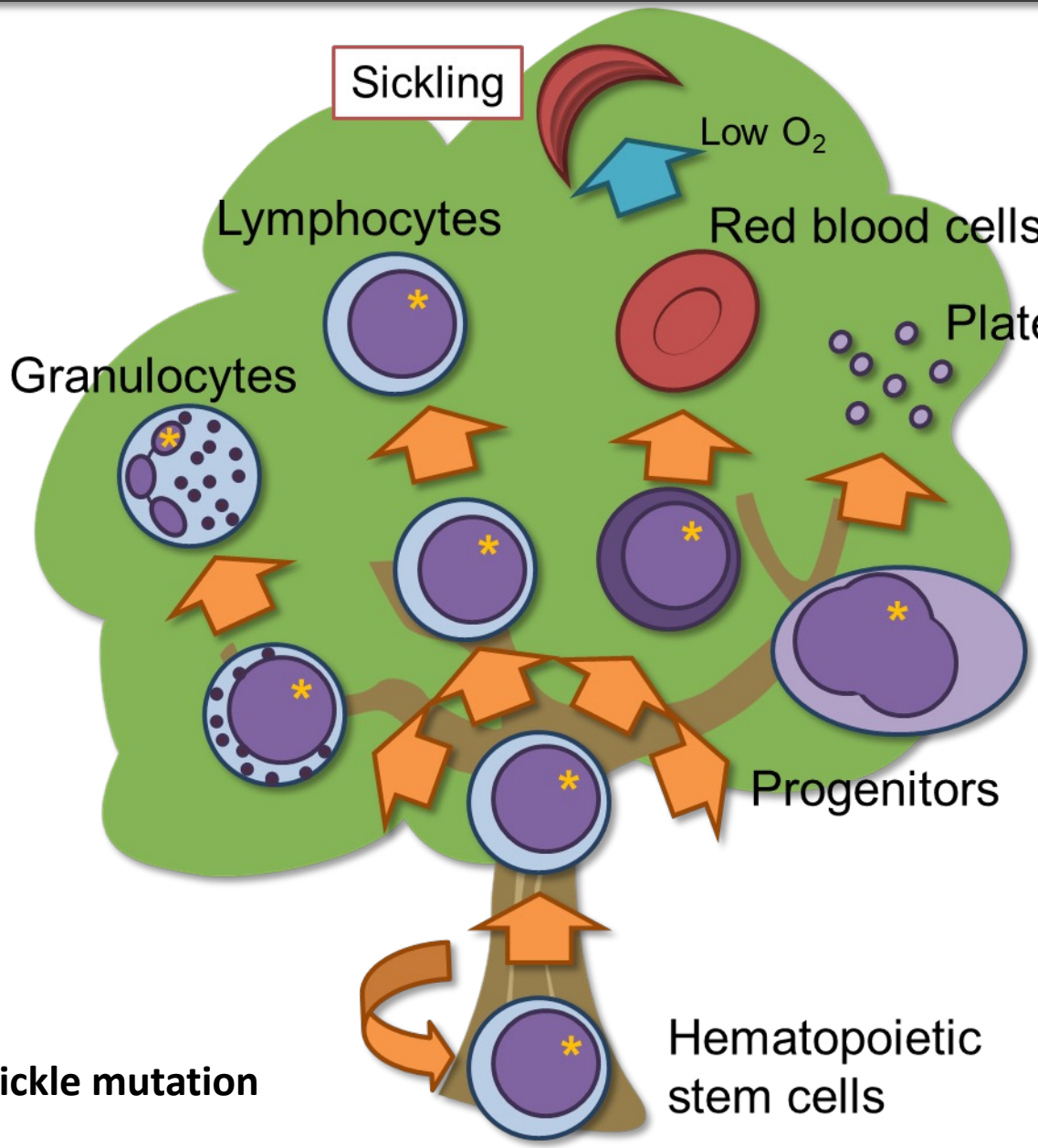
“Whole genome therapy” cured the first patient of SCD through allogeneic bone marrow transplantation in 1984

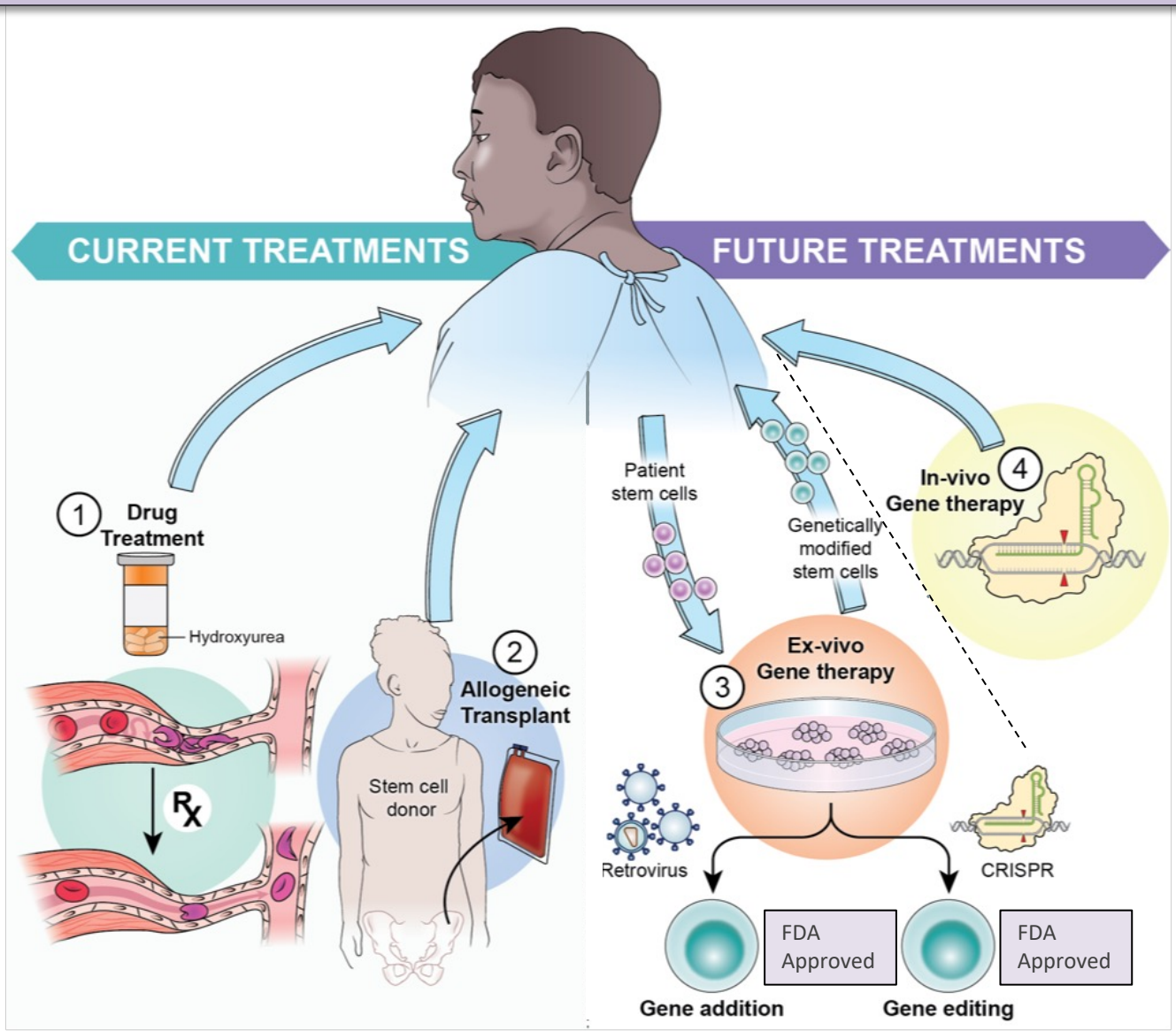
Bone marrow transplants cure by replacing the seeds of the blood



Hematopoietic stem cells (HSCs) produce all types of hematopoietic cells long-term.

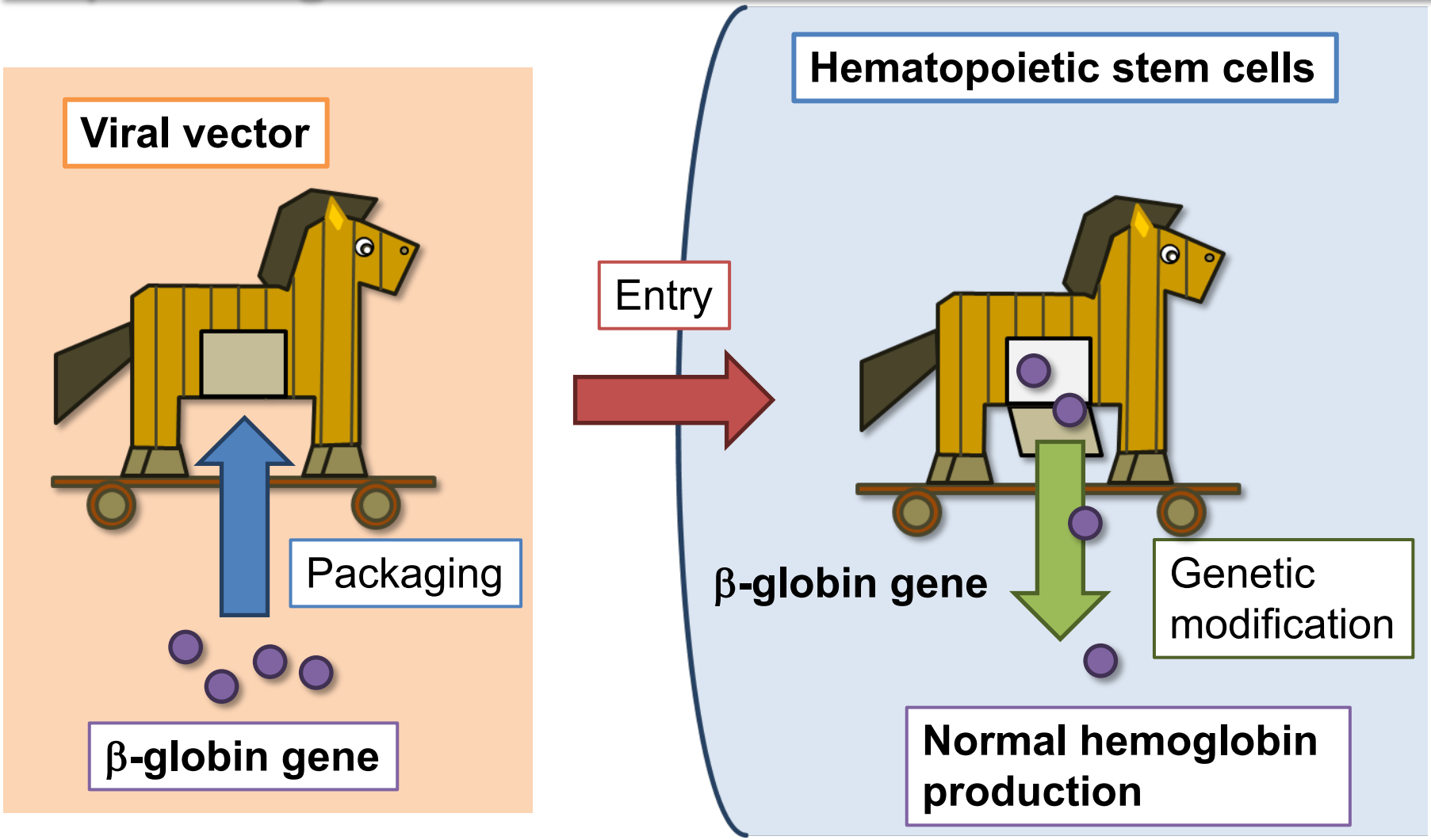
We have sought to develop curative strategies based upon replacing or repairing bone marrow stem cells.

Strategies for the treatment of sickle cell disease



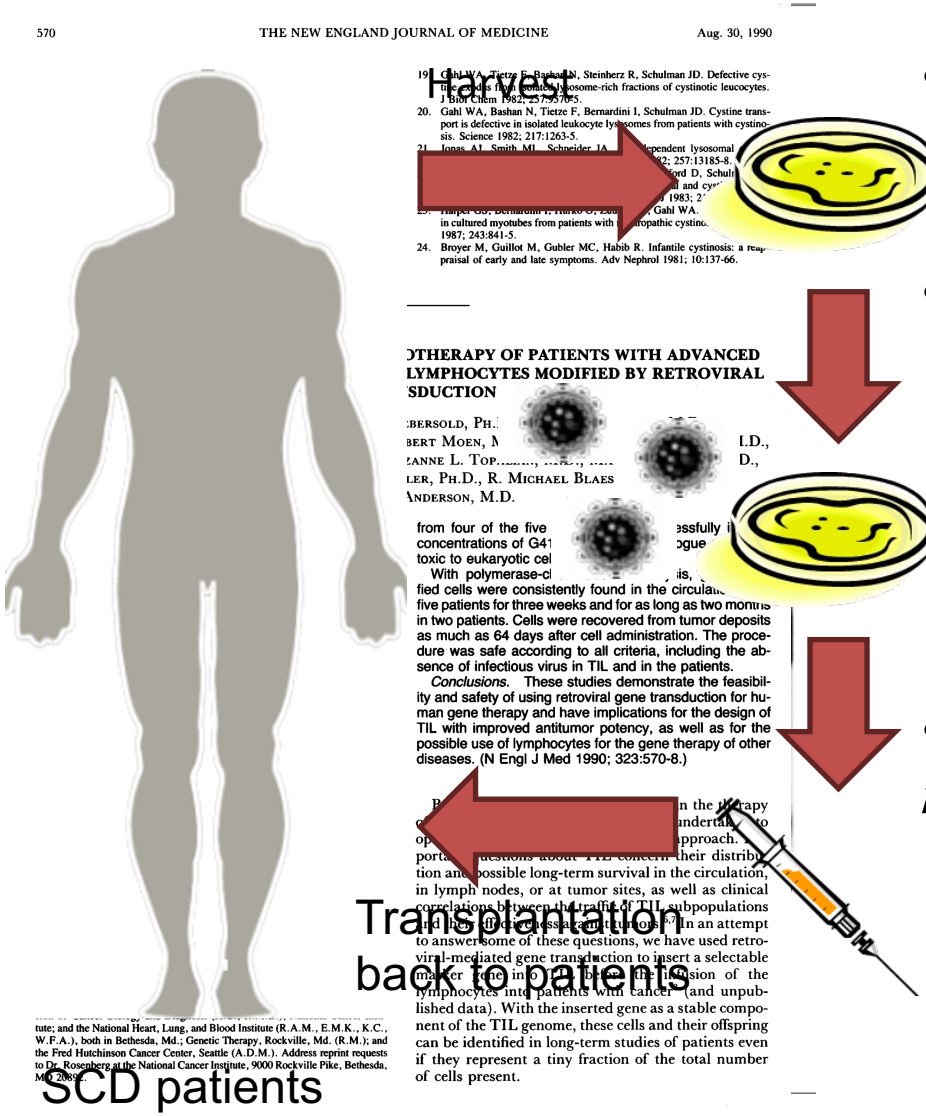
Tisdale, Thein and Eaton, Science, 13 Mar, 2020

Gene transfer for “gene addition” gene therapy require integration



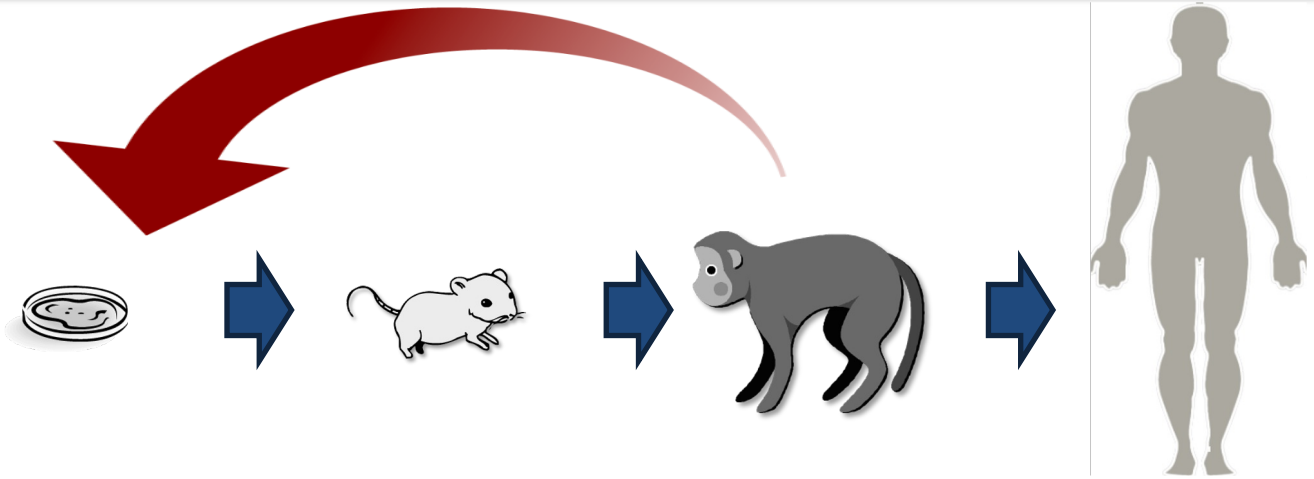
Gene transfer for “gene addition” therapies

National Institutes of Health



- ***Preclinical models***
  - –High gene transfer rates easily achieved in mouse models *in vivo*
- ***Early human clinical***
  - –Equally high gene transfer rates estimated by *in vitro* assays
  - –In vivo levels of <1/100,000 cells
  - – Too low to expect clinical benefit in SCD
- ***Predictive human HSC assays needed***
  - –Methods optimized over time in the nonhuman primate competitive repopulation model

Translational research milestones to develop gene therapy for sickle cell disease



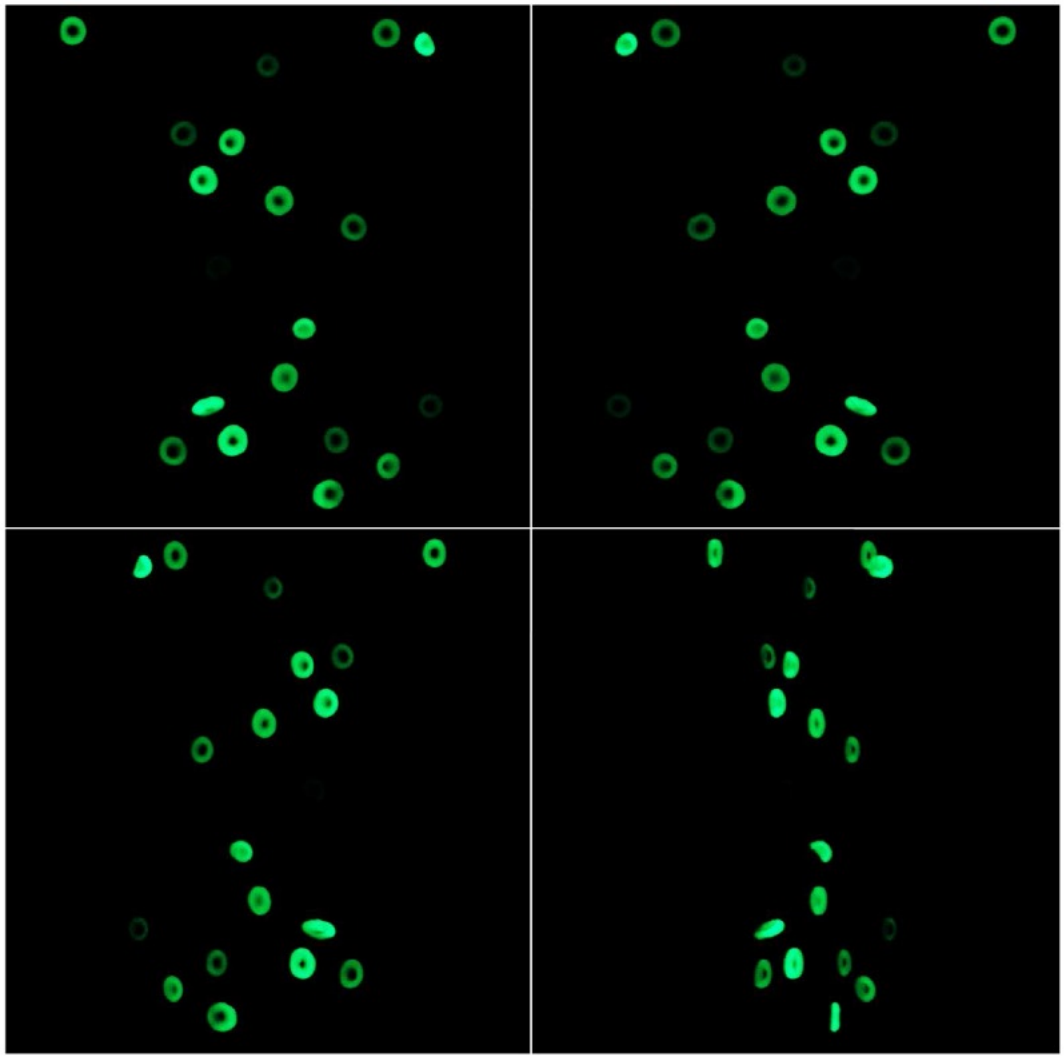
Cell culture	Small animal	Large animal	Clinical trial
Cell lines iPS cells	Mice Disease model mice Humanized mice	Non-human primates	Phase I Phase II Phase III Phase IV

Efficiency

Cell lines > Mouse HSCs >> Rhesus HSCs ≈ Human HSCs

Maximize benefit/minimize risks

HIV1-based lentiviral vectors allow gene transfer at levels sufficient to correct SCD



Uchida, J Virol. 2009

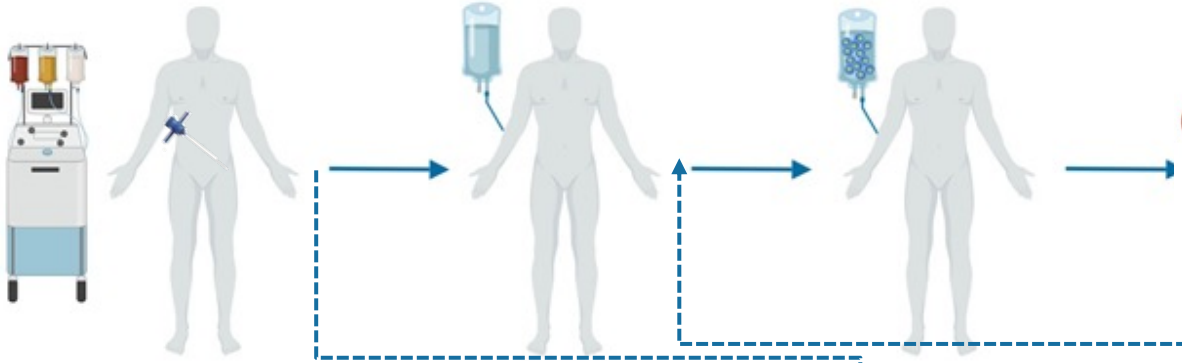
HGB-206: study of HIV-based lentiviral vector gene therapy for severe sickle cell disease

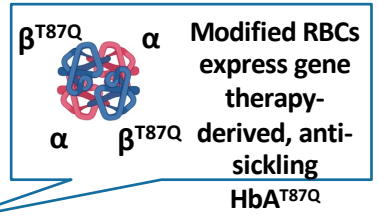
HSC collection
Bone marrow harvest or mobilization with plerixafor & apheresis

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 (12+ in group C)
- 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

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DOI: 10.1002/ajh.26741

AJH WILEY

RESEARCH ARTICLE

Lovo-cel gene therapy for sickle cell diseas process evolution and outcomes in the init HGB-206 study

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Matthew Hsieh[5] | Naoya Uchida[5] | Philippe Leboulch[6,7]
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Abstract

lovo-cel (bb1111; LentiGlobin for sickl
(GT) comprises autologous transplantation
cells transduced with the BB305 lentiviral v
($\beta^{A\text{-}T87Q}$) to produce anti-sickling hemoglob
lovo-cel for SCD are being evaluated in t
(ClinicalTrials.gov: NCT02140554). The trea
learnings from outcomes in the initial patien
file. Following modest expression of HbA^{T8}
alterations were made to the treatment proc
Group B ($n = 2$, patients B1 and B2), inclu
lovo-cel manufacturing. After 6 months, m
copy number (≥0.08 c/dg) and HbA^{T87Q} leve
stantial clinical effect but stable and su
improved in Group B (patient B1: ≥0.53 c/d
and ≥6.40 g/dL, respectively) and generated
Group B, including higher total hemoglobin
the lovo-cel for SCD treatment regimen lar
HSPC collection, busulfan conditioning regi
leukemia was observed in two patients in
insertional oncogenesis. Changes made du
ment process were associated with impro
future SCD GT studies.

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The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease

J. Kanter, M.C. Walters, L. Krishnamurti, M.Y. Mapara, J.L. Kwiatkowski, S. Rifkin-Zenenberg, B. Aygun, K.A. Kasow, F.J. Pierciey, Jr., M. Bonner, A. Miller, X. Zhang, J. Lynch, D. Kim, J.-A. Ribeil, M. Asmal, S. Goyal, A.A. Thompson, and J.F. Tisdale

ABSTRACT

BACKGROUND
Sickle cell disease is characterized by the painful recurrence of vaso-occlusive events. Gene therapy with the use of LentiGlobin for sickle cell disease (bb1111; lovotibeglogene autotemcel) consists of autologous transplantation of hematopoietic stem and progenitor cells transduced with the BB305 lentiviral vector encoding a modified β-globin gene, which produces an antisickling hemoglobin, HbA^{T87Q}.

METHODS
In this ongoing phase 1–2 study, we optimized the treatment process in the initial 7 patients in Group A and 2 patients in Group B with sickle cell disease. Group C was established for the pivotal evaluation of LentiGlobin for sickle cell disease, and we adopted a more stringent inclusion criterion that required a minimum of four severe vaso-occlusive events in the 24 months before enrollment. In this unprespecified interim analysis, we evaluated the safety and efficacy of LentiGlobin in 35 patients enrolled in Group C. Included in this analysis was the number of severe vaso-occlusive events after LentiGlobin infusion among patients with at least four vaso-occlusive events in the 24 months before enrollment and with at least 6 months of follow-up.

RESULTS
As of February 2021, cell collection had been initiated in 43 patients in Group C; 35 received a LentiGlobin infusion, with a median follow-up of 17.3 months (range, 3.7 to 37.6). Engraftment occurred in all 35 patients. The median total hemoglobin level increased from 8.5 g per deciliter at baseline to 11 g or more per deciliter from 6 months through 36 months after infusion. HbA^{T87Q} contributed at least 40% of total hemoglobin and was distributed across a mean (±SD) of 85±8% of red cells. Hemolysis markers were reduced. Among the 25 patients who could be evaluated, all had resolution of severe vaso-occlusive events, as compared with a median of 3.5 events per year (range, 2.0 to 13.5) in the 24 months before enrollment. Three patients had a nonserious adverse event related or possibly related to LentiGlobin that resolved within 1 week after onset. No cases of hematologic cancer were observed during up to 37.6 months of follow-up.

CONCLUSIONS
One-time treatment with LentiGlobin resulted in sustained production of HbA^{T87Q} in most red cells, leading to reduced hemolysis and complete resolution of severe vaso-occlusive events. (Funded by Bluebird Bio; HGB-206 ClinicalTrials.gov number, NCT02140554.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Tisdale can be contacted at johntis@mail.nih.gov or at the Cellular and Molecular Therapeutics Branch NHLBI–NIDDK, National Institutes of Health, Bethesda, MD 20814.

Drs. Kanter and Walters contributed equally to this article.

This article was published on December 12, 2021, at NEJM.org.

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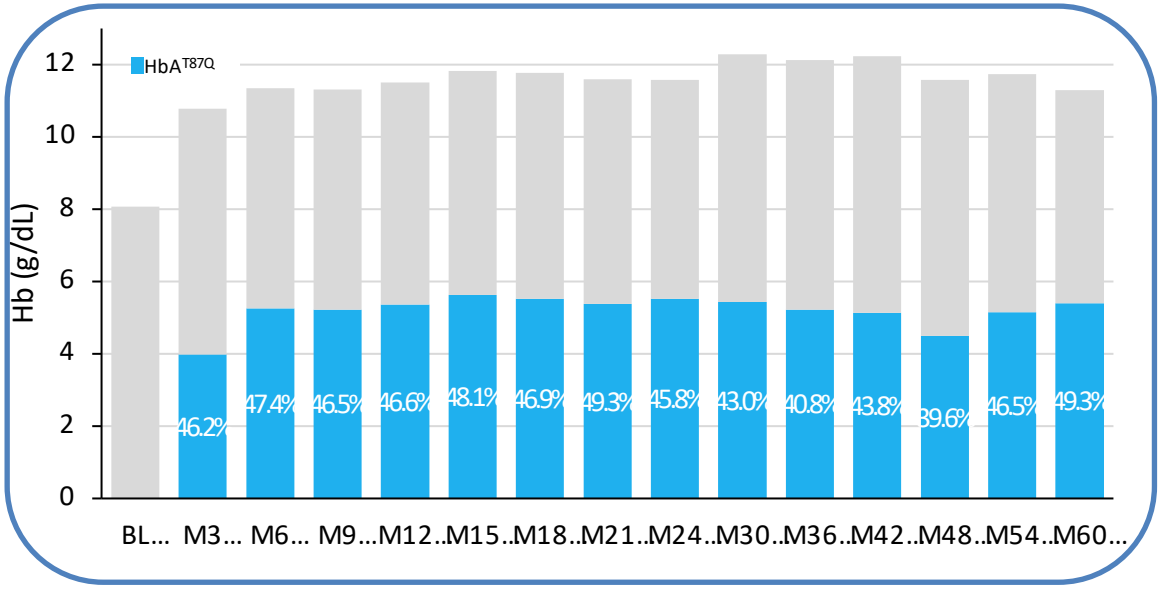
Updated results of the HGB206 and HGB210 studies: HbA^{T87Q} levels and globin response maintained over time



	BL…	M3…	M6…	M9…	M12…	M15…	M18…	M21…	M24…	M30…	M36…	M42…	M48…	M54…	M60…
Total Hb (g/dL):	8.70	11.20	11.40	11.60	11.70	11.70	11.85	11.90	11.90	12.10	12.00	11.80	11.35	11.95	11.60
% HbS:	59.5	48.5	49.2	51.0	49.5	49.8	49.8	48.5	51.0	50.1	51.3	50.4	52.9	48.5	44.4

Median percent HbA^{T87Q} of nontransfused total Hb was ≥40%

- **86.8%** (33/38) of patients[a] achieved globin response
  (Globin response defined as: weighted average HbA^{T87Q} ≥30% of nontransfused total Hb; AND weighted average increase in nontransfused total Hb of ≥3 g/dL vs baseline total Hb OR weighted average nontransfused total Hb of ≥10 g/dL).
- **100%** (33/33) of patients demonstrated a durable globin response through last follow up[b]
- All patients maintained stable HbA^{T87Q} levels from 6 months to last follow-up and as far out as month 60
- No patients with a history of stroke experienced a stroke post treatment

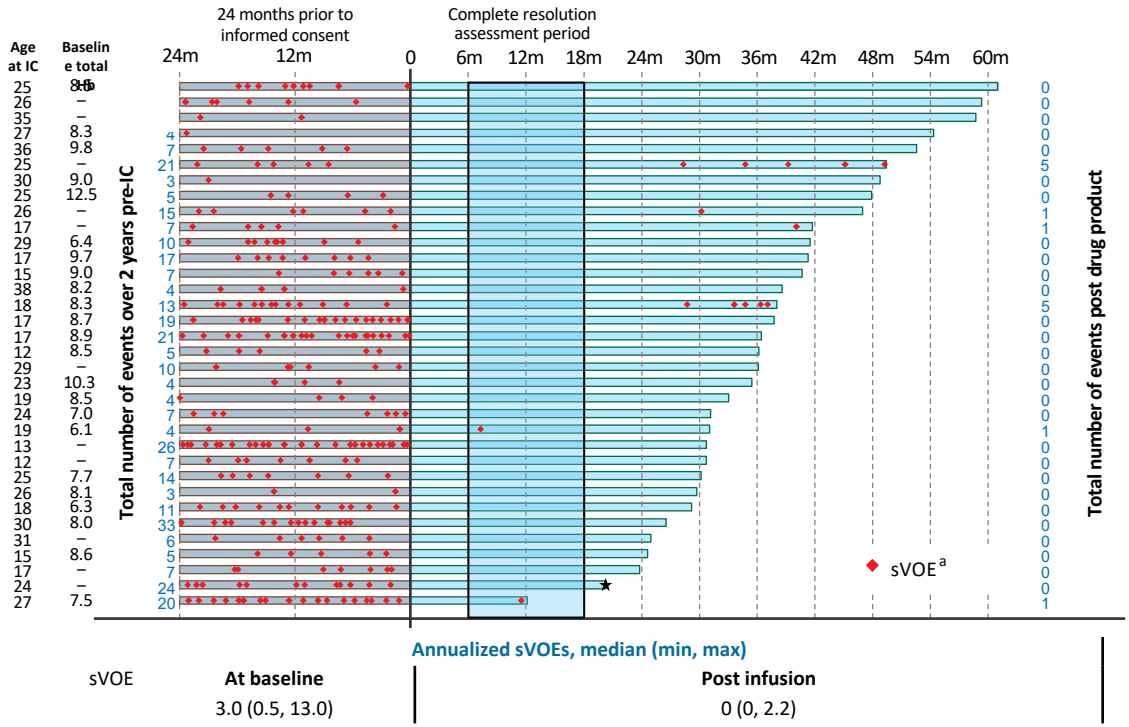
Percentages represent the median HbA^{T87Q} fraction as a percentage of nontransfused total Hb. Values above each bar represent the median total Hb or HbS % of nontransfused total Hb at each visit and are not equivalent to the sum of the individual Hb fraction medians. The baseline was an average of 2 qualified, total Hb values (measured in g/dL) during the 24 months before study enrollment. [a]Assessed in patients who had ≥18 months follow-up or achieved globin response during the assessment period (months 6 to 18 post DPI). [b]Three patients achieved globin response but later did not meet globin response criteria due to: transfusions due to an unrelated accident or illness (N = 2), or death (N = 1).

BL, baseline; DP, drug product infusion; Hb, hemoglobin; HbA, adult Hb; HbA^{T87Q}, anti-sickling Hb; HbS, sickle cell hemoglobin; M, month.

Population: Evaluable for globin response Data as of Feb 13, 2023

Kanter, et al., ASH 2023 Congress - Abstract # 1051

Updated results of the HGB206 and HGB210 studies: 94% (32/34) achieved complete resolution of severe VOEs



sVOE Resolution

- **94.1%** (32/34; 95% CI, 80.3-99.3) of patients experienced **complete resolution of sVOEs**[b]
  (sVOE defined as: A VOE requiring ≥24-hour hospital or ER observation unit visit or ≥2 visits to a day unit or ER over a 72-hour period, with both visits requiring intravenous treatment)

Hospital Admissions & Days

- **85.3%** (29/34) of patients had no VOE[c]-related hospital admissions from 6 months post infusion to last follow-up

- Among patients with VOEs post lovo-cel infusion, annualized median (min, max):
  - **Hospital admissions** were reduced from **2.5** (1, 13) to **0.41** (0, 2)
  - **Hospital days** were reduced from **15.75** (3.5, 136.0) to **2.20** (0.0, 25.4)

★ Death, due to significant baseline SCD-related cardiopulmonary disease; not considered related to lovo-cel.
An Independent Event Adjudication Committee confirmed VOEs met protocol criteria. [a]Defined as a VOE requiring ≥24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over a 72-hour period, with both visits requiring intravenous treatment; all VOEs of priapism were also considered sVOEs. [b]Maintained for a median of XX months (min, max). [c]Any of the following: acute episodes of pain with no medically determined cause other than a vaso-occlusion lasting 2 hours and requiring care at a medical facility; acute chest syndrome requiring oxygen treatment and/or blood transfusion; acute hepatic sequestration; acute splenic sequestration; or acute priapism lasting 2 hours and requiring care at a medical facility.

Hb, hemoglobin; IC, informed consent; SCD, sickle cell disease; sVOE, severe vaso-occlusive event; VOE, vaso-occlusive event. Population: Evaluable for VOE-CR and sVOE-CR Data as of Feb 13, 2023

Kanter, et al., ASH 2023 Congress - Abstract # 1051

CRISPR/Cas9 system for genome editing?

- Arose from basic science studies of yogurt, bacteria viruses
- Achieves targeted editing of genomes with enzyme + guide RNA
  - Initial approaches created knockouts; expanded to induce repair by homologous recombination
  - Can serve like a “find and replace” function in a word processor
  - Base editing technologies can correct point mutations
- Has accelerated production of mouse models – and revolutionized basic molecular biology
- Paves the way for new therapeutics

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

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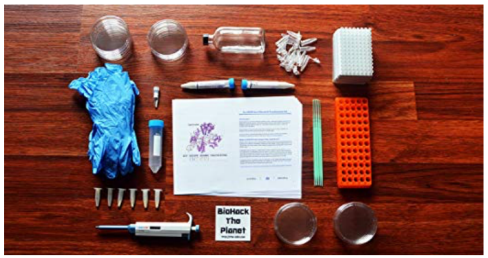

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DIY Bacterial Genome Engineering CRISPR Kit

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
- Actual Genetic Engineering
- Learn CRISPR Technology through Hands-On experimentation

Specifications for this item

Part Number	CRISPR1
Number of Items	10
Brand Name	The ODIN
EAN	0636391770750
UNSPSC Code	41000000
UPC	636391770750

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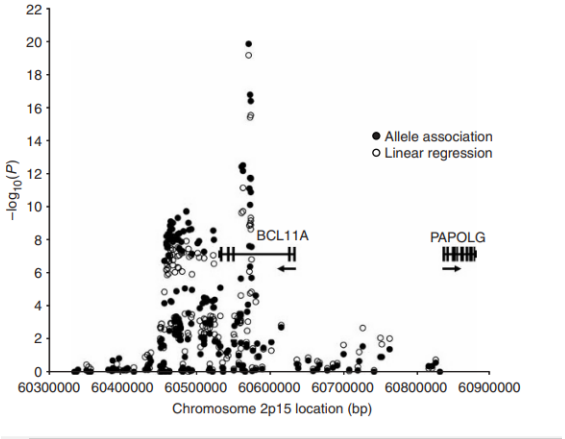
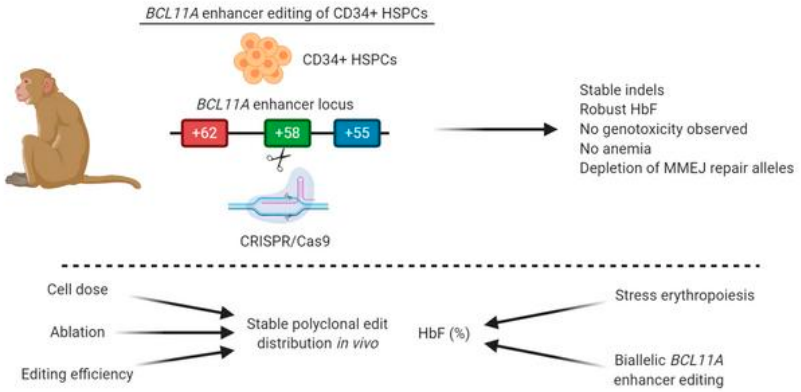
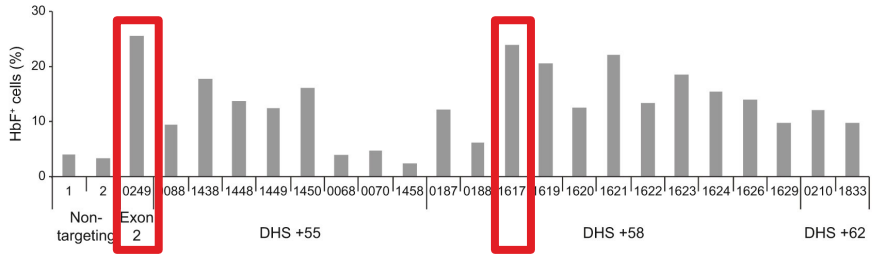
BCL11A Enhancer Editing Strategy to Reactivate Fetal Hemoglobin to treat SCD



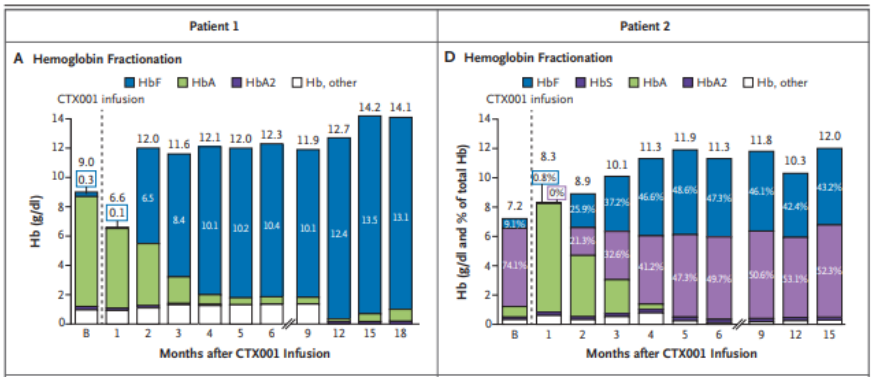
Menzel et al., Nat Gen, 2007: Uda et al., PNAS, 2008



Demirci et al., JCI, 2020



Bauer et al., Science, 2013; Canver et al., Nature, 2015; Brendel et al., JCI, 2016



Frangoul et al., NJEM, 2021

First FDA approval of CRISPR, December 2023

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Exagamglogene Autotemcel for Severe Sickle Cell Disease

H. Frangoul, F. Locatelli, A. Sharma, M. Bhatia, M. Mapara, L. Molinari, D
R.I. Liem, P. Telfer, A.J. Shah, M. Cavazzana, S. Corbacioglu, D. Rond
R. Meisel, L. Dedeken, S. Lobitz, M. de Montalembert, M.H. Steinbe
M.C. Walters, M.J. Eckrich, S. Imren, L. Bower, C. Simard, W. Zhou, F. X
P.K. Morrow, W.E. Hobbs, and S.A. Grupp, for the CLIMB SCD-121 Study

ABSTRACT

BACKGROUND

Exagamglogene autotemcel (exa-cel) is a nonviral cell therapy designed to
vate fetal hemoglobin synthesis by means of ex vivo clustered regularly inte
short palindromic repeats (CRISPR)–Cas9 gene editing of autologous
hematopoietic stem and progenitor cells (HSPCs) at the erythroid-specific e
region of *BCL11A*.

METHODS

We conducted a phase 3, single-group, open-label study of exa-cel in pati
to 35 years of age with sickle cell disease who had had at least two sever
occlusive crises in each of the 2 years before screening. CD34+ HSPCs were
with the use of CRISPR-Cas9. Before the exa-cel infusion, patients und
myeloablative conditioning with pharmacokinetically dose-adjusted busulf
primary end point was freedom from severe vaso-occlusive crises for at l
consecutive months. A key secondary end point was freedom from inpatie
pitalization for severe vaso-occlusive crises for at least 12 consecutive mont
safety of exa-cel was also assessed.

RESULTS

A total of 44 patients received exa-cel, and the median follow-up was 19.3
(range, 0.8 to 48.1). Neutrophils and platelets engrafted in each patient.
30 patients who had sufficient follow-up to be evaluated, 29 (97%; 95% con
interval [CI], 83 to 100) were free from vaso-occlusive crises for at least
secutive months, and all 30 (100%; 95% CI, 88 to 100) were free from hosp
tions for vaso-occlusive crises for at least 12 consecutive months (P<0.001 f
comparisons against the null hypothesis of a 50% response). The safety pr
exa-cel was generally consistent with that of myeloablative busulfan condi
and autologous HSPC transplantation. No cancers occurred.

CONCLUSIONS

Treatment with exa-cel eliminated vaso-occlusive crises in 97% of patients wit
cell disease for a period of 12 months or more. (CLIMB SCD-121; ClinicalTr
number, NCT03745287.)

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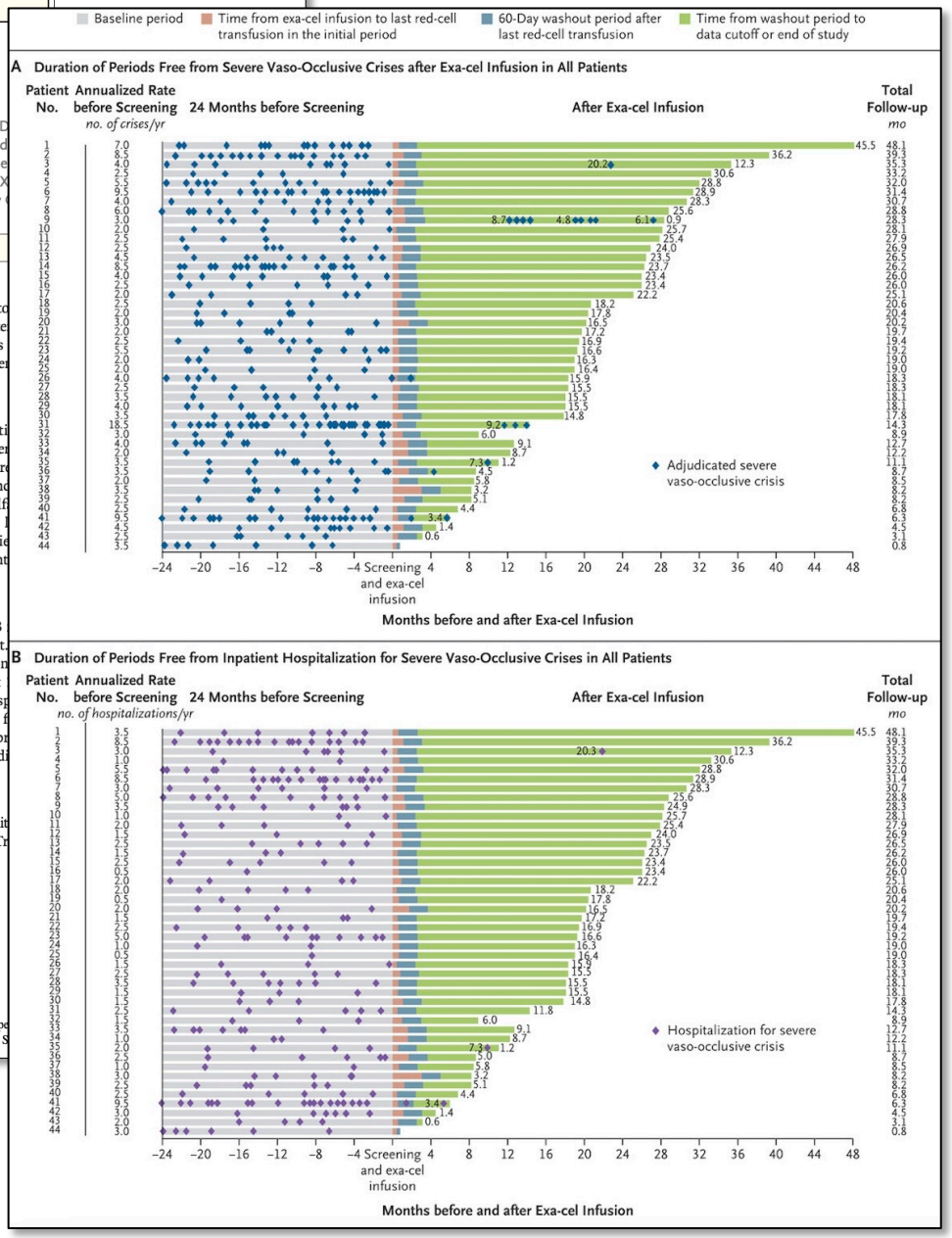

The New York Times

First Patient Begins Newly Approved Sickle Cell Gene Therapy

A 12-year-old boy in the Washington, D.C., area faces months of procedures to remedy his disease. “I want to be cured,” he said.

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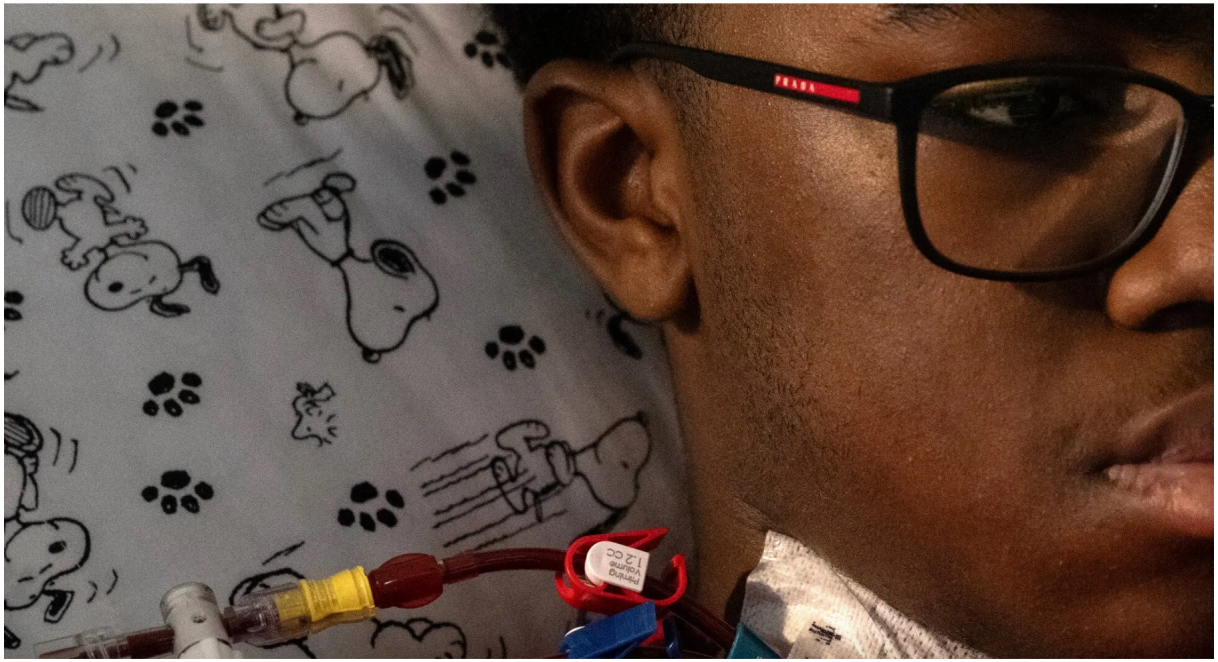


Kendric Cromer, 12, the first commercial patient for Bluebird Bio’s gene therapy to cure his sickle cell disease, in the hospital as his bone marrow stem cells were being removed for gene editing.


By Gina Kolata Photographs by Kenny Holston

Gina Kolata visited Kendric and his parents at the hospital in Washington, D.C., when he was having his stem cells extracted

Published May 6, 2024 Updated May 7, 2024

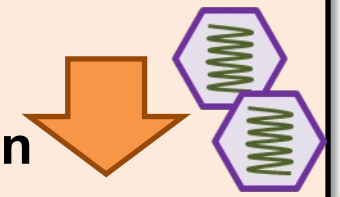
In vivo HSC-targeted gene addition/gene editing gene therapy: a future goal for broad application

***Ex vivo* gene addition/editing**

Cell processing center

Harvest

Hematopoietic stem cells (HSCs)



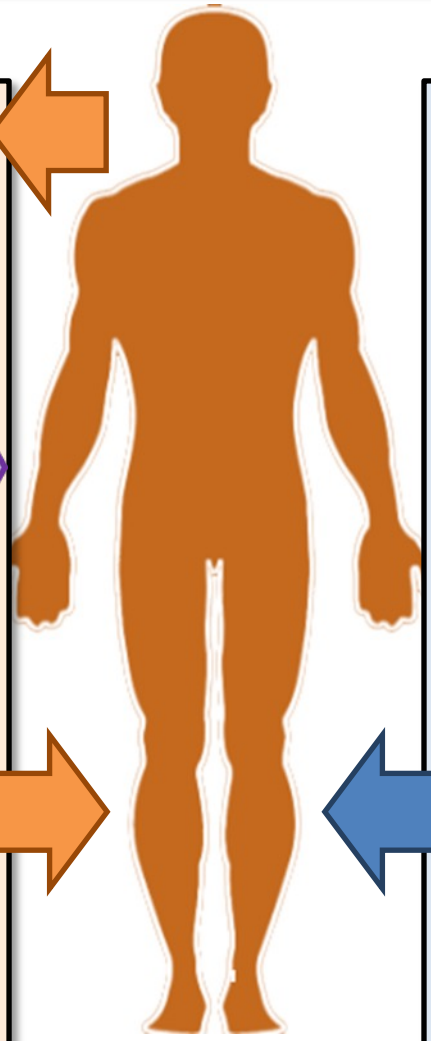
β-globin **gene addition** with a lentiviral vector

or

Gene editing with an engineered nuclease

Transplantation

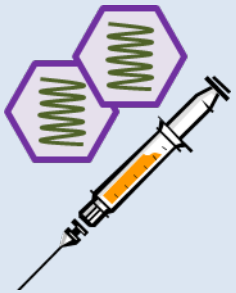
- **Efficacy and safety confirmed**
- Hospitalization needed
- **Cell processing center needed**
- High cost



***In vivo* gene addition/editing**

HSC-targeted gene delivery system

- Viral vectors
- Nanoparticles



Injection

- Under development
- Simple method
- **Walk-in basis**
- **Low cost**

RESEARCH SUMMARY

CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis

Gillmore JD et al. DOI: 10.1056/NEJMoa2107454

CLINICAL PROBLEM

In transthyretin amyloidosis, misfolded transthyretin (TTR) protein accumulates, primarily in the nerves and heart, and is ultimately fatal. Current therapies reduce amyloid formation through repeated infusions that can have serious adverse effects or require infusion premedications. These treatments slow but do not stop disease progression.

CLINICAL TRIAL

Study Design: An open-label, phase 1 clinical study evaluated the safety and pharmacodynamic effects of NTLA-2001, a CRISPR-Cas9–based in vivo gene-editing therapy targeting *TTR* in human hepatocytes, in adults with hereditary transthyretin amyloidosis and polyneuropathy with or without cardiomyopathy.

Intervention: 6 patients received a single intravenous infusion of NTLA-2001 at a dose of either 0.1 or 0.3 mg per kilogram of body weight.

RESULTS

Efficacy: At 28 days after infusion, TTR levels were reduced from baseline with both doses; the reduction was greater with the larger dose.

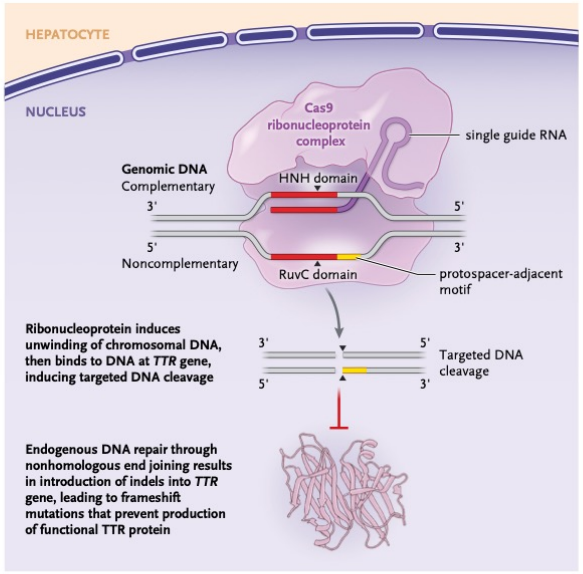
Safety: Adverse effects occurred in 3 patients and were mild.

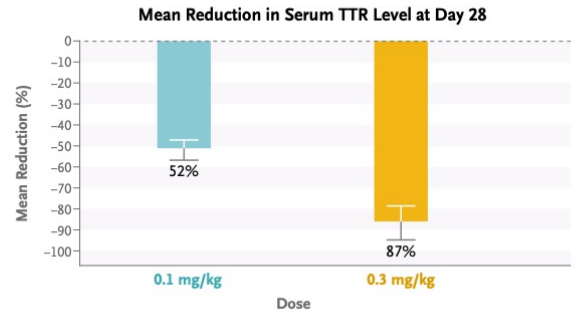
LIMITATIONS AND REMAINING QUESTIONS

Further study is required to understand the following:

- The duration of TTR reduction after a single infusion of NTLA-2001 at the doses used in this study and at higher doses
- Clinical outcomes in these 6 patients and in larger trials
- Whether other adverse effects, including off-target gene editing, occur in the longer term

Links: Full Article | NEJM Quick Take | Editorial



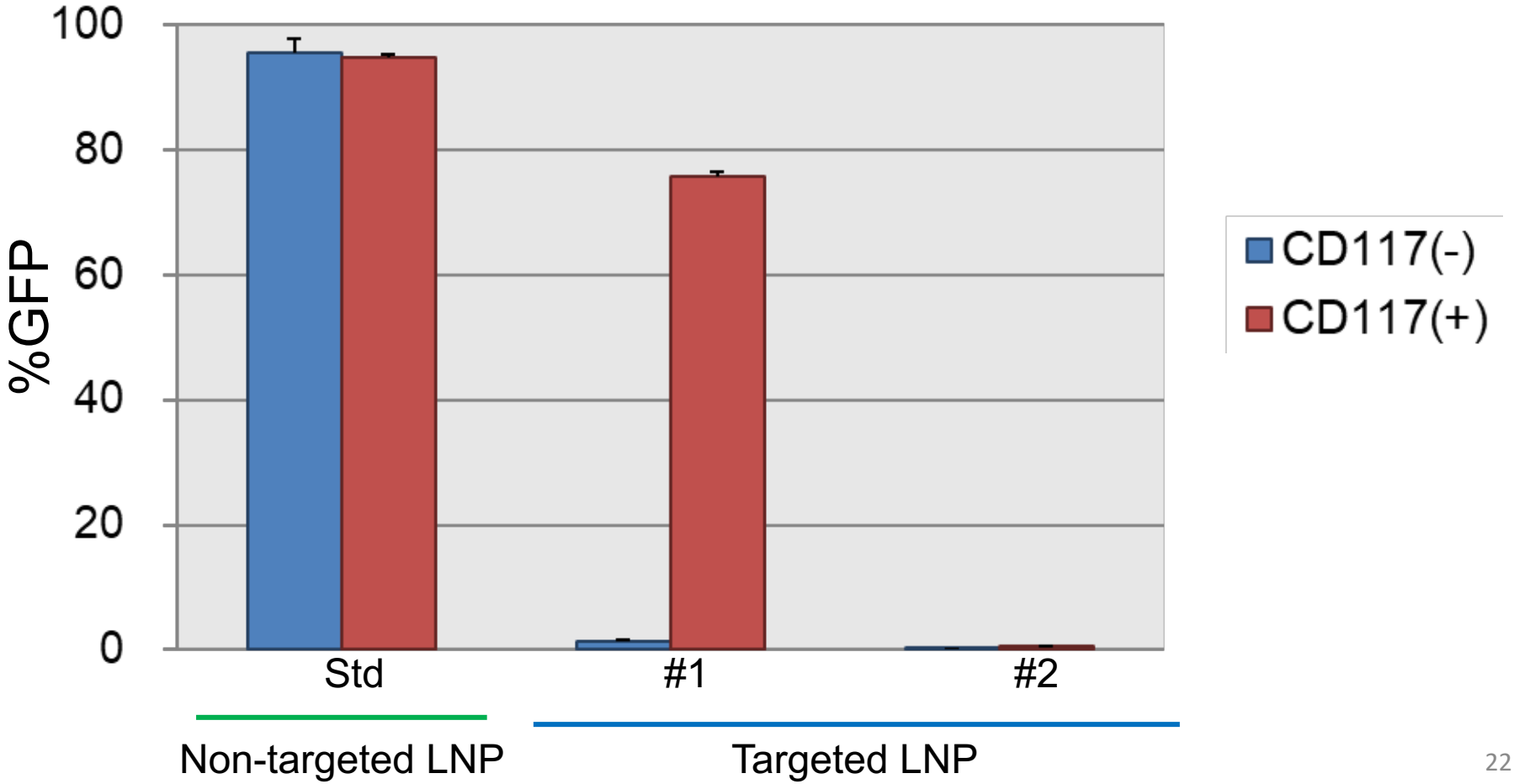


CONCLUSIONS

This trial involving a small number of patients with hereditary transthyretin amyloidosis provides proof-of-concept evidence that CRISPR-Cas9–based gene editing with NTLA-2001 greatly reduces TTR levels after a single infusion, with only mild adverse events.

Targeting blood stem cells through peptide conjugation of LNPs might allow delivery exclusively to HSCs

1:1 mix of CD117(+) and (-) K562 cells



This is complicated, why should I care?

1. Gene addition and gene editing gene therapy using patient HSCs appear promising as a curative strategies with similar short-term outcomes in SCD.
   - *Ex vivo* approaches in SCD have provided proof concept.
   - The costs of these approaches are staggering, further limiting application.
   - Resources and infrastructure required limit application to highly resourced settings.

1. Methods to deliver genetic tools *in vivo* should improve access to curative therapies and reduce cost.
   - *In vivo* approaches in other diseases have provided proof of concept.
   - Delivery through antibody or peptide methods linked to vectors or LNPs a goal.
   - Global distribution of vaccines based on LNPs has paved a viable path for SCD and other genetic diseases.

CREW

HGB-206 Study

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- Julie Kanter
- Lakshmanan Krishnamurti
- Janet Kwiatkowski
- Mark Walters
- Markus Mapara
- Monica Bhatia

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