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

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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

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

“Whole genome therapy” cured the first patient of SCD through allogeneic bone marrow transplantation in 1984
Hematopoietic stem cells (HSCs) produce all types of hematopoietic cells long-term.

*Sickle mutation

Bone marrow transplants cure by replacing the seeds of the blood.

We have sought to develop curative strategies based upon replacing or repairing bone marrow stem cells.
Strategies for the treatment of sickle cell disease
Gene transfer for “gene addition” gene therapy require integration.

- **Viral vector**
  - Packaging
  - β-globin gene

- **Entry**

- **Hematopoietic stem cells**
  - Genetic modification
  - Normal hemoglobin production
  - β-globin gene
• 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
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Cell lines | Mice | Non-human primates | Phase I
iPS cells | Disease model mice | | Phase II
| Humanized mice | | Phase III
| | | Phase IV

Efficiency

| Cell lines | Mouse HSCs | Rhesus HSCs | Human HSCs |
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Maximize benefit/minimize risks
HIV1-based lentiviral vectors allow gene transfer at levels sufficient to correct SCD

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

Transduced HSCs engraft and contribute to reconstitution of functional RBCs

Modified RBCs express gene therapy-derived, anti-sickling HbA

2-yr follow-up
Long-Term Follow-Up Study

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
Lovo-cel gene therapy for sickle cell disease: process evolution and outcomes in the initial HGB-206 study

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Abstract

Lovo-cel (bb1111; LentiGlobin for sickle cell disease (bb1111;lovoblogene autorexed) consists of autologous transplantation of hematopoietic stem and progenitor cells transduced with the BB803 lentiviral vector encoding a modified β-globin gene, which produces an antisickling hemoglobin, HbA109). The treatment, called Lovo-cel for SCD, is being evaluated in the Lovo-cel-204 trial (ClinicalTrials.gov: NCT02140554). The treatment resulted in an improved clinical and laboratory profile. Following modest expression of HbA109 levels, the HbA109 levels were improved and sustained at levels above 30%.

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 A was treated in the initial phase and Group B in the initial patients in the initial phase. Group B included 8 patients in Group B and 20 patients in Group B, including a higher total hemoglobin level. The lovo-cel for SCD treatment regimen included an initial total hemoglobin level, an HSPC collection, and a conditioning regimen. Each patient was treated with a single dose of 30 mg/kg of Lovo-cel. Changes made during the treatment process were associated with improved SCD GT outcomes.

RESULTS

As of February 2021, cell collection had been initiated in 43 patients in Group C. Sixty patients had received Lovo-cel infusion, with a median follow-up of 17.3 months (range, 3.7 to 37.6). Erythrocytosis occurred in all 60 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. HbA109 contributed at least 40% of total hemoglobin and was distributed across a mean (±SD) of 8.5% 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 Lovo-cel that resolved within 1 week after onset. No cases of hematologic cancer were observed during up to 376 months of follow-up.

CONCLUSIONS

One-time treatment with Lovo-cel resulted in sustained production of HbA109 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.)
Updated results of the HGB206 and HGB210 studies: HbAT87Q levels and globin response maintained over time

- **86.8% (33/38)** of patients achieved globin response (Globin response defined as: weighted average HbAT87Q ≥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.

- All patients maintained stable HbAT87Q 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.

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

Percentages represent the median HbAT87Q 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. *Assessed in patients who had ≥18 months follow-up or achieved globin response during the assessment period (months 6 to 18 post DPI). *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; HbAT87Q, anti-sickling Hb; HbS, sickle cell hemoglobin; M, month.

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.
  (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-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)**

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**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....
**BCL11A Enhancer Editing Strategy to Reactivate Fetal Hemoglobin to treat SCD**

*Canver et al., Nature, 2015; Brendel et al., JCI, 2016; Bauer et al., Science, 2013; Menzel et al., Nat Gen, 2007; Uda et al., PNAS, 2008; Demirci et al., JCI, 2020; Frangoul et al., NJEM, 2021*

First FDA approval of CRISPR, December 2023
Exagamglogene Autotemcel for Severe Sickle Cell Disease


ABSTRACT

BACKGROUND
Exagamglogene autotemcel (exacel) is a nonviral cell therapy designed to correct fetal hemoglobin synthesis by means of ex vivo clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing of autologous hematopoietic stem and progenitor cells (HSPCs) at the erythroid-specific enhancer region of BCL11A.

METHODS
We conducted a phase 3, single-arm, open-label study of exa-cell in patients aged 55 years of age with sickle cell disease who had had at least two severe vaso-occlusive crises in each of the 2 years before screening. CD34+ HSPCs were collected, and CD34+ HSPCs were used with the use of CRISPR-Cas9. Before the exa-cell infusion, patients underwent myeloablation conditioning with pharmacokinetically dose-adjusted busulfan. The primary end point was freedom from severe vaso-occlusive crises for at least 12 consecutive months. A key secondary end point was freedom from hospitalization for severe vaso-occlusive crises for at least 12 consecutive months. Safety of exa-cell was also assessed.

RESULTS
A total of 44 patients received exa-cell, and the median follow-up was 15.3 months (range, 0.8 to 48.1). Neutrophils and platelets engrafted in each patient. At 30 months, 29 patients who had sufficient follow-up to be evaluated, 29 (97%, 95% confidence interval [CI], 83 to 100) were free from vaso-occlusive crises for at least 12 months, and all 30 (100%, 95% CI, 88 to 100) were free from hospitalizations for vaso-occlusive crises for at least 12 consecutive months (P<0.001 for comparisons against the null hypothesis of a 50% response). The safety profile of exa-cell was generally consistent with that of myeloablative busulfan conditioning and autologous HSPC transplantation. No cancers occurred.

CONCLUSIONS
Treatment with exa-cell eliminated vaso-occlusive crises in 97% of patients with sickle cell disease for a period of 12 months or more. (CLIMB SCD-121; ClinicalTrials.gov number, NCT03765287.)
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.

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

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

Ex vivo gene addition/editing

Cell processing center
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

CD117 (+)/(-) cells → LNP addition → 2 days → %GFP

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<th>%GFP</th>
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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.
HGB-206 Study
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Patients and their caregivers