Poster 1864

CEDAR Trial in Progress:

A First-in-Human, Phase 1/2 Study of the Correction of a Single Nucleotide Mutation in Autologous HSCs (GPH101) to Convert HbS to HbA for Treating Severe SCD

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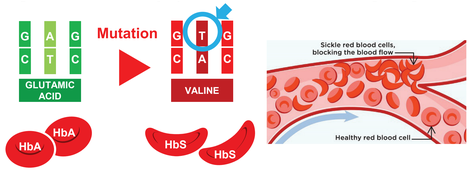
INTRODUCTION

Sickle Cell Disease

- Sickle cell disease (SCD) is an autosomal recessive disease resulting from a point mutation in the human beta-globin gene (**Figure 1**)
  - The mutation causes the red blood cells (RBCs) to develop an abnormal sickle shape
  - Sickle-shaped cells can occlude blood vessels, blocking blood flow, leading to other lifelong complications

Figure 1. SCD is caused by a point mutation in the human beta-globin gene[1,2]



HbA, adult hemoglobin; HbS, hemoglobin sickle cell; RBC, red blood cell; SCD, sickle cell disease.

- SCD is associated with lifelong complications and is associated with high morbidity and mortality[1-5]
  - SCD results in hemolytic anemia, chronic pain, vaso-occlusive crises (VOC), acute chest syndrome (ACS), progressive end-organ damage, and, ultimately, shorter lifespan[6,7]
- Patients with SCD have limited treatment options
  - Allogeneic hematopoietic stem cell transplant (HSCT), the only available cure for SCD, carries significant risk and substantial burden[8-10]
    - Lack of well-matched donors
    - Need for immunosuppression
    - Risk of graft-versus-host disease and graft rejection
- People with one sickle cell gene and one normal gene have Sickle Cell Trait (SCT), and do not have any health complications
  - Provides a comparator for the efficacy of future curative therapies
- Nontransplant-based treatments fail to address the underlying cause of SCD, which is a genetic mutation[1,8,7]
- There is a high unmet need for a treatment option with curative potential that restores normal adult hemoglobin (HbA) expression

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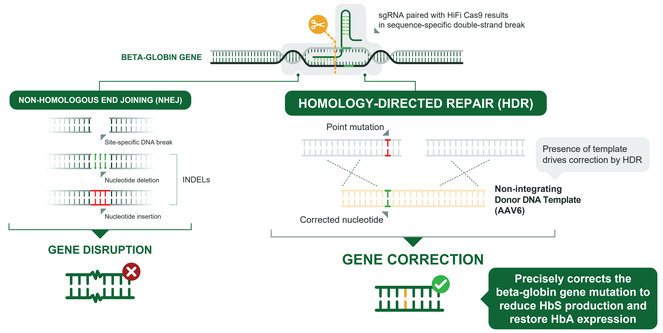
GPH101 Mechanism of Action and Preclinical Data

- GPH101 is an investigational drug product (DP) intended for the treatment of patients with severe SCD
- GPH101 is intended to repopulate the blood and immune system of SCD patients by restoring a healthy RBC compartment and eliminating the sickling erythrocytes responsible for SCD-related morbidity and mortality
- GPH101 harnesses the natural homology-directed repair (HDR) pathway to precisely correct the beta-globin gene mutation (**Figure 2**)
  - Single-strand guide RNA (sgRNA) paired with HiFi Cas9 results in a sequence-specific double-strand break
  - Most gene editing approaches follow the non-homologous end joining (NHEJ) pathway, which disrupts gene expression through the creation of insertions and/or deletions (INDELs)
  - Utilizing the HDR pathway, the CRISPR Cas9 complex in combination with adeno-associated virus type 6 (AAV6) delivery template corrects beta-globin gene mutation to reduce HbS production and restore HbA expression

Figure 2. High efficiency HDR via CRISPR/HiFi Cas9 precisely corrects the SCD mutation

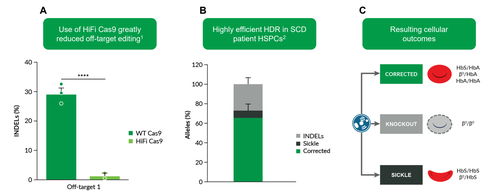


AAV6, adeno-associated virus type 6; Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; HbA, adult hemoglobin; HbS, hemoglobin sickle cell; HDR, homology-directed repair; HiFi, high-fidelity; INDEL, insertion and/or deletion; NHEJ, non-homologous end joining; SCD, sickle cell disease; sgRNA, single-strand guide RNA.

HiFi Cas9 Reduces Off-Target Editing

- HiFi Cas9 retains high-frequency editing capability and reduces off-target editing by as much as 30-fold when compared with Wild-Type Cas9[1] (**Figure 3A**)
- A preclinical study using 2 SCD donors showed that the HDR pathway is highly efficient in correcting alleles compared with INDEL creation[2] (**Figure 3B,C**)
  - Beta-globin gene sequencing reveals the 3 categories of resulting cells and can be used to measure the rate of INDELs and corrected alleles

Figure 3. GPH101 gene correction protocol optimized in preclinical studies



HiFi Cas9 reduces off-target editing by as much as 30-fold, when compared with Wild-Type Cas9[1,a]

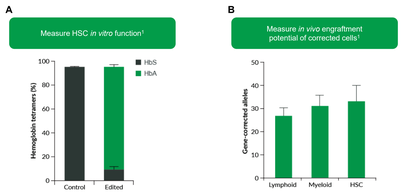
Sequencing of beta-globin gene to measure rate of INDELs and corrected alleles[2] (n=6 experiments, 2 patient donors)

Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; HbA, adult hemoglobin; HbS, hemoglobin sickle cell; HDR, homology-directed repair; HiFi, high-fidelity; HSPC, hematopoietic stem and progenitor cell; INDEL, insertion and/or deletion; SCD, sickle cell disease; WT, wild-type.
[a]Bars represent mean ± SEM, n=3 SCD patients. ****$P < 0.0001$.
1. Reprinted from Vakulskas CA, et al. *Nat Med*. 2018;24(8):1216-1224; 2. Reprinted from Lattanzi A, et al. *Sci Transl Med*. 2021;13(598):eabf2444.

Optimized GPH101 Gene Correction Protocol

- The GPH101 gene correction protocol was optimized in healthy donor-derived hematopoietic stem and progenitor cells (HSPCs) and results in efficient correction of SCD patient HSPCs
- Erythroid differentiation *in vitro* demonstrated >90% HbA production (**Figure 4A**)
- Long-term engraftment of gene-corrected HSCs with multilineage repopulation was demonstrated *in vivo* following transplantation into immunodeficient mice (**Figure 4B**)

Figure 4. Translational data show robust and reproducible gene correction of the beta-globin gene



HbA, adult hemoglobin; HbS, sickle hemoglobin; HSC, hematopoietic stem cell; NSG, NOD-scid IL2Rgammanull; RBC, red blood cell.
[a]Background fetal hemoglobin not included for ease of comparison.
1. Reprinted from Lattanzi A, et al. *Sci Transl Med*. 2021;13(598):eabf2444.

GPH101 Patient Journey

Clinical trial site

- Participants are screened for trial eligibility, including screening for pretreatment cytogenetic abnormalities (**Figure 5**)
- Participants will undergo plerixafor mobilization and apheresis at the clinical site. CD34+ cell selection and cryopreservation of the apheresis product will be undertaken locally at each trial site before shipment to a centralized manufacturer for GPH101 production

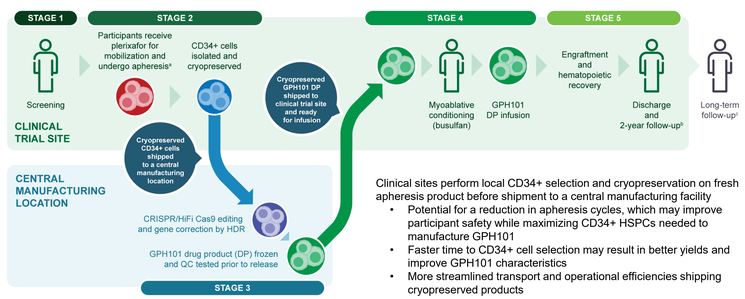
Central manufacturing facility

- CD34+ cells will undergo gene correction at the central manufacturing facility
- GPH101 will be cryopreserved and will undergo quality control testing before being released for shipment back to the trial site

Clinical trial site

- Patients will undergo eligibility reconfirmation before busulfan conditioning and GPH101 infusion
- Patients will undergo safety, efficacy, and pharmacodynamic measurements for 2 years post-infusion including physical exams, laboratory and imaging assessments, and adverse event evaluations
- Patients who receive GPH101 will be invited to enroll in a long-term follow-up study that will monitor participants for an additional 13 years

Figure 5. GPH101 treatment process



Clinical sites perform local CD34+ selection and cryopreservation on fresh apheresis product before shipment to a central manufacturing facility
- Potential for a reduction in apheresis cycles, which may improve participant safety while maximizing CD34+ HSPCs needed to manufacture GPH101
- Faster time to CD34+ cell selection may result in better yields and improve GPH101 characteristics
- More streamlined transport and operational efficiencies shipping cryopreserved products

Cas9, CRISPR-associated protein 9; CD34, cluster of differentiation 34; CRISPR, clustered regularly interspaced short palindromic repeats; DP, drug product; HDR, homology-directed repair; HiFi, high-fidelity; QC, quality control.
[a]Backup cells kept at site as a safety measure. [b]Patients will be followed for 24 months after GPH101 infusion with physical exams, laboratory and imaging assessments, and adverse event evaluations. [c]Patients who receive GPH101 will be followed for 13 years in a long-term follow-up study.

Disclosures

Julie Kanter reports consultancy from Forma, GLG, Graphite Bio, and Novartis; membership on advisory committees for Agios, Beam, Forma, Novartis, and Sanofi; and honoraria from Agios, Beam, Forma, Guidepoint Global, Novartis, and Sanofi. **John F. DiPersio** reports consultancy and membership on the board of directors for RiverVest Venture Partners; current equity holder of stock options in Magenta Therapeutics, current holder of stock options in Magenta Therapeutics as a privately held company, and membership on board of directors or advisory committees for Magenta Therapeutics; current equity holder of stock options in Wugen, current holder of stock options in Wugen as a privately held company, and membership on board of directors or advisory committees for Wugen; and research funding from BioLine Rx and MacroGenics. **Alexis A. Thompson** reports research funding from Bavaltia, BioMarin, CRISPR Therapeutics, Vertex, Graphite Bio, and Novartis; consulting and research funding from bluebird bio and Celgene; consultancy for Agios Pharmaceuticals and Beam Therapeutics; and current equity holder in Global Blood Therapeutics. **Matthew H. Porteus** reports current equity holder of stock options in CRISPR Therapeutics, Allogene Therapeutics, Ziopharm, and Graphite Bio and consultancy for Versant ventures. **Allison Intondi** reports current employment and current equity holder in Graphite Bio; current equity holder in Global Blood Therapeutics; and employment with Global Blood Therapeutics in the past 24 months. **Premanjali Lahiri** reports current employment and current equity holder in Graphite Bio. **Daniel P. Dever** reports current employment and current equity holder in Graphite Bio. **Alexandria Petrusich** reports current employment and current equity holder in Graphite Bio; current equity holder in bluebird bio; and employment with bluebird bio in the past 24 months. **Joshua Lehrer-Graiwer** reports current employment and current equity holder in Graphite Bio; current equity holder in Global Blood Therapeutics; and employment with Global Blood Therapeutics in the past 24 months. All other authors had nothing to disclose.

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CEDAR Trial Info

Trial Design

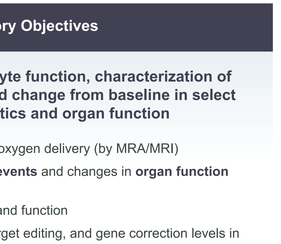
- CEDAR (NCT04819841) is a first-in-human, single-arm, open-label, single-dose, multi-site Phase 1/2 clinical trial in participants with severe SCD (**Figure 6**)
- The study will evaluate safety, efficacy, and pharmacodynamics (PD) of human autologous CRISPR/HiFi Cas9 edited and sickle mutation-corrected HSPCs (GPH101)
- Approximately 15 adult (18 to 40 years; n≈9) and adolescent (12 to 17 years; n≈6) participants will be enrolled across 5 sites, with adolescent enrollment proceeding after a favorable assessment of adult safety data by a Safety Monitoring Committee

Figure 6. CEDAR trial design (NCT04819841)

Participants with severe SCD (≈15 participants)

Key Inclusion Criteria
- ≥12 to ≤40 years
- Diagnosis of severe SCD (βS/βS)
  - ≥4 severe VOCs in the 2 years prior and/or
  - ≥2 episodes of ACS in the 2 years prior with at least 1 episode in the past year
  - **Note: Participants on chronic transfusions may be eligible if documentation of the above severity criteria is available for 2 years prior to starting transfusions**

Key Exclusion Criteria
- Available 10/10 HLA-matched sibling donor
- Prior receipt of HSCT or gene therapy



ACS, acute chest syndrome; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplant; SCD, sickle cell disease; SMC, Safety Monitoring Committee; VOC, vaso-occlusive crisis.

Figure 7. CEDAR key study endpoints

Primary Objectives

Evaluate the safety of treatment with GPH101 in participants with severe SCD

- Kinetics of **HSC engraftment**
- Transplant-related mortality
- Overall survival
- Frequency and severity of **AEs and SAEs**, including lab abnormalities

Secondary Objectives

Evaluate the efficacy and pharmacodynamics of treatment with GPH101 in participants with severe SCD

- Assessment of the following over time
  - **Levels of HbA**, **HbS**, and total hemoglobin
  - Peripheral myeloid **gene correction and globin chain expression** in cells
- Frequency and severity of painful episodes of **VOC** and **ACS** following GPH101 DP infusion
- Changes in **pRBC transfusion needs** (volume and frequency)

Exploratory Objectives

Evaluate PROs, erythrocyte function, characterization of gene correction rates, and change from baseline in select SCD characteristics and organ function

- **Cerebral hemodynamics** and oxygen delivery (by MRA/MRI)
- Improvements in **SCD-related events** and changes in **organ function** (e.g. heart, brain, liver)
- Measurements of **RBC health** and function
- Measurement of INDELs, off-target editing, and gene correction levels in peripheral immune cells
- Correlation of GPH101 DP characteristics with clinical outcomes

ACS, acute chest syndrome; AE, adverse event; DP, drug product; HbA, adult hemoglobin; HbS, hemoglobin sickle cell; HSC; hematopoietic stem cell; INDEL, insertion and/or deletion; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; pRBC, packed red blood cell; PRO, patient-reported outcome; RBC, red blood cell; SAE, serious adverse event; SCD, sickle cell disease; VOC, vaso-occlusive crisis.