Development of a Beta-globin Gene Replacement Strategy as a Therapeutic Approach for β-Thalassemia

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All authors are current employees and equity holders in Graphite Bio. Christopher Bandoro reports equity holdings in Sana Biotechnology and James Partridge reports equity holdings in Global Blood Therapeutics.
Our goal: Restore normal genotype and physiology in people with hemoglobinopathies

Sickle Cell Disease (SCD)¹,²

β-Thalassemia²

Annually 330,000 infants are born with hemoglobinopathies worldwide, of which sickle cell disease and β-thalassemia are the most common³

The UltraHDR™ platform allows for gene correction and gene replacement in sickle cell disease and β-thalassemia.

**Gene Correction**
- Correct point mutations or short DNA stretches in endogenous locus

**Gene Replacement**
- Gene replacement driven by endogenous promoter

**Dysfunctional β-globin gene**
- Repair by homology directed repair (HDR)
- Donor Vector (adeno-associated virus serotype 6 [AAV6])
- Homology Arms (HA) (>400 base pairs [bp]) (nuclease resistant)

**β-thalassemia (GPH102)**

**Sickle Cell Disease (GPH101)**

Trial in Progress – Poster 806
β-thalassemia is a genetic disorder with high unmet need characterized by reduced production of β-globin¹


Figures created with BioRender.com

- More than 300 mutations are known to cause β-thalassemia²
- Globally, 1.5% of the population are carriers of β-thalassemia mutations³
- Approximately 68,000 symptomatic individuals are born annually⁴
- β-globin (HBB) gene replacement would be an ideal therapeutic strategy with the potential to reproduce a normal genotype and restore adult hemoglobin (HbA) expression

Bone marrow expansion
Iron overload (from transfusions)
Destruction of red blood cells (RBCs)

Skeletal deformities
Organ damage
Anemia

Insoluble α-globin aggregates
Abnormal erythroblast
Ineffective erythropoiesis (most erythroblasts die in bone marrow)

Destruction of red blood cells (RBCs)
Iron overload (from transfusions)

Bone marrow expansion

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β-globin (HBB) gene replacement would be an ideal therapeutic strategy with the potential to reproduce a normal genotype and restore adult hemoglobin (HbA) expression
How do β-globin expression levels from a replaced β-globin coding sequence compare to physiological β-globin levels?

Replaced β-globin gene

- Diverged HBB exons (ex)
- Point mutation

Physiological β-globin expression

- Point mutation

HiFi Cas9

Guide RNA (gRNA)

Electroporation

Hematopoietic stem and progenitor cells (HSPCs) (CD34+)

AAV6 DNA donor

Gene-corrected HSPCs (CD34+)

RBC differentiation

Hemoglobin expression (high-performance liquid chromatography [HPLC])

*To distinguish β-globin produced from the HDR allele from endogenously produced β-globin, a sickle point mutation was introduced in the DNA donor.
The β-globin gene requires introns for physiological protein expression

- Replacement with a diverged β-globin coding sequence reduces hemoglobin expression
- Introns are necessary for physiological expression of β-globin
- Requirement for non-homologous DNA donor prevents use of β-globin introns
- We sought to develop a non-homologous AAV6 DNA donor that results in physiological β-globin expression levels
A T2A-EGFP reporter system allows screening for high-expressing DNA donors.

Physiological β-globin control:

- **HBB-EGFP (ctrl)**
- Cas9 only
- AAV6 only
- No introns
- HBA1 introns
- HBG2 introns
- HBD introns
- Primate introns

β-globin gene replacement:

- HBB gRNA
- **β-globin**
- Diverged exons
- No introns
- α-globin (HBA1) introns
- γ-globin (HBG2) introns
- δ-globin (HBD) introns
- Primate introns

Incorporating heterologous introns restores β-globin expression to physiological levels.
Heterologous intron donors produce hemoglobin tetramers

HBB coding sequence with HBG2 introns results in up to 40% HDR and physiological hemoglobin protein levels
Can the HBG2 β-globin DNA donor be further optimized?

**Intron length:**
- Shorter intron sequences, and thereby a shorter gene cassette, may result in higher rates of HDR
- Shorter intron sequences reduce the potential for homologous recombination with γ-globin gene

**Polyadenylation (pA) sequences:**
- We assessed if different pA sequences could increase protein expression
- Screened a total of 11 different pA tails
- The original bovine growth hormone (bGH) pA performed best
Optimization of the HBG2 β-globin DNA donor identified an additional β-globin DNA donor with truncated introns

- Most intron deletions resulted in a relative loss of expression
- We identified one truncated intron 2 DNA donor that resulted in sustained HBB-EGFP expression

Two high-expressing DNA donors

Truncated Introns
β-globin gene replacement is effective in both healthy and SCD patient HSPCs

HSPCs from sickle-cell patients serve as a surrogate for β-thalassemia patient HSPCs

Similar frequencies of HDR are achieved in HSPCs derived from healthy volunteers and from patients with sickle cell disease
β-globin gene replacement restores adult hemoglobin (HbA) expression in patient SCD-HSPCs

SCD-HSPCs (CD34+)

Gene-corrected SCD-HSPCs (CD34+)

RBC differentiation

HbA/Sickle hemoglobin (HbS)
levels (HPLC)

SCD-HSPC donors = 2
Replicates = 6-8

HPLC

% Globin tetramers

Untreated
SNP-donor
HBG2-WT
HBG2-12x2

0 50 100

Absorbance Units [AU]

Time, min

HbS
HbA

HBG2-WT

HBB gene replacement using the optimized DNA donors restored HbA expression to a level comparable to a SCD point mutation-correction strategy
Conclusions

• β-thalassemia is a genetic disorder with high unmet need
• Using our UltraHDR™ platform, we developed a precise β-globin gene replacement strategy that restores HbA expression, offering a differentiated approach for treating β-thalassemia
• Additional preclinical studies are planned to further study this approach and advance the GPH102 program
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<td>AAV6</td>
<td>adeno-associated virus serotype 6</td>
</tr>
<tr>
<td>AU</td>
<td>absorbance unit</td>
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<tr>
<td>bGH</td>
<td>bovine growth hormone</td>
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<td>EGFP</td>
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