GBT021601 Improves the Pathophysiology of Sickle Cell Disease in a Murine Model

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BACKGROUND

- Sickle cell disease (SCD) is characterized by hemolytic anemia, vaso-occlusion, and progressive end-organ damage. The underlying mechanism of SCD involves polymerization of intracellular sickle hemoglobin (HbS) following deoxygenation in the microvasculature, leading to decreased red blood cell (RBC) deformability, morphologic sickling of RBCs, decreased RBC survival, microvascular obstruction, and clinical complications.[1]
- Voxelotor, a HbS polymerization inhibitor recently approved for the treatment of SCD, is an allosteric modifier of hemoglobin (Hb) that increases the proportion of oxygenated Hb in all RBCs. In clinical studies in patients with SCD, voxelotor at 1500 mg daily (QD) dosing led to Hb occupancies of ~27%, was well tolerated, and resulted in reduced hemolytic anemia.[2,3]
- GBT021601 is a potent, next-generation HbS polymerization inhibitor with the potential to achieve even higher Hb occupancies in patients with SCD at lower doses and therefore with less pill burden.

OBJECTIVE

- To evaluate the effect of GBT021601, a second-generation HbS polymerization inhibitor, on the pathophysiology in SCD using the Townes mouse model.

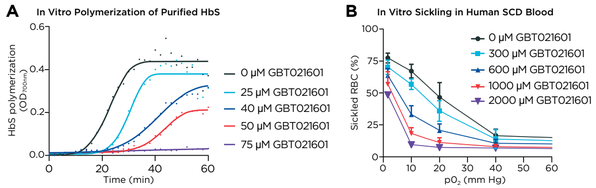
METHODS

- **HbS polymerization:** GBT021601-modified, purified HbS in 1.8 M K_2PO_4 buffer (pH 7.4) was deoxygenated at 4 °C with sodium dithionite, followed by a temperature jump to 37 °C to initiate polymerization, and monitored at an optical density of 700 nm.
- **Dosing of Townes (SS) mice:** GBT021601 (20, 40, 75, or 150 mg/kg QD) or vehicle (0.5% methylcellulose/phosphate-buffered saline [PBS] pH 7.4/0.01% Tween-80) was administered via oral gavage to homozygous SCD (SS) mice as a repeat dose for 21 days. Blood was drawn on day 21 for determination of GBT021601 concentration and pharmacodynamic analysis. To evaluate the effects of dosing duration, GBT021601 was administered at 40 mg/kg via oral gavage to SS mice as a repeat dose for 21 or 42 days, followed by pharmacodynamic analysis.
- **Hematological measurements:**
  - **Serum erythropoietin (EPO)** was measured by enzyme-linked immunosorbent assay using the Mouse EPO Quantikine kit (R&D Systems).
  - **Complete blood count** was determined using a Sysmex XT-2000iV (IDEXX BioResearch Corp.).
  - **Mitochondria⁺ RBCs** were determined by staining RBCs with tetramethylrhodamine (Invitrogen) and analyzing for fluorescence on a BD Accuri C6 cell analyzer.
  - **Mature CD71⁻ RBCs** were determined by staining RBCs with anti-CD71 antibody/anti-Ter119 antibody (BD Biosciences) followed by flow cytometry.
  - **RBC half-life** was determined by intravenous injection of 50 mg/kg *N*-hydroxysuccinimide biotin (ThermoScientific) via the tail vein into SS mice on day 9 to produce a pulse label. Mice were then bled each subsequent day, and biotinylated Ter119⁺ live singlet scatter-gated RBCs were identified with fluorescently labeled streptavidin using an LSRII flow cytometer (BD Biosciences). The percentage of biotinylated RBCs remaining over time was calculated, and RBC half-life was determined using a plateau followed by an exponential decay model.
  - **Circulating sickled RBCs** were determined by counting sickled cells in blood drawn from SS mice under anesthesia (using isoflurane in 100% oxygen) and immediately fixed in 2% glutaraldehyde/PBS.
  - **Oxygenscan** was performed under controlled deoxygenation using nitrogen in a Lorrca instrument.[4]
- **Oxygen equilibrium curves (OECs) and sickling:** OECs were obtained by deoxygenation of O_2-equilibrated blood samples in Hemox buffer at 37 °C, using a Hemox Analyzer (TCS Scientific). For ex vivo sickling of SS mouse blood, RBCs were collected at 20 mm Hg from the Hemox Analyzer and immediately fixed in 2% glutaraldehyde/PBS. For in vitro sickling of GBT021601-modified blood from individuals with SCD, RBCs were collected at 40, 20, 10, and 1.6 mm Hg from the Hemox Analyzer and immediately fixed in 2% glutaraldehyde/PBS. The percentage of sickled cells was determined via manual counting of bright-field images of fixed RBCs.
- **Statistical analysis:** Data were analyzed via Mann–Whitney test and Hochberg's method for multiple testing.

RESULTS

- In vitro modification of purified HbS (50 µM) with GBT021601 followed by deoxygenation resulted in a concentration-dependent increase in the delay time leading to the onset of HbS polymer formation. These results indicate that GBT021601 dose-dependently inhibits HbS polymerization by extending the polymerization delay time (**Figure 1A**).
- In vitro modification of blood (20% hematocrit) from individuals with SCD with the indicated concentrations of GBT021601 followed by deoxygenation resulted in a concentration-dependent decrease in the percentage of sickled RBCs (**Figure 1B**).

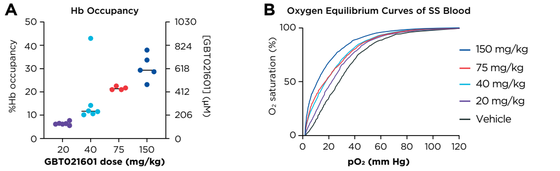
Figure 1. GBT021601 Inhibits In Vitro HbS Polymerization and Reduces RBC Sickling in Blood From Individuals With SCD



HbS, sickle hemoglobin; pO_2, partial pressure of oxygen; OD_{700nm}, optical density at 700 nm; RBC, red blood cell; SCD, sickle cell disease.

- GBT021601 was administered at 20, 40, 75, and 150 mg/kg QD via oral dosing in SS mice. After 21 days of dosing:
  - GBT021601-treated SS mice achieved median Hb occupancies (at minimum concentration in blood) of 6%, 12%, 21%, and 29%, corresponding to 20, 40, 75, and 150 mg/kg doses, respectively (**Figure 2A**).
  - There was a dose-dependent left-shift of the OEC of blood from GBT021601-treated SS mice relative to that of vehicle-treated SS mice (**Figure 2B**).

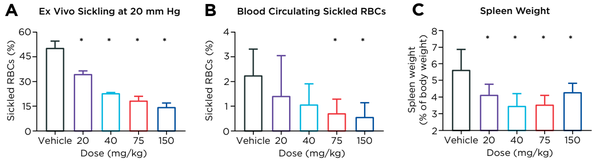
Figure 2. GBT021601 Demonstrated Dose-Dependent Increases in Hb Occupancy and Hb-O_2 Affinity in Blood of SS Mice



Hb, hemoglobin; pO_2, partial pressure of oxygen; SS, homozygous sickle cell disease.

- GBT021601-treated SS mice showed a dose-dependent reduction in the percentage of sickled RBCs in blood exposed to hypoxic conditions (20 mm Hg) ex vivo (**Figure 3A**).
- GBT021601-treated SS mice demonstrated a dose-dependent reduction in the percentage of circulating sickled RBCs in vivo (**Figure 3B**).
- GBT021601-treated SS mice showed a significant reduction in spleen weight, indicating improved splenic function (**Figure 3C**).

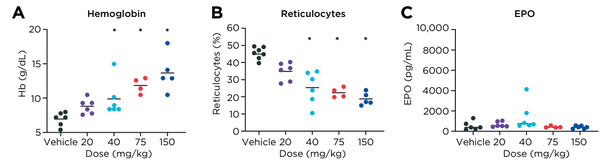
Figure 3. GBT021601 Reduced Sickled RBCs and Spleen Weight in SS Mice



*P<0.05.
RBC, red blood cell; SS, homozygous sickle cell disease.

- GBT021601-treated SS mice showed a dose-dependent increase in Hb levels up to the normal range in wild-type mice (**Figure 4A**).
- GBT021601-treated SS mice showed a dose-dependent reduction in the percentage of reticulocytes (**Figure 4B**).
- There was no increase in serum EPO levels in GBT021601-treated SS mice relative to vehicle-treated SS mice (**Figure 4C**).

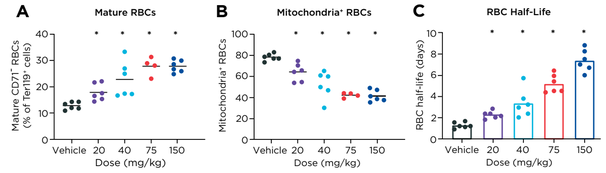
Figure 4. GBT021601 Increased Hb Levels and Reduced Reticulocytes in SS Mice



*P<0.05.
EPO, erythropoietin; Hb, hemoglobin; SS, homozygous sickle cell disease.

- GBT021601-treated SS mice showed a dose-dependent increase in mature CD71⁻ RBCs (**Figure 5A**).
- GBT021601-treated SS mice showed a dose-dependent reduction in the percentage of mitochondria⁺ RBCs (**Figure 5B**).
- GBT021601-treated SS mice showed a dose-dependent increase in RBC half-life (**Figure 5C**).

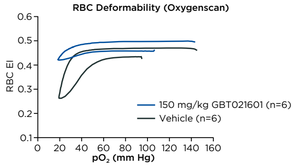
Figure 5. GBT021601 Improved RBC Health and Prolonged RBC Half-Life in SS Mice



*P<0.05.
RBC, red blood cell; SS, homozygous sickle cell disease.

- The maximum dosage, 150 mg/kg GBT021601, was used to observe RBC deformability over a similar treatment period as described previously.
- GBT021601-treated SS mice showed an improvement in RBC deformability in the Oxygenscan, as captured by an increase in maximal elongation index (EI_{max}) and minimal elongation index (EI_{min}) and a decrease in the partial oxygen pressure of the point of sickling (POS) (**Figure 6**).
- Curves from blood samples (n=6) in each treatment group were averaged and are presented in **Figure 6**. Mean 3 standard deviation are shown for the parameters in **Table 1**.

Figure 6. GBT021601 Improved RBC Deformability in SS Mice



EI, elongation index; pO_2, partial pressure of oxygen; RBC, red blood cell; SS, homozygous sickle cell disease.

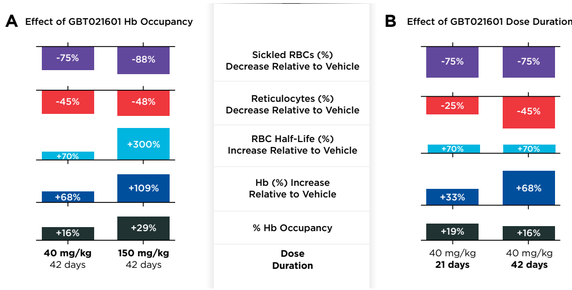
Table 1. Measurements of Elongation of Deformed RBC Samples

Parameter	Vehicle	150 mg/kg GBT021601
EI_{max}	0.47 3 0.01	0.49 3 0.01
EI_{min}	0.26 3 0.04	0.44 3 0.01
POS (mm Hg)	35 3 2.4	32 3 2.2

EI_{max}, maximal elongation index; EI_{min}, minimal elongation index; POS, point of sickling; RBC, red blood cell.

- GBT021601 was administered at 40 or 150 mg/kg QD via oral dosing in SS mice for 21 or 42 days to observe changes in the pathophysiology of RBCs, reticulocytes, and Hb over different dosages and dose durations compared to vehicle-treated animals (n=3-6).
  - GBT021601 improved SCD pathophysiology in a Hb occupancy–dependent manner (mean percentage changes are shown, **Figure 7A**).
  - GBT021601 improved SCD pathophysiology in a dose duration–dependent manner (mean percentage changes are shown, **Figure 7B**).

Figure 7. GBT021601 Hb Occupancy and Treatment Duration Drive Improvements in SCD Pathophysiology in SS Mice



Hb, hemoglobin; RBC, red blood cell; SCD, sickle cell disease.

CONCLUSIONS

- **GBT021601 is a potent, next-generation HbS polymerization inhibitor with anti-sickling properties demonstrated in vitro in blood from individuals with SCD and in vivo in SS mice.**
- **Treatment of SS mice with GBT021601 led to dose-dependent increases in Hb occupancy, with corresponding increases in Hb-O_2 affinity.**
- **Treatment of SS mice with GBT021601 led to dose-dependent and treatment duration–dependent improvements in hemolysis markers, including substantial increases in Hb levels (without increases in EPO) and concurrent reduction in reticulocytes.**
- **Treatment of SS mice with GBT021601 led to dose-dependent improvements in RBC health, prolongation of RBC half-life, and reduction of spleen weight.**
- **These results support the clinical development of GBT021601 as a potential best-in-class HbS polymerization inhibitor for the treatment of SCD.**

References

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Disclosures

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