GBT021601 Inhibits HbS Polymerization, Prevents RBC Sickling and Improves the Pathophysiology of Sickle Cell Disease in a Murine Model

Kobina Dufu, PhD; Carsten Alt, MSc, PhD; Steven Strutt, PhD; Tzechiang Tang; Hilary Liao-Zou; Yue Yuan; Brian E. Cathers, PhD; Donna Oksenberg, PhD

Global Blood Therapeutics, South San Francisco, CA, USA

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Disclosures

Kobina Dufu, Carsten Alt, Steven Strutt, Tzechiang Tang, Hilary Liao-Zou, Yue Yuan, Brian E. Cathers, Donna Oksenberg

- Employee, equity ownership: Global Blood Therapeutics

Introduction

- 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]

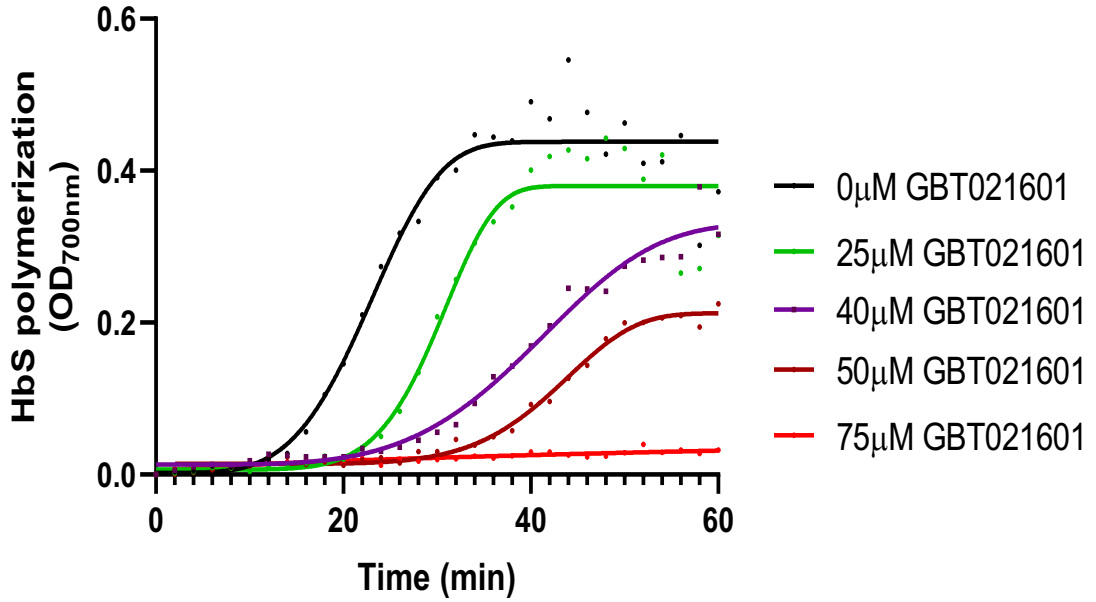
- Voxelotor, a HbS polymerization inhibitor recently approved for the treatment of SCD, is an allosteric modifier of Hb that increases the proportion of oxygenated Hb in all RBCs. In clinical studies in patients with SCD, voxelotor at 1500 mg daily dosing achieved Hb occupancies of ~27%, was well tolerated, 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.

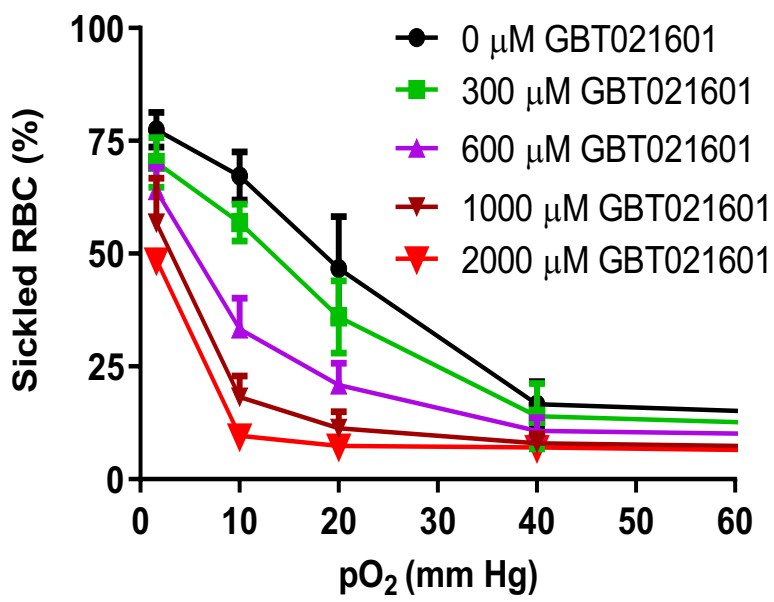
1. Bunn H. *N Engl J Med.* 1997;337(11):762-769. 2. Howard J, et al. *Blood.* 2019;133(17):1865-1875. 3. Vichinsky E, et al. *NEJM*. 2019;381:509-519.

GBT021601 Inhibits *In Vitro* HbS Polymerization and Reduces RBC Sickling in Blood from Patients with SCD

A.



B.

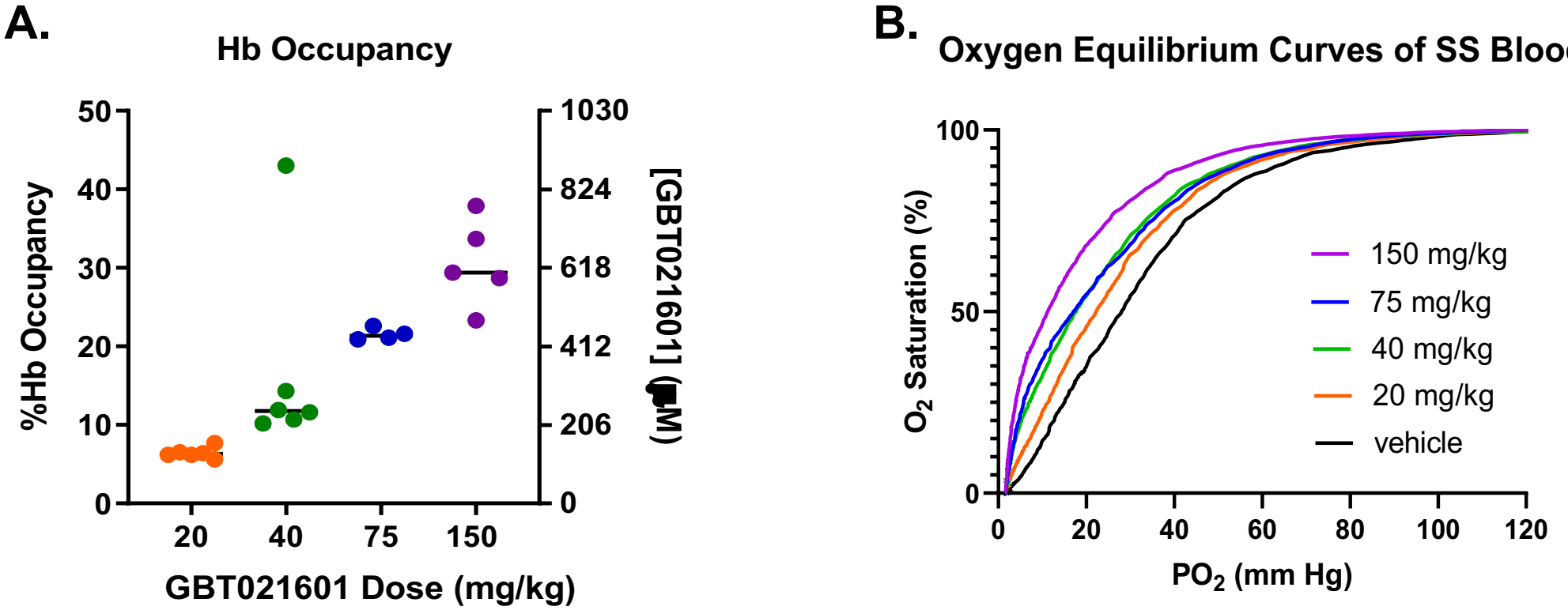


A. *In vitro* modification of purified HbS (50 μM) with GBT021601 followed by deoxygenation resulted in a concentration-dependent increase in the delay time leading to the onset of HbS polymer formation. These results indicate that GBT021601 dose-dependently inhibits HbS polymerization by extending the polymerization delay time.

B. *In vitro* modification of blood (20% hematocrit) from patients (n=3) with SCD with the indicated concentrations of GBT021601 followed by deoxygenation resulted in a concentration-dependent decrease in the percentage of sickled RBCs.

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

Repeated Dosing with GBT021601 Demonstrated Dose-dependent Increases in Hb Occupancy and Hb-O_2 Affinity in SS Mice Blood



GBT021601 was administered at 20, 40, 75, and 150 mg/kg QD via oral dosing in SS mice. After 21 days of dosing:

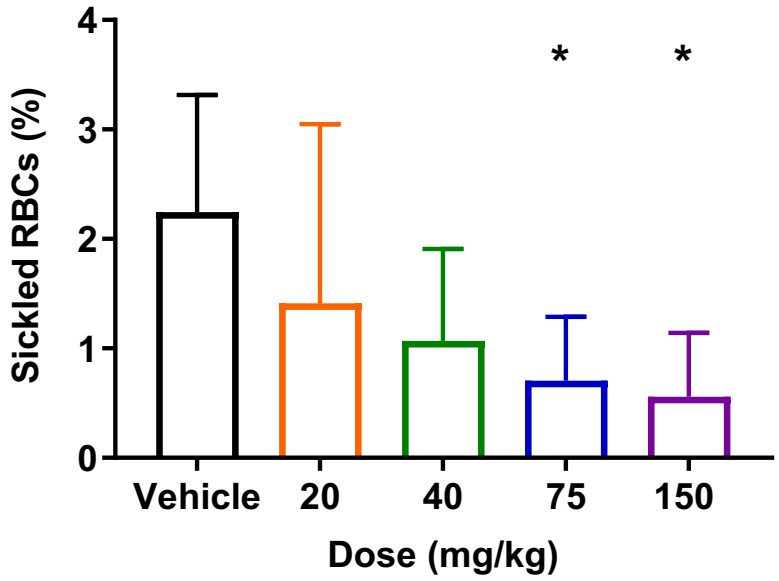
A. GBT021601-treated SS mice achieved median Hb occupancies (at C_{min}) of 6%, 12%, 21%, and 29% corresponding to 20, 40, 75, and 150 mg/kg doses, respectively.

B. 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.

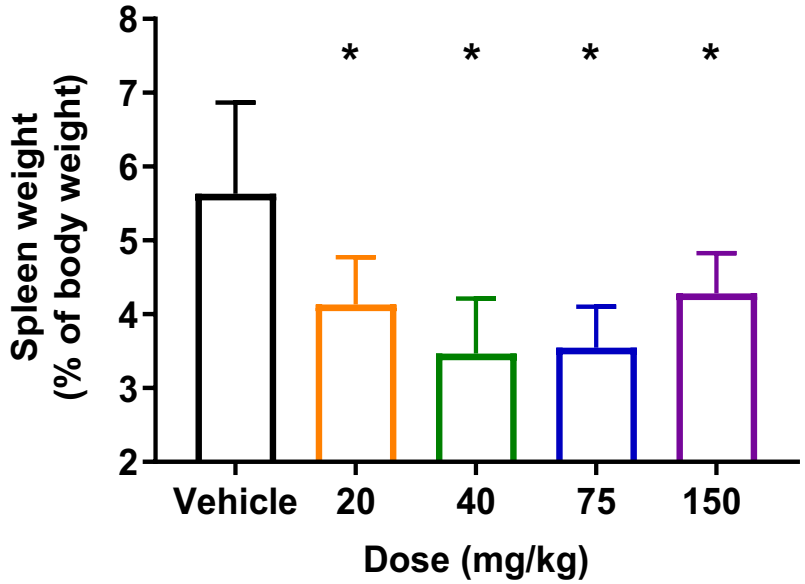
C_{min}, minimum concentration in plasma; Hb, hemoglobin; OEC, oxygen equilibrium curve; QD, once daily; SS, homozygous sickle cell disease.

Repeated Dosing with GBT021601 Reduced Circulating Sickled RBCs and Spleen Weight in SS Mice Blood

A.



B.



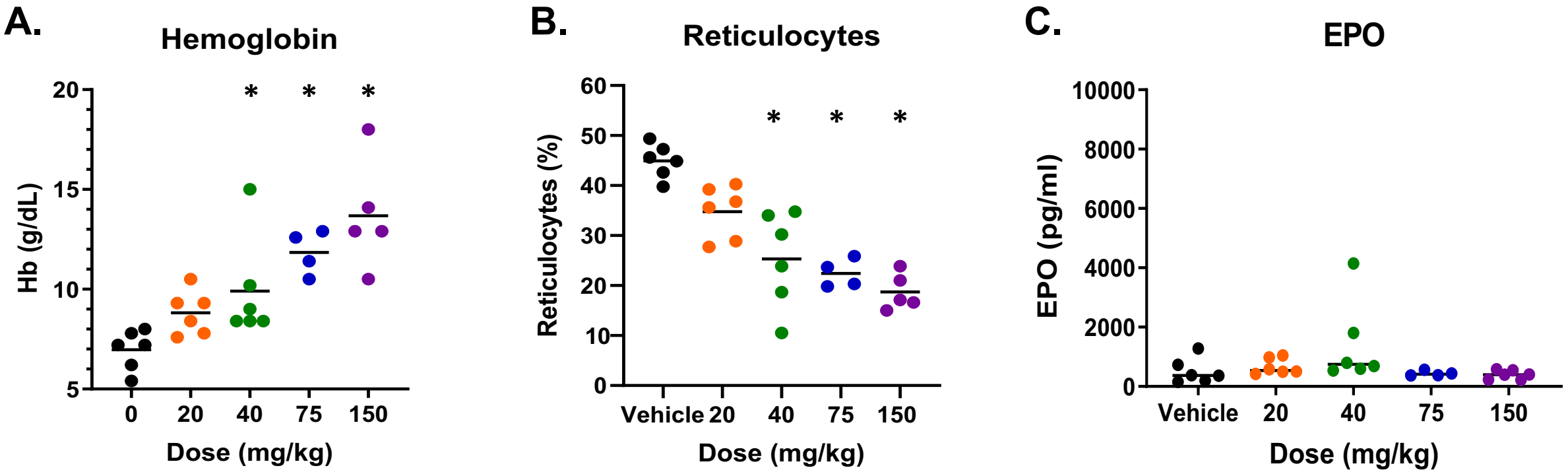
GBT021601 was administered at 20, 40, 75, and 150 mg/kg QD via oral dosing in SS mice. After 21 days of dosing:

A. GBT021601-treated SS mice demonstrated a dose-dependent reduction in the percentage of *in vivo* circulating sickled RBCs (*P<0.05).

B. GBT021601-treated SS mice demonstrated a significant reduction in spleen weight (*P<0.05), indicating improved splenic function.

QD, once daily; RBC, red blood cell; SS, homozygous sickle cell disease.

Repeated Dosing with GBT021601 Increased Hemoglobin Levels and Reduced Reticulocytes in SS Mice



GBT021601 was administered at 20, 40, 75, and 150 mg/kg QD via oral dosing in SS mice. After 21 days of dosing:

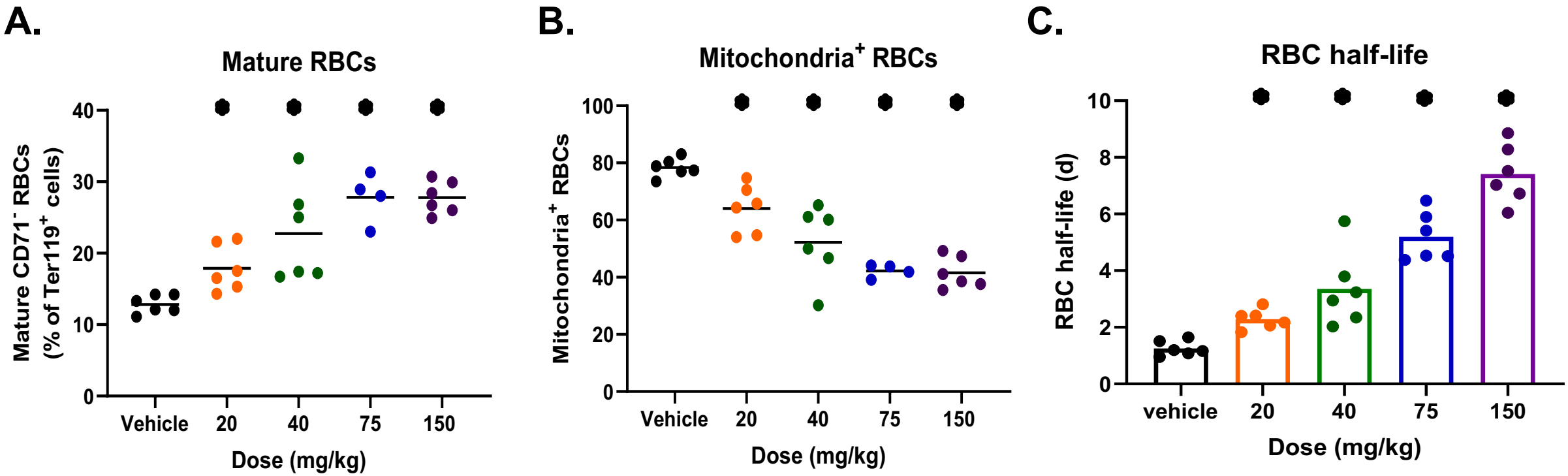
A. GBT021601-treated SS mice demonstrated a dose-dependent increase in Hb levels up to the normal range in wildtype mice (*P<0.05).

B. GBT021601-treated SS mice demonstrated a dose-dependent reduction in the percentage of reticulocytes (*P<0.05).

C. There was no increase in serum EPO levels in GBT021601-treated SS mice relative to vehicle-treated SS mice.

EPO, erythropoietin; Hb, hemoglobin; QD, once daily; SS, homozygous sickle cell disease.

Repeated Dosing with GBT021601 Improved RBC Health and Prolonged RBC Half-Life in SS Mice



GBT021601 was administered at 20, 40, 75, and 150 mg/kg QD via oral dosing in SS mice. After 21 days of dosing:

A. GBT021601-treated SS mice demonstrated a dose-dependent increase in mature $CD71^-$ RBCs (*P<0.05).

B. GBT021601-treated SS mice demonstrated a dose-dependent reduction in the percentage of mitochondrial$^+$ RBCs (*P<0.05).

C. GBT021601-treated SS mice demonstrated a dose-dependent increase RBC half-life (*P<0.05).

QD, once daily; RBC, red blood cell; SS, homozygous sickle cell disease.

Repeated Dosing with GBT021601 Improved RBC Deformability in SS Mice

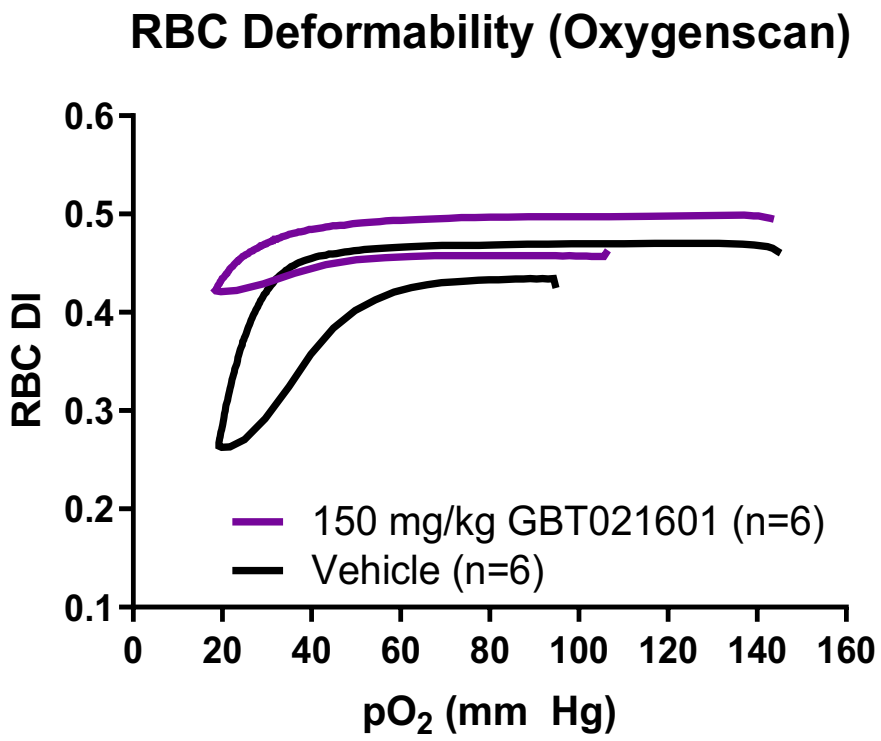


Table 1

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

GBT021601 was administered at 150 mg/kg QD via oral dosing for 21 days in SS mice.

- GBT021601-treated SS mice demonstrated an improvement in RBC deformability in the Oxygenscan, as captured by an increase in EI_{max} (maximal elongation index) and EI_{min} (minimal elongation index) and a decrease in the pO_2 of the point of sickling (POS).
- Curves from blood samples (n=6) in each treatment group were averaged and are presented in the graph. Mean ± standard deviation are shown for the parameters in Table 1.

QD, once daily; RBC, red blood cell; SS, homozygous sickle cell disease.

Summary

GBT021601 is a potent HbS polymerization inhibitor with anti-sickling properties demonstrated both *in vitro* in blood from patients with SCD and *in vivo* in SS mice.

Repeated dosing of GBT021601 in SS mice led to dose-dependent increases in Hb occupancy with corresponding increases in Hb-O_2 affinity.

Repeated dosing of GBT021601 in SS mice led to dose-dependent improvements in hemolysis markers, including substantial increases in Hb levels (without increases in EPO) and concurrent reduction in reticulocytes.

Repeated dosing of GBT021601 in SS mice led to dose-dependent improvements in RBC health and prolongation of RBC half-life, and reduction of spleen weight.

These results support the clinical development of GBT021601 as a potential best-in-class sickle hemoglobin polymerization inhibitor for the treatment of SCD.

EPO, erythropoietin; Hb, hemoglobin; RBC, red blood cell; SCD; sickle cell disease; SS, homozygous sickle cell disease.