GTx011, A POTENT ALLOSTATIC MODIFIER OF HEMOGLOBIN OXYGEN AFFINITY, PREVENTS RBC SICKLING IN WHOLE BLOOD AND PROLONGS RBC HALF-LIFE IN VIVO IN A MURINE MODEL OF SICKLE CELL DISEASE

Goal: To develop an orally available small molecule that enables chronic preventative therapy for sickle cell disease

Approach:

• Design a long-lived, once daily-dosing, direct acting HbS modifier

• Inhibit HbS polymerization by increasing the fraction of oxy-Hb within RBCs

• Chronically and consistently achieve anti-sickling activity

PROPHYLACTIC THERAPY FOR SICKLE CELL DISEASE
GBT440 (GTX011) ALLOSTERICALLY MODIFIES HbS AND INCREASES Hb-OXYGEN AFFINITY

Crystal structure shows one GBT440 molecule per Hb tetramer

SCD Blood at 20% Hct (~1 mM Hb)

%O₂ Saturation

pO₂ (mm Hg)

1000 µM
600 µM
300 µM
No cmpd
Persistent expression of HbF in the range of 15-35% prevents virtually all clinical manifestations of SCD.

GBT440 modified-HbS has similar in vitro anti-polymerization activity as HbF.

Hypothesis: A 20-30% HbS modification with GBT440 will be sufficient to prevent sickling.
# DRAMATIC RBC PARTITIONING AND SUSTAINED EXPOSURE FOLLOWING SINGLE DOSE

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Dog</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IV Dose (mg/kg)</strong></td>
<td>1.6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>PO Dose (mg/kg)</strong></td>
<td>7.2</td>
<td>2.5</td>
<td>4.3</td>
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<tr>
<td><strong>Oral bioavailability (% F)</strong></td>
<td>60</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td><strong>Blood/Plasma Ratio</strong></td>
<td>69</td>
<td>74</td>
<td>71</td>
</tr>
<tr>
<td><strong>T1/2 (hr)</strong></td>
<td>19</td>
<td>78</td>
<td>39</td>
</tr>
</tbody>
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![Graphs showing concentration over time for Rat, Dog, and Monkey](image-url)
MEASUREMENT OF THE HB-OXYGEN DISSOCIATION CURVES AND SICKLED CELLS

- Hand counting
- Image analysis
- Image cytometry
GBT440 INHIBITS IN VITRO SICKLING OF SCD BLOOD

- SCD blood was treated with GBT440 prior to deoxygenation.

- GBT440 dose-dependently inhibits sickling and shows antisickling activity at 30% HbS modification (300 µM)
GBT440 REDUCES *IN VITRO* SICKLING AT 30% Hb MODIFICATION

pO2 (40mm Hg)

- **No cmpd**: 50% Sickled Cells
- **GBT440 (300 µM)**: 11% Sickled Cells

Representative images shown
GBT440 REDUCES IN VITRO SICKLING AT 30% Hb MODIFICATION

pO2 (40mm Hg)

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<th>No cmpd</th>
<th>GBT440 (300 µM)</th>
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<td>50% Sickled Cells</td>
<td>11% Sickled Cells</td>
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Sickled cells are circled
Representative images shown
MURINE SICKLE CELL MODEL

- Townes knock-in sickle mice were used (Wu 2006)
  - Express only the human globins (α, γ and β^S)

- Mice were administered single oral doses GBT440 (100 mg/kg)
  - Blood samples: Pharmacokinetics, CBC, Oxygen dissociation curves & % Sickled cells.

- Repeat dosing of GBT440 (100 mg/kg), po BID for 9-12 days
  - Following repeat dosing:
    - RBC half life: pulsed labeled with biotin, flow cytometry.
    - Reticulocyte count: thiazole orange, flow cytometry.
GBT440 PROTECTS RBC AGAINST SICKLING FOLLOWING SINGLE ORAL DOSE IN TOWNES SS MICE

- Single oral dose of GBT440 at 100 mg/kg
- Blood concentration at Cmax was 158 µM (11% Hb occupancy)
GBT440 INCREASES Hb-OXYGEN AFFINITY FOLLOWING REPEAT ORAL DOSING IN TOWNES SS MICE

![Graphs showing O2 Saturation vs pO2 and p50 vs Hb Occupancy for SS vehicle and SS GBT440](image-url)
GBT440 DECREASES EX VIVO RBC SICKLING FOLLOWING REPEAT ORAL DOSING IN TOWNES SS MICE

Representative results from N= 14 are shown
GBT440 PROLONGS RBC HALF LIFE FOLLOWING REPEAT ORAL DOSING IN TOWNES SS MICE

Vehicle-treated: 2.4 ± 0.1 days
GBT440-treated: 3.8 ± 0.1 days
GBT440 DECREASES RETICULOCYTE COUNT FOLLOWING REPEAT ORAL DOSING IN TOWNES SS MICE

Animals with >30% Hb occupancy show 30 % decrease in Reticulocyte count
SUMMARY AND CONCLUSIONS

- *In vitro*, GBT440 increases Hb-oxygen affinity, delays HbS polymerization and prevents sickling in blood from SCD patients

- *In vivo*, in a murine model of SCD
  - Single oral doses of GBT440 demonstrate increased Hb-oxygen affinity and *ex vivo* anti-sickling activity
  - Repeat oral GBT440 dosing shows
    - Increased Hb-oxygen affinity
    - *Ex vivo* anti-sickling activity
    - Prolongation of RBC half-life and decreased reticulocyte counts in mice where 20-40% target Hb occupancy was achieved

- GBT440 shows promise as a disease-modifying treatment for chronic management of patients with sickle cell disease
ACKNOWLEDGEMENT

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DISCLOSURES

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  Global Blood Therapeutics

Consultancy
  Global Blood Therapeutics