GTx011, A POTENT ALLOSTERIC MODIFIER OF HEMOGLOBIN OXYGEN AFFINITY, DELAYS POLYMERIZATION AND PREVENTS SICKLING

ABSTRACT # 316

DUFU, OKSENBERG, METCALF & SINHA
SICKLE CELL DISEASE – SEVERE DISEASE CAUSED BY TENDENCY OF DEOXYGENATED HbS TO POLYMERIZE
ALLOSTERIC MODIFICATION OF HbS WITH GTx011

Goal: To develop an oral small molecule agent that restores normal hemoglobin dynamics in SCD and enables chronic prophylactic therapy

Hypothesis:

• The oxygenated state of Hb is capable of inhibiting HbS polymerization
• Anti-sickling activity can be achieved chronically and consistently by a long-lived, direct acting HbS modifier

Crystal structure shows one GTx011 per Hb tetramer
To confirm that GTx011:

• Allosterically modifies HbS to maintain an oxygenated state under hypoxia

• Inhibits polymerization of HbS

• Prevents sickling of RBCs from SCD patients

• Achieves and maintains appropriate levels in RBCs following oral dosing

• Has anti-sickling activity when dosed orally in Townes SS mice
GTx011 INCREASES Hb AFFINITY FOR OXYGEN AND MAINTAINS AN OXY-Hb STATE UNDER PROLONGED HYPOXIA

A.

\[ [\text{Hb}] = 25 \, \mu M \]

B.

\[ \text{Hb} \]

\[ \text{Hb} + \text{GTx011} \]
GTx011 INCREASES DELAY TIME OF HbS POLYMERIZATION

HbS + GTx011
(in 1.8M K₂HPO₄)

Temperature jump to 37°C initiates polymerization measured spectrophotometrically.

NATURAL Hb VARIANTS, HbF AND HbA REDUCE HbS POLYMERIZATION AND PREVENT DISEASE AT SUB-STOICHIOMETRIC CONCENTRATIONS

• **Clinical**
  – HbS carriers (sickle cell trait, expressing both HbA and HbS) rarely manifest SCD
  – Individuals with coinheritance of HbS with hereditary persistence of fetal hemoglobin (S/HPFH) are clinically asymptomatic

• **In vitro**
  – HbA and HbF act as potent sub-stoichiometric inhibitors of HbS polymerization *in vitro*\(^2,3,4\)

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3. Franklin et al, Br J Haem, 1983
GTx011-MODIFIED HbS DELAYS POLYMERIZATION OF HbS ANALOGOUS TO HbF AND HbA-CO

<table>
<thead>
<tr>
<th>% Inhibitor</th>
<th>Δ Delay time (min)</th>
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<tr>
<td>20% HbF</td>
<td>9</td>
</tr>
<tr>
<td>20% HbA-CO</td>
<td>7</td>
</tr>
<tr>
<td>20% GTx011-HbS</td>
<td>10</td>
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Table shows representative Δ delay time per % inhibitor
ACUTE AND SEVERE RBC SICKLING PROTOCOL

SSRBCs in Phosphate Buffered Saline + GTx011 → NORMOXIA (21% O₂) for 1 hr → HYPOXIA (4% O₂, 96% N₂) for 0.5 hr

SSRBCs are imaged and quantified by software.
GTx011 INHIBITS SICKLING OF HUMAN SSRBC

Representative images
GTx011 REVERSES SICKLING OF PREVIOUSLY SICKLED HUMAN SSRBC

Representative images

**Normoxia**

- Hypoxia 0.5 hr; INPUT
  - 80% Sickled

- Hypoxia 2.5 hr; No compound
  - 97% Sickled

- Hypoxia 2.5 hr; 5 mM GTx011
  - 33% Sickled

- 18% Sickled

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GTx011 SHOWS HIGH ORAL BIOAVAILABILITY, SUSTAINED EXPOSURE AND DRAMATIC PARTITIONING INTO RBCs FOLLOWING SINGLE ORAL DOSE

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Dog</th>
<th>Monkey</th>
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</thead>
<tbody>
<tr>
<td>IV: Dose (mg/kg)</td>
<td>1.6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PO: Dose (mg/kg)</td>
<td>7.2</td>
<td>2.5</td>
<td>4.25</td>
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<tr>
<td>Oral bioavailability (% F)</td>
<td>59.8</td>
<td>36.6</td>
<td>36.1</td>
</tr>
<tr>
<td>Blood/Plasma Ratio</td>
<td>69.0</td>
<td>74.4</td>
<td>70.9</td>
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GTx011 INCREASES Hb-OXYGEN AFFINITY AND PROTECTS RBC AGAINST SICKLING FOLLOWING SINGLE ORAL DOSE IN TOWNES SS MICE

- Single dose of 100 mg/kg po achieved average RBC and plasma concentrations of 470 µM and 21 µM, respectively
  - RBC: plasma ratio = 23:1
- Ex vivo sickling assay following blood sample harvested at different oxygen tensions (Hemox)
SUMMARY & CONCLUSIONS

GTx011:

• Increases Hb-oxygen affinity and maintains oxy-Hb state under hypoxia

• Inhibits HbS polymerization at sub stoichiometric concentrations

• Inhibits sickling of human SSRBC, consistent with GTx011 inhibiting HbS polymerization within RBCs
  - Reverses sickling of human SSRBC

• Shows high oral bioavailability, sustained exposure and dramatic partitioning into RBCs following single oral dose in animals

• Protects RBC against sickling following single oral dose in Townes SS mice
ACKNOWLEDGEMENT

HbS Project Team at Global Blood Therapeutics

Collaborator
Dr. David Archer, Emory University School of Medicine

SCD blood obtained from
UNC Comprehensive Sickle Cell Program at Chapel Hill
Children’s Hospital Oakland Research Institute
• Back Up
Dose Response of GTx011 in human blood at 35% hematocrit

At 492uM will elicit at 50% change with respect to p20
At 802uM will elicit a 50% change with respect to p50