Sickle cell disease (SCD) is caused by a point mutation in the β-globin gene leading to production of hemoglobin S (HbS), which polymerizes upon deoxygenation with subsequent formation of sickled red blood cells (RBCs). GBT440 modulates O₂ affinity of hemoglobin (Hb) by binding to the N-terminal chain of Hb via a reversible Schiff base. We previously demonstrated that GBT440 prevented sickling of RBCs from sickle cell patients in vitro. Also, in a murine model of sickle cell disease (Talen™ mice), GBT440 prevented ex vivo sickling of RBCs and prolonged RBC half-life. In this presentation, ADME profiles and pharmacodynamic/pharmacokinetic (PK/PO) relationship in nonclinical species are described.

An analysis of Blood and Plasma GBT440 Concentrations
Blood and plasma samples were analyzed for GBT440 concentrations using LCMS. The analytical range was 50 to 100,000 ng/mL for blood and 10 to 20,000 ng/mL for plasma samples.

Hematology
Blood samples were kept at 4°C until analysis. The hematocrit (Hct) for each individual sample was determined prior to analysis with a HemoAnalysys (TCS Scientific Corporation, New Hope, PA). After appropriate dilution in buffer, the samples were transferred to the hematometer sample chamber where they were first saturated with compressed air for 20 minutes and then deoxygenated with pure nitrogen. The absorbance at wavelengths that correspond to the isosbestic point (570 nm) and the end of the HbO2 (600 nm) was recorded as a function of the sample’s oxygen level (pO2). During deoxygenation the pO2 and N2 saturation are recorded to obtain an OEC as well as a pO2 value.

Rat Mass Balance Study
A mass balance study of 14C-GBT440 (40 mg/kg; 150 µCi/g PO) was conducted in 3 male Sprague-Dawley rats to determine the route of elimination of GBT440. Urine, feces, and expired air samples were collected separately up to 240 hours post-dose. Animal carcasses was collected at the end of the study. After appropriate processing, all samples were analyzed for radioactivity with a liquid scintillation counter.

Rat QBWA
A quantitative whole body autoradiography study (QBWA) to determine tissue distribution of GBT440 in pigmented and nonpigmented rats was conducted following an oral dose of 14C-GBT440 (10 mg/kg; 150 µCi/g PO). One non-pigmented rat (Sprague-Dawley) and one pigmented rat (Long Evans) were sacrificed per QBWA at 0.5, 1, 2, 4, 8, 24, and 72 hr. One pigmented rat (Long Evans) was sacrificed per QBWA at 0.5, 2, 4, 8, 24, and 72 hr. One pigmented rat (Long Evans) was sacrificed per QBWA at 0.5, 2, 4, 8, 24, and 72 hr. One pigmented rat (Long Evans) was sacrificed per QBWA at 0.5, 2, 4, 8, 24, and 72 hr. One pigmented rat (Long Evans) was sacrificed per QBWA at 0.5, 2, 4, 8, 24, and 72 hr.

Predicted vs. Actual Human PK Profiles
PK parameters in rat, dog, and monkey were used to predict PK parameters in human using simple allometric scaling method, assuming a one compartment model. Blood concentration-time profile was simulated assuming an absorption rate constant (K0) of 1.15/hr and oral bioavailability of 37% (similar to dog). Actual human PK data was obtained from the ongoing clinical trial, GBT440-001 [ClinicalTrials.gov NCT02285080].

Results and Discussion

GBT440 Prevented the Production of Hemoglobin S (HbS)
Crude RBC lysates were obtained from Sprague-Dawley rats and incubated with GBT440 to determine if GBT440 would bind to hemoglobin and modulate O₂ affinity. CRD chromatography was used to separate HbS (unmodified) and HbS with GBT440 bound. HbS with bound GBT440 was then analyzed for OEC and color, which corresponded to the OEC of HbO2 (2,3-bisphosphoglycerate (2,3-BPG)).

Pharmacokinetic study

Table 1. Pharmacokinetic Parameters of GBT440 in Rat, Dog, and Monkey Following IV/PO Administration

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>V1 (mL/kg)</th>
<th>V2 (mL/kg)</th>
<th>CL (mL/min/kg)</th>
<th>Cmax (µg/mL)</th>
<th>T1/2 (hr)</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Blood</td>
<td>1</td>
<td>0.015</td>
<td>0.017</td>
<td>15.2</td>
<td>2.0</td>
<td>1.2</td>
<td>88.0 ± 152</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td>1.80</td>
<td>0.78</td>
<td>7.27</td>
<td>9.0</td>
<td>1.0</td>
<td>74.4 ± 164</td>
</tr>
<tr>
<td>Dog Blood</td>
<td>1</td>
<td>0.013</td>
<td>0.017</td>
<td>0.81</td>
<td>0.5</td>
<td>4.2</td>
<td>70.9 ± 156</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td>1.29</td>
<td>0.45</td>
<td>5.56</td>
<td>0.1</td>
<td>0.9</td>
<td>5.4 ± 0.871</td>
</tr>
<tr>
<td>Monkey Blood</td>
<td>1</td>
<td>0.016</td>
<td>0.017</td>
<td>0.33</td>
<td>0.1</td>
<td>4.5</td>
<td>70.9 ± 156</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td>1.84</td>
<td>0.45</td>
<td>4.25</td>
<td>0.2</td>
<td>0.5</td>
<td>70.9 ± 156</td>
</tr>
</tbody>
</table>

GBT440 was well absorbed and rapidly excreted after oral administration. By 240 hours post-dose, mean values of 79.0 ± 3.86 and 9.74 ± 0.32% of the administered radioactivity were excreted in feces and urine, respectively. The mean overall recovery of radioactivity was 92.4 ± 0.875%. Metabolism via both Phase I and Phase II pathways was the major route of elimination of GBT440. These data indicate that despite its high affinity binding with Hb, GBT440 could be released from the hemoglobin-complex and completely eliminated from the body.

The quantitatively whole body autoradiography (QBWA) data (Figure 5) suggest that GBT440 is primarily eliminated via the renal route and to a lesser extent through the fecal route. The distribution of radioactivity between the plasma and tissues is consistent with the known pharmacokinetic properties of GBT440, with the highest concentration of drug-related radioactivity corresponding to contents in the gastrointestinal tract. GBT440-derived radioactivity was not selectively associated with melanin-containing tissues.

Conclusions

• GBT440 showed high specificity for red blood cells with RBC/plasma ratio of ~150:1.
  - Pharmacodynamic data showed:
    - GBT440 was well absorbed in all animal species tested
    - Dose-dependent and linear pharmacokinetics
    - Half-life which is suitable for once daily dosing (human data, ~72 hr).

• Pharmacodynamic data showed good correlation between GBT440 blood concentration and an increase in hemoglobin O₂ affinity, as measured via hemoglobination.

• Metabolism is the major route of elimination involving both Phase I and Phase II metabolism pathways, with complete elimination from the body.

• GBT440 distributes to hematopoietic tissues as expected for a molecule whose target is hemoglobin, including blood, spleen, liver and bone marrow.

These data support that GBT440 preferentially partitions to RBCs, binds specifically to Hb, modulates Hb-O₂ affinity and has a half-life suitable for once daily dosing.

GBT440 is in clinical trials for the treatment of sickle cell disease.