In vitro characterization of FTX-6058, a novel small molecule fetal hemoglobin inducer for sickle cell disease

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Disclosure

All authors are current or former employees and equity holders of Fulcrum Therapeutics.
Therapeutic Rationale: Inducing fetal hemoglobin can reduce mortality and morbidity risks in SCD

SCD Patient

- RBC sickling
- VOCs
- Hemolysis

SCD Patient with High Fetal Hemoglobin (HbF)

- Increased F-cells*
- Reduced VOCs
- Reduced hemolysis

Pancellular HbF Expression and Induction

- Pancellular HbF Expression and Induction
- Asymptomatic presentation
- Reduced recurring events (VOCs, ACS, Hospitalization)
- Reduced mortality
- Increased F-cells*

HbF Level

30%
20%
10%

*F-cells - fetal hemoglobin expressing cells

References:
Parallel CRISPR and chemical probe screens in HUDEP-2 cells identify targets which increase HBG1/2

Target identification using parallel strategies in genetically relevant human cells

HEMATOLOGY FRANCHISE

HUDEP-2 Cells

Parallel Target ID Screening

Gene Regulation Phenotype
Disease Gene Activation Readout

Functional Validation
Disease Modeling in Primary CD34⁺ Cells

HBG1/2 and HbF Elevation
Chemical probe and CRISPR screens converge on published and novel drug targets

Gene Regulation Drug Targets

- BCL11A, NuRD, HDACs, LSD1, DNMT1, IKZF1, IKZF3, SPOP

Structure-Based Drug Design

- EED $K_D = 0.163$ nM
- PRC2 $IC_{50} < 5$ nM
- Highly Selective
- Clean Off-target Profile

FTX-6058

Identified Embryonic Ectoderm Development (EED) as a critical regulator of HbF

FTX-6058 is a Chemical Probe that inhibits EED with $K_D = 0.163$ nM and has an $IC_{50} < 5$ nM for PRC2. It is highly selective and has a clean off-target profile.
FTX-6058 in vitro target engagement is consistent across donors in differentiated primary CD34⁺ cells

FTX-6058 reduces H3K27me3 with an IC80 12-40 nM across 4 healthy CD34⁺ donors
FTX-6058 increases % HbF in CD34+ cells: HPLC quantitation

Quantification of %HbF in differentiated primary CD34+ cells

- **DMSO**: 12.6%
- **33µM Hydroxyurea**: 13.1%
- **100nM FTX-6058**: 27.4%

Hemoglobin HPLC: CD34+ cells differentiated and treated for 7 days

with Gerd Blobel laboratory (UPenn/CHOP)
FTX-6058 induces pancellular distribution of HbF

Left: pancellular HbF distribution in blood of patient with HPFH & SCD - asymptomatic SCD

FTX-6058 induced pancellular distribution of HbF

SCD Mother

FTX-6058 - 100 nM

HbF: ~30%

SCD Child w/HPFH

HbF: ~11%

HbF Flow cytometry: CD34+ cells differentiated and treated for 7 days; Gated and quantified for HbF+/CD235a+/CD71+


From 50% to 80% HbF hi
FTX-6058 elevates % HbF in differentiated primary CD34+ cells from SCD and healthy donors

- FTX-6058 induces superior HbF protein expression over HU across all CD34+ donors (healthy and SCD)

- Higher doses of FTX-6058 can further induce HbF protein expression, whereas higher HU concentrations were toxic
FTX-6058 Robustly Induces Fetal Hemoglobin in CD34+ Cells from Healthy and SCD Donors

- Observe an absolute 8 – 18% increase in HbF upon treatment with FTX-6058, which has the ability to address mortality risk and recurring events in SCD patients
- Small increases in HbF (1 – 5%) have the potential to provide clinical benefits to all SCD patients

### HbF Induction with FTX-6058

<table>
<thead>
<tr>
<th>Donor</th>
<th>Baseline %HbF</th>
<th>Maximal %HbF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Donor 2</td>
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</tr>
<tr>
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<td>Donor 5</td>
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</tr>
<tr>
<td>Donor 6 (SCD)</td>
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</tbody>
</table>

- Asymptomatic presentation
- Reduced recurring events (VOCs, ACS, Hospitalization)
- Reduced mortality
FTX-6058 has potential to be a transformative therapy for SCD

- FTX-6058 is a potent, selective and orally active small molecule EED inhibitor.

- Proof of concept studies provided *in vitro* evidence for FTX-6058 in inhibiting PRC2 activity, which leads to elevation of HbF in human primary CD34+ cells.

- FTX-6058 demonstrated potent TE and HbF induction *in vivo* in animal models at plasma concentrations reasonably expected to be achieved in the clinic. (See abstract #789)

- FTX-6058 pharmacological activity in target cells can be readily monitored in the clinic since TE in bone marrow correlates with TE in peripheral monocytes in animals. (See abstract #789)

- FTX-6058 demonstrated an impressive preclinical pharmacological profile with the potential to be a disease-modifying therapeutic for sickle cell patients. (See abstract #1548)
Additional questions:
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