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

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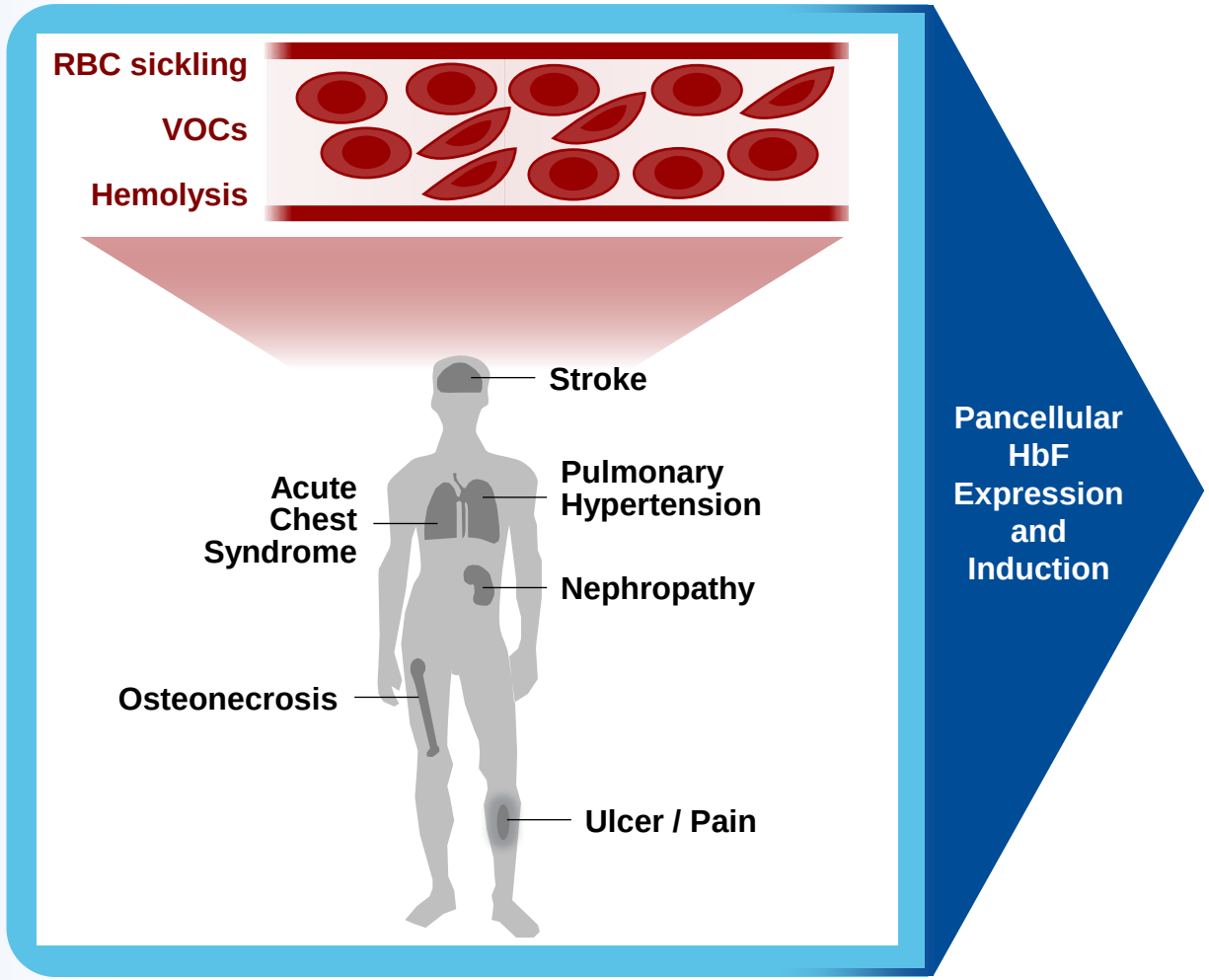
Disclosure

All authors are current or former employees and equity holders of Fulcrum Therapeutics.

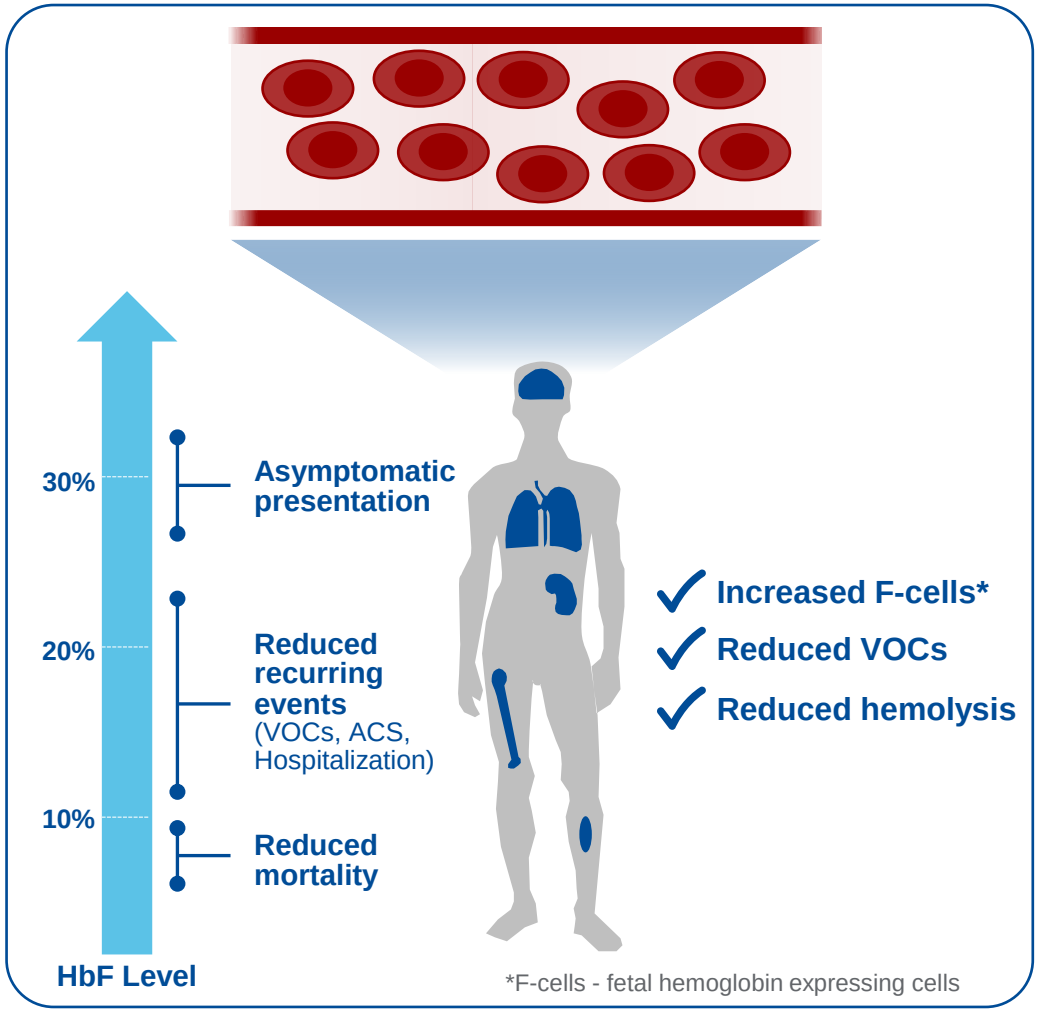
Therapeutic Rationale: Inducing fetal hemoglobin can reduce mortality and morbidity risks in SCD

SCD Patient



Pancellular HbF Expression and Induction

SCD Patient with High Fetal Hemoglobin (HbF)



Powars, DR. Blood. 1984; Estepp, JH. Br J Haematol. 2013; Platt, OS. NEJM. 1994; Akinsheye, I. Blood. 2011.

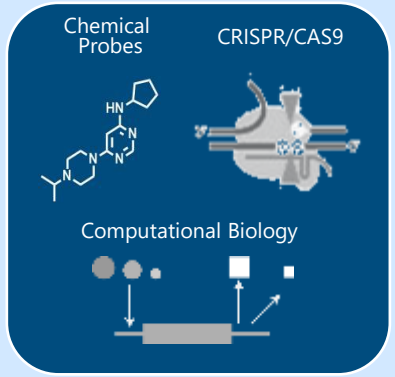
Parallel CRISPR and chemical probe screens in HUDEP-2 cells identify targets which increase HBG1/2

Target identification using parallel strategies in genetically relevant human cells

HEMATOLOGY FRANCHISE

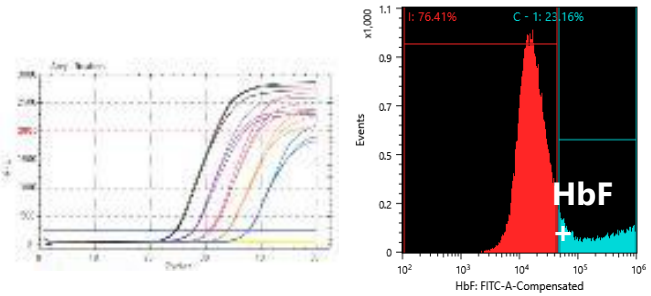
HUDEP-2 Cells

Parallel Target ID Screening



Gene Regulation Phenotype

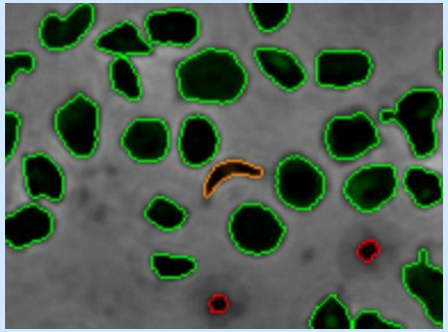
Disease Gene Activation Readout



HBG1/2 and HbF Elevation

Functional Validation

Disease Modeling in Primary CD34⁺ Cells



Chemical probe and CRISPR screens converge on published and novel drug targets

Chemical Probe Screening

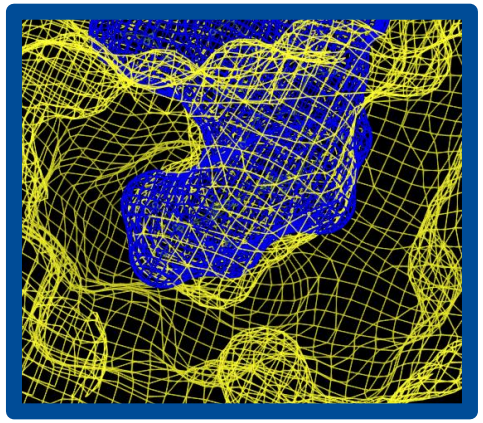
CRISPR Screening

Gene Regulation Drug Targets

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

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

Structure-Based Drug Design

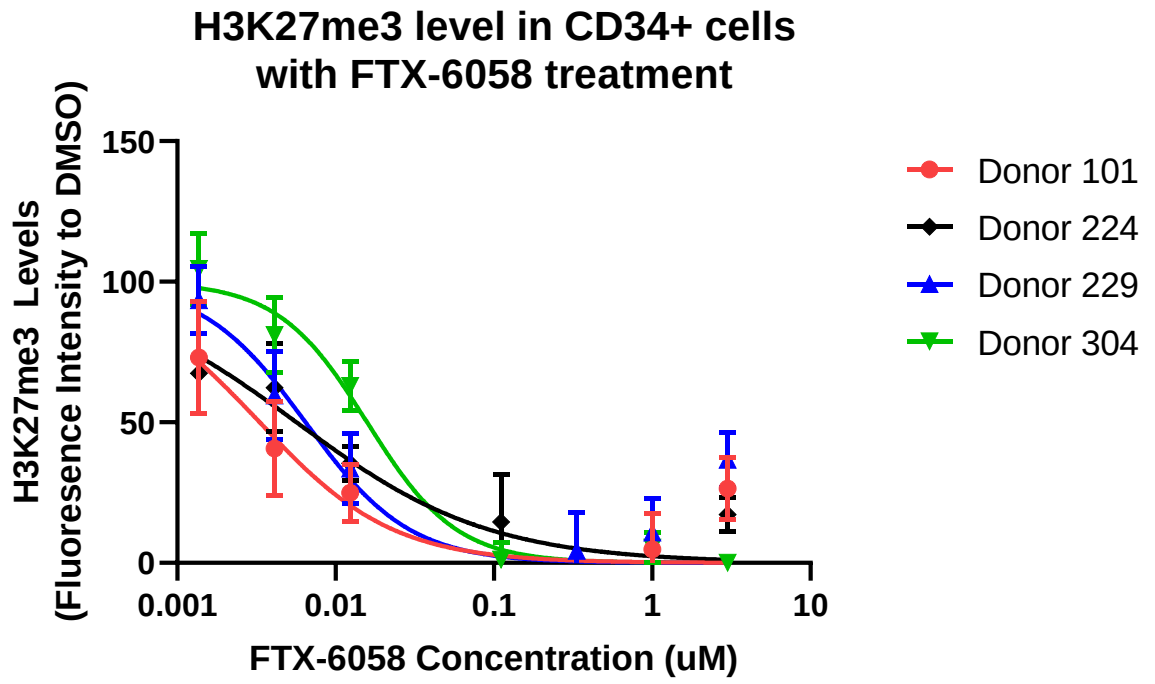


FTX-6058

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

FTX-6058 *in vitro* target engagement is consistent across donors in differentiated primary CD34+ cells

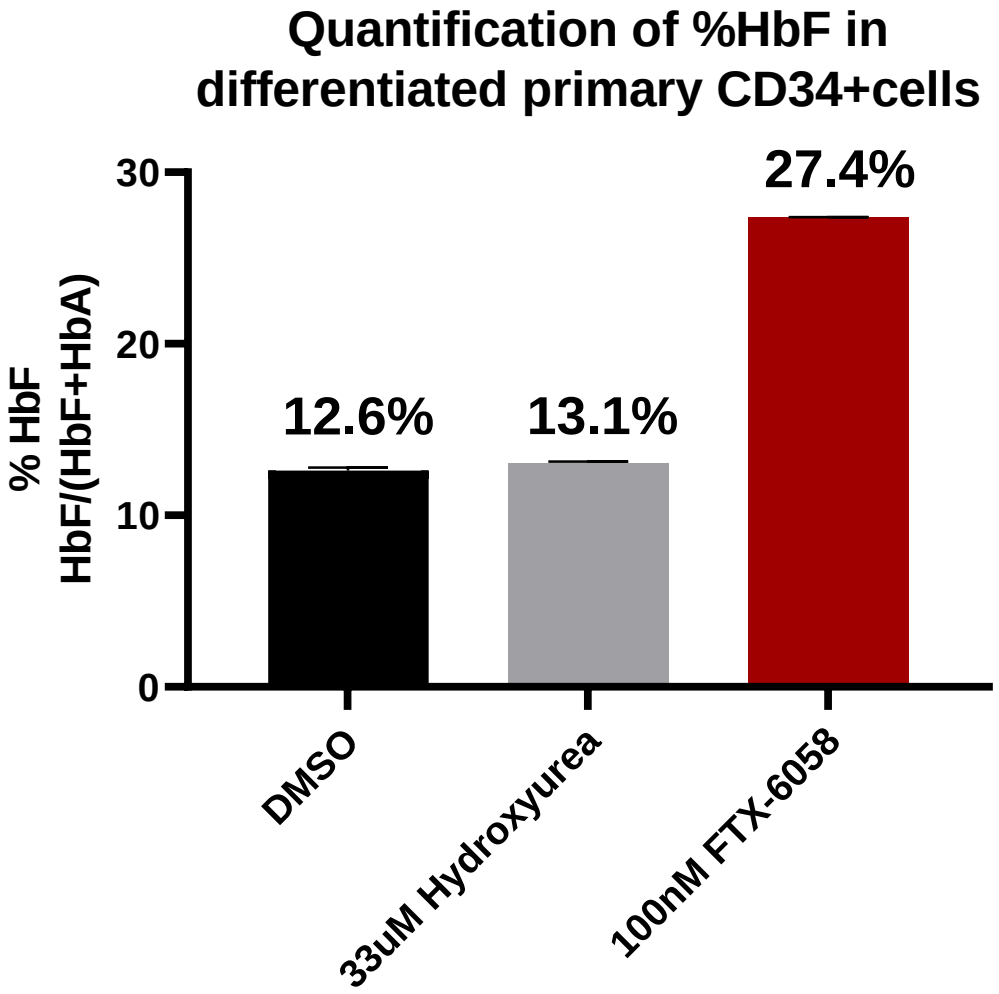
FTX-6058 target engagement in primary CD34+ cells is similar across 4 donors

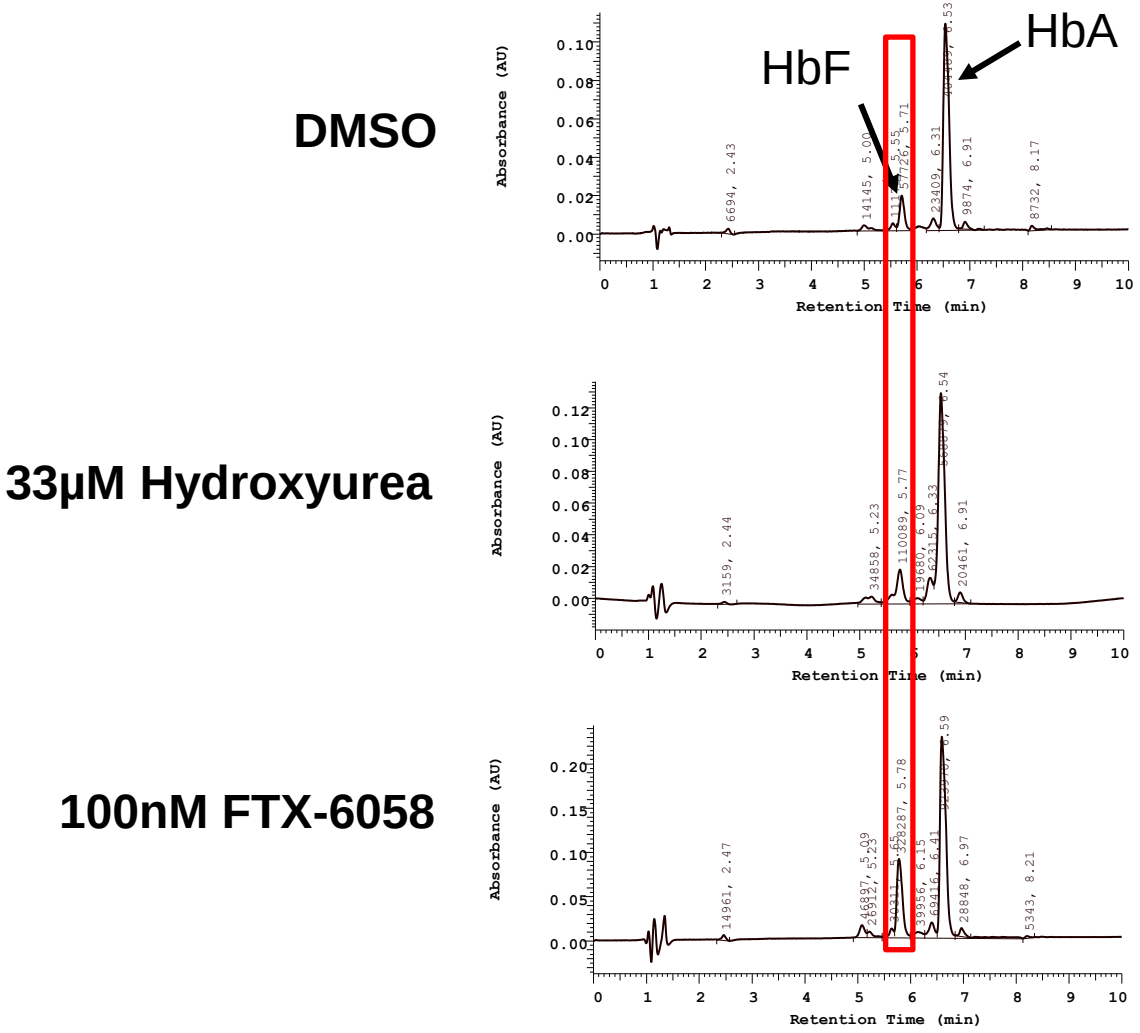


Target engagement (ICC): Primary CD34+ cells; 4 days treatment and differentiation

- FTX-6058 reduces H3K27me3 with an IC80 12-40 nM across 4 healthy CD34+ donors

FTX-6058 increases % HbF in CD34⁺ cells: HPLC quantitation





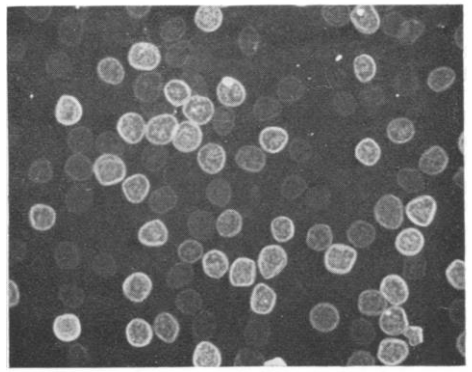
Hemoglobin HPLC: CD34⁺ cells differentiated and treated for 7 days

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

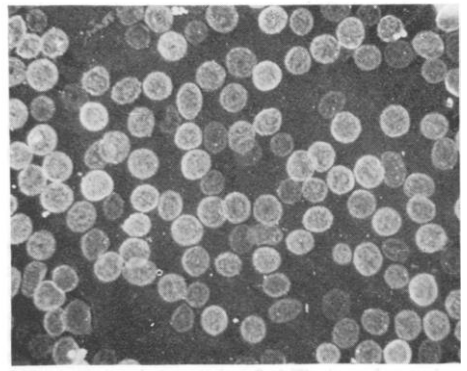
SCD Mother



(b)

HbF: ~11%

SCD Child w/HPFH



(a)

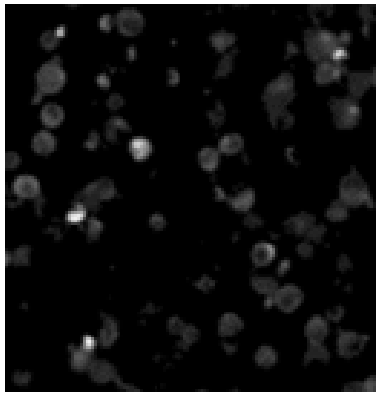
HbF: ~30%

Wood WG *et al.* J Med Gen, **14**:237 1977

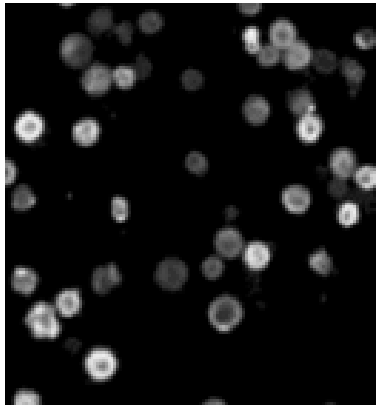
Fig. 2 *Peripheral blood smears from (a) the propositus M.K. and (b) his mother S.C., stained with FITC labelled anti Hb F antibodies, demonstrating in the former that virtually all the cells are stained, but with considerable intercellular heterogeneity, while in the latter, two distinct populations are evident.*

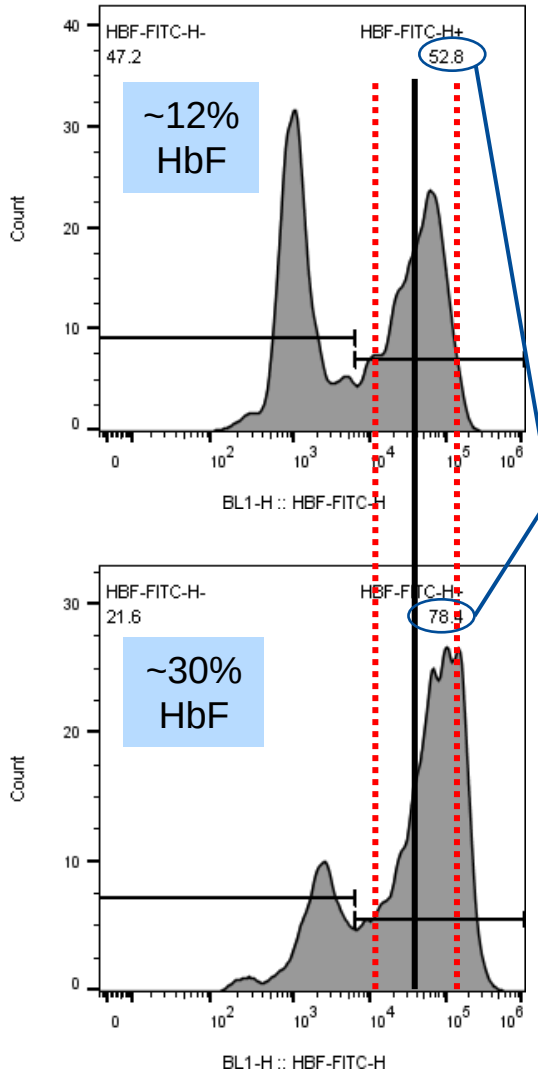
FTX-6058 induced pancellular distribution of HbF

DMSO



100 nM FTX-6058





From 50% to 80% HbF^{hi}

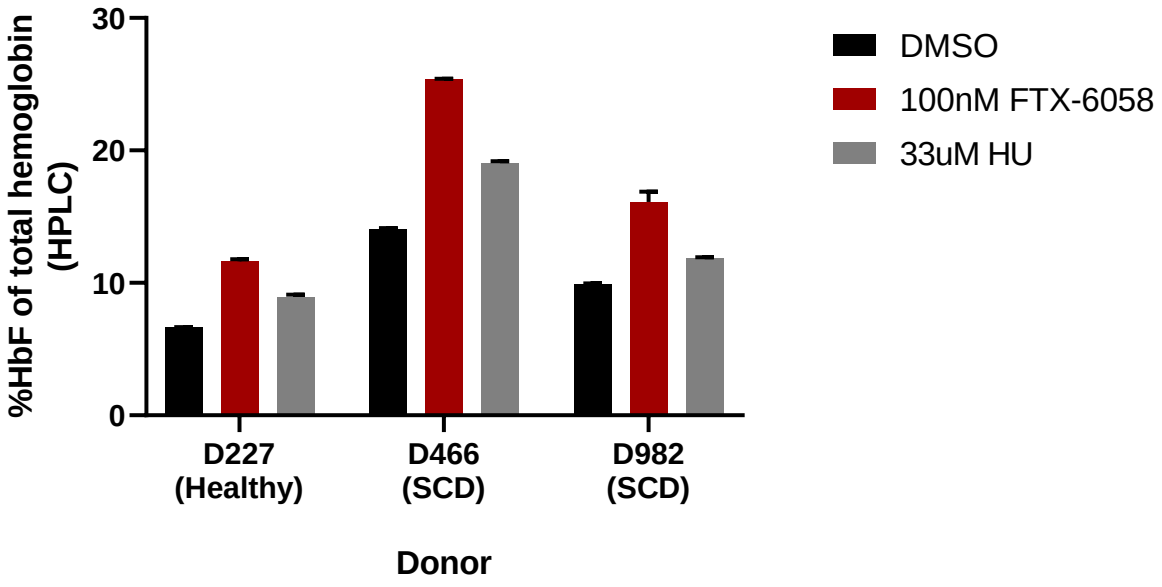
Counts

HbF

HbF flow cytometry: CD34^{+} cells differentiated and treated for 7 days; Gated and quantified for HbF+/CD235a+/CD71+

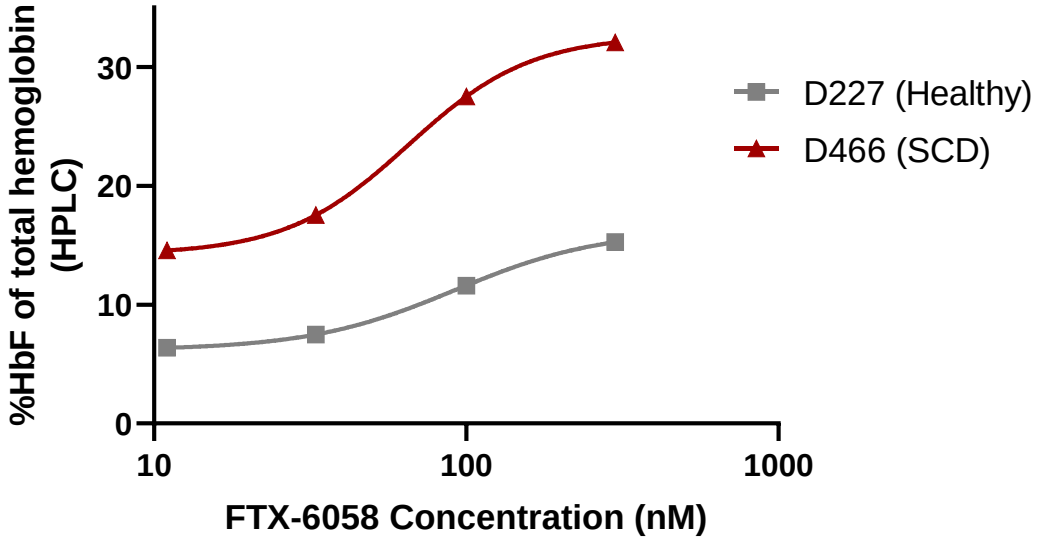
FTX-6058 elevates % HbF in differentiated primary CD34+ cells from SCD and healthy donors

%HbF elevation with FTX-6058 and HU



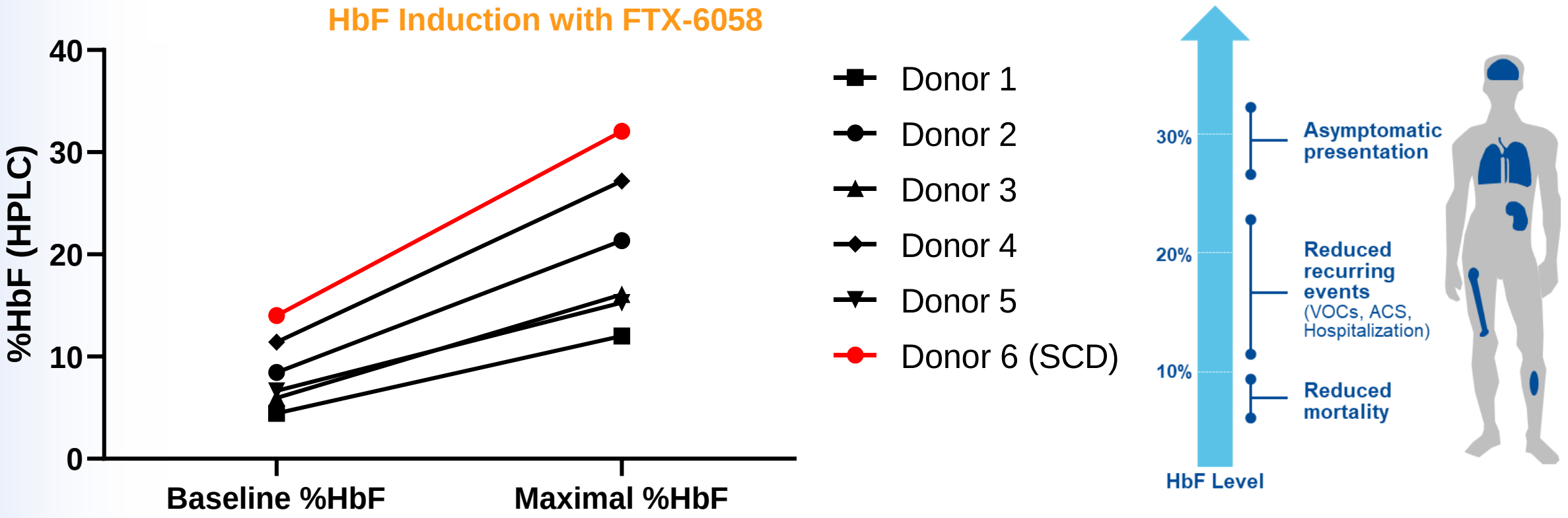
- FTX-6058 induces superior HbF protein expression over HU across all CD34+ donors (healthy and SCD)

Concentration-dependent increase in %HbF in CD34+ cells from SCD and healthy donors



- 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**

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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