REAWAKENING OF HUMAN FETAL HEMOGLOBIN AND AN EPIGENETIC PATH TO THE CLINIC FOR SICKLE CELL DISEASE AND BETA-THALASSEMIA: IDENTIFICATION OF AN ORALLY-AVAILABLE, POTENT, AND SELECTIVE EUCHROMATIC HISTONE LYSINE METHYLTRANSFERASE 1 AND 2 (EHMT1/2) INHIBITOR


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DISCLOSURES

I have the following financial relationships to disclose:

• Stockholder in and Employee of: Epizyme, Inc
HISTONE METHYLTRANSFERASES EHMT1 AND EHMT2 ARE INDUCERS OF FETAL HEMOGLOBIN: OFFERS A THERAPEUTIC APPROACH TO SICKLE CELL DISEASE

- It is well-established that SCD patients exhibit fewer or no clinical symptoms if they carry hereditary persistence of fetal hemoglobin (HPFH) mutations that lead to increased blood levels of HbF
  - Re-expression of g-genes and increasing HbF is a powerful disease modifying treatment approach for SCD patients

- The gene responsible for HbF formation, HBG, is negatively regulated by EHMT1/2 post-birth

- EHMT2 (G9a) and EHMT1 (GLP) function in a heterodimeric complex to di-methylate H3K9, a repressive mark of transcription

- EHMT1/2 play roles in multiple biological processes including hematopoiesis, imprinting, and immunity

EHMT1/2 INHIBITION PREVENTS DI-METHYLATION OF H3K9 AT A SINGLE GENE LOCUS LEADING TO RE-EXPRESSION OF HbF THAT HAS DISEASE MODIFYING POTENTIAL FOR SCD

- HbF is a known modulator of SCD clinical symptoms
EPZ035544 is a potent and selective EHMT1/2 inhibitor

- **Potent**, orally available EHMT1/2 inhibitor
- MOI: Peptide competitive, SAM non-competitive
- **Highly selective** vs. off-targets
  - HMT Panel: >2,000-fold by $K_i$
  - Diversity Kinase Panel: >1,000-fold by $K_i$

EPZ035544
EHMT1/2 $K_i$: 4 nM, 9 nM
Cellular H3K9 IC$_{50}$: 67 nM
LogP: 2.5, LogD: 0.5, TPSA: 73

DOSE DEPENDENT MOUSE PK
Plasma exposure required for IC$_{50}$
EHMT1/2-MEDIATED FETAL HEMOGLOBIN RE-EXPRESSION

Human CD34+ erythroid progenitor cells were differentiated in the presence of EPZ035544 and after 14 days, CD235+CD71+ cells were tested for the following readouts:

At Chr11 β-Locus:

1. EHMT1/2 inhibition
2. Decrease of H3K9me2
   - % H3K9me2 from Control
3. Selective expression of HBG genes
4. Increase of HbF
   - %HbF producing cells
   - Protein levels

% HBG/(HBG+HBB) = % HbF/(HbF + HbA)

ROBUST AND ON-TARGET IN VITRO ACTIVITY OF TOOL COMPOUND IN BLOOD PROGENITORS

• FACS analysis shows that EPZ035544 treatment of erythroid progenitors leads to dose-dependent decreases in H3K9me2 together with increases in % HbF+ cells

• 1:1 Correlation between the induction of HBG mRNA (PCR) and HBG protein (highly quantitative mass spec) is evident

• EPZ035544 shows no effects on in vitro erythroid differentiation as measured by CD235+ and CD71+ cell populations from differentiated CD34+ cells
EHMT1/2 inhibition in human CD34+ cells leads to the decrease of H3K9me2 together with an increase of H3K9Ac and Pol II occupancy at the HBG locus

**H3K9me2**
- Insignificant changes observed at Chr 16 HbA promoters
- At β-Locus on Chr 11
  - General H3K9me2 reduction across the locus upon compound treatment

**H3K9Ac**
- Treatment causes an increase at HBG promoters only, HBA and other Hb gene loci are unaffected

**RNA Pol II**
- Treatment causes recruitment of RNA Pol II to HBG promoters only, confirming active replication

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**De-repression**  |  **Activation**  |  **Active Transcription**
HBG GENES ARE THE MOST SIGNIFICANTLY UPREGULATED GENES UPON EPZ035544-TREATED HUMAN CD34+ CELLS

EPZ035544 vs DMSO GENE EXPRESSION PROFILE
RNA Seq Analysis

Selective up-regulation of HBG genes

Confirms RNA Pol II CHIP Seq Results

MOA for EHMT1/2-mediated HbF induction is fully validated

Expression of erythroid transcription factors and known HBG repressors was unchanged
TESTING THE RE-ACTIVATION OF DEVELOPMENTAL HEMOGLOBINS IN VIVO

Tool compound studies

1. **In vitro**: Human Erythroid Progenitors CD34+
   - EHMT1/2 inhibition
   - Decrease of H3K9me2
   - Selective expression of HBG genes
   - HbF protein increase (Hbβγ in mouse)

   Solid understanding of MOA

2. **In vivo**: C57BL6 Mice
   - Robust correlation between target engagement and efficacy
LONG TERM DOSING OF EPZ035544 IN NAÏVE MICE IS WELL TOLERATED AND SHOWS NO SIDE EFFECTS

Well tolerated at all dose group after 90 days of continuous dosing

- Bodyweight
- Spleen weight
- RBC
- WBC
- Platelets
- Neutrophils
EPZ035544 TREATMENT OF NAÏVE MICE INDUCES HBεγ PROTEIN IN VIVO

- Solid reduction of H3K9me2 observed in PBMCs (peripheral blood mononuclear cells) from EPZ035544-treated naïve mice
- Dose-dependent induction of mouse embryonic Hbεγ mRNA with steady state achieved after 30 days
- Increase of Hbεγ protein over time in whole blood

Lost due technical issues with sample collection

~100X Hbεγ/Total (Protein)
SUMMARY: EPZ035544 CAN INDUCE DEVELOPMENTAL HEMOGLOBINS IN IN VITRO AND IN VIVO SYSTEMS

- EHMT1/2 inhibition
- Decrease of H3K9me2
- Selective expression of HBG genes
- Increase of HbF protein (Hbεγ in mouse)

Enables a rational clinical path

Solid understanding of MOA

In vitro and in vivo induction of developmental hemoglobins
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