Abbreviated Best of ASH 2016

Sickle cell anemia
Red cells/iron

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Johns Hopkins University School of Medicine
Abstract #0322

Metformin Induces FOXO3-Dependent Fetal Hemoglobin Production in Primary Erythroid Cells

Vivien Sheehan, Yankai Zhang, Carly, Ginter Summarell, Mitchell Weiss, Pavel Sumazin
Background

Induction of HbF is an important strategy for treating sickle cell disease (SCD).

Additional HbF inducing drugs are urgently needed.

We analyzed WES to identify genetic variants that regulate HbF.

FOXO3 variants are associated with reduced HbF levels in patients with SCD.

HbF = α2γ2
HbA = α2β2

Zhang et al, ASH 2015
Hypothesis: Metformin induces HbF through FOXO3

Transcription factor characterized by a distinct forkhead DNA-binding domain

Querioz et al, PLOSOne 2014
Metformin Increases g-globin Expression in Primary Erythroid Culture

- Day 0: Human CD34+ Expansion
- Day 7: Epo added, Differentiation
- Day 14: Collect cells for RT-qPCR or HPLC

**FOXO3 mRNA**

- μM Metformin: 0, 50, 100
- FOXP3 mRNA levels: 0, 50, 100

**γ/(β+γ) globin ratio**

- μM Metformin: 0, 50, 100
- γ/(β+γ) globin ratio: 0, 50, 100

* p < 0.05; ** p < 0.01; *** p < 0.001; n=3
<table>
<thead>
<tr>
<th>Condition</th>
<th>HbF (%)</th>
<th>HbS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µM Met</td>
<td>18%</td>
<td>82%</td>
</tr>
<tr>
<td>100 µM Met</td>
<td>45%</td>
<td>55%</td>
</tr>
<tr>
<td>100 µM Met + 30 µM HU</td>
<td>67%</td>
<td>33%</td>
</tr>
</tbody>
</table>
Conclusions

• FOXO3 is a positive regulator of $\gamma$-globin.
  • FOXO3 upregulation via metformin increases the $\gamma/\beta$ globin ratio.

• Metformin is a promising HbF inducing agent
  • It does not delay erythroid maturation.
  • It acts additively with HU to increases HbF.
Abstract #001

SUSTAIN: A Multicenter, Randomized, Placebo-Controlled, Double-Blind, 12-Month Study to Assess Safety and Efficacy of SelG1 with or without Hydroxyuria Therapy in Sickle Cell Disease Patients with Sickle Cell-Related Pain Crises

Kenneth Ataga, Abdullah Kutlar, Julie Kanter, Darla Liles, Rodolfo Cancado, Joao Friedrisch, Troy Guthrie, Jennifer Knight-Madden, Ofelia Alvarez, Victor Gordeuk, Sandra Gualandro, Marina Pereira Colella, Wally Smith, Scott Rollins, Jonathan Stocker and Russell Rother for The SUSTAIN Investigators
Background

• Sickle cell pain crises (SCPC) cause substantial morbidity and mortality
• Hydroxyurea only approved drug:
  • P-selectin: an adhesion molecule on activated endothelial cells and platelets
    • Key molecule in initiation of leucocyte rolling on vessel wall

SelG1: first in class humanized anti-P-selectin Ab.
  ➢ Dosed as an IV infusion over 30 minutes
SUSTAIN Study Inclusion Criteria

Study Population (Key Inclusion Criteria):

• 16 to 65 years of age, male or female

• Diagnosis of Sickle Cell Disease (including the genotypes of HbSS, HbSC, HbSB$^0$-thalassemia, HbSB$^+$-thalassemia)

• Having had at least 2 but not more than 10 acute sickle-related painful crisis events within the 12 months prior to enrollment into the study

• Could either be receiving concomitant hydroxyurea (on a stable dose) or not

• Could not be on chronic transfusion therapy
Randomization

High-Dose SelG1 (5.0 mg/kg) - 67 patients
Low-Dose SelG1 (2.5 mg/kg) - 66 patients
Placebo - 65 patients

1 year of monthly dosing with a loading dose in first two weeks
Primary End Point - Annual Rate of SCPC

<table>
<thead>
<tr>
<th>Primary End point</th>
<th>High-Dose SelG1 (N=67)</th>
<th>Low-Dose SelG1 (N=66)</th>
<th>Placebo (N=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median rate of SCPC per year</td>
<td>1.63</td>
<td>2.01</td>
<td>2.98</td>
</tr>
<tr>
<td>Reduction vs. placebo</td>
<td>45.3%</td>
<td>32.6%</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.010</td>
<td>0.180</td>
<td></td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>(0.00 - 3.97)</td>
<td>(1.00 – 3.98)</td>
<td>(1.25 – 5.87)</td>
</tr>
<tr>
<td>Number of patients with SCPC rate of zero at end of study</td>
<td>24</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>
Time to First SCPC Event

Median Time to 1st SCPC
High-Dose 4.1 months
Low-Dose 2.2 months
Placebo 1.4 months

Adapted from Ataga KI, et al. NEJM [epub December 3, 2016].
Conclusions

• Treatment with high-dose SelG1 resulted in a clinically meaningful reduction in the frequency of SCPC in patients with SCD

• 41% reduction in frequency of SCPC was achieved with high-dose SelG1 treatment vs. placebo regardless of concomitant HU usage or SCD genotype

• Median times to 1st and 2nd crises with high-dose SelG1 were extended 2 – 3 fold compared to patients who received placebo

• The incidence of adverse events with SelG1 treatment was low
## ErythroMer Design Strategy

<table>
<thead>
<tr>
<th>Table 1</th>
<th>HBOC Flaw</th>
<th>NanoCrit Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxygen (O₂) Transport from Lungs to Tissue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Design Issue</strong></td>
<td>O₂ affinity is fixed, not context-responsive</td>
<td>pH-responsive 2,3-DPG shuttle</td>
</tr>
<tr>
<td><strong>Consequence</strong></td>
<td>Adequate O₂ capture (lungs), poor O₂ release (tissue)</td>
<td>O₂ affinity shifts during transit (lungs ↔ tissue) and limits amount of O₂ release to tissue need</td>
</tr>
<tr>
<td><strong>Interference with Normal Regulation of Blood Vessel Caliber</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Design Issue</strong></td>
<td>Traps the endogenous vasodilator NO</td>
<td>Novel, 'tunable' PEI polymer shell</td>
</tr>
<tr>
<td><strong>Consequence</strong></td>
<td>Intense vasoconstriction, tissue ischemia</td>
<td>Permits O₂ diffusion, retards NO trapping, vascular tone unaltered</td>
</tr>
<tr>
<td><strong>Maintain Hemoglobin (Hb) Functionality During Circulation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Design Issue</strong></td>
<td>Hb auto-oxidizes, generating methHb</td>
<td>Leuko MB packaged in particle payload</td>
</tr>
<tr>
<td><strong>Consequence</strong></td>
<td>Drastically limits effective circulation time</td>
<td>Simple reduction system regenerates oxidized Hb</td>
</tr>
<tr>
<td><strong>Storage and Ease of Use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Design Issue</strong></td>
<td>Incompatible with dry storage</td>
<td>Particle can be lyophilized</td>
</tr>
<tr>
<td><strong>Consequence</strong></td>
<td>Limits shelf life and versatility</td>
<td>Extends shelf life, facile use</td>
</tr>
</tbody>
</table>

**Polyethyleneimine (PEI)**

**Amphiphilic PEI**

**Hemoglobin**

**2,3-DPG (allosteric factor)**

**Methylene Blue** (inhibits auto-oxidation of Hb)

**Leuko Methylene Blue**
In Vivo Efficacy Rat model 40% blood volume removed and resuscitated with equal vol. of EM.
Whole Animal Bioassay for Tissue $O^2$ Delivery HIF-1$\alpha$ (ODD)-luciferase mouse

**Whole body HIF Luc**

![Graph and images showing hemoglobin concentration and body radiance vs. anemia stages.]

Relationship of Hb levels and whole body HIF Luc

- Hemoglobin threshold levels: 130g/L, 90g/L, 70g/L, 50g/L
- N=5
In Vivo Efficacy (hemodilution, HIF-bioluminescence, mice fast ErythroMer kinetics (t1/2 ~ 30 min)

Manipulation:
- Murine RBC [Hb]:
  - 70% vol X (HES) 70% vol X (EM)
  - 5 4 4 4 5
- EM [Hb]:
  - 0 10 2 0 0

anemia Hb restored post EM clearance
ErythroMer Summary

• **Strengths**
  • Physiologic O₂ capture/release
  • Does not trap NO (no vasospasm)
  • Shell is synthetic, immune silent? (no crossmatching)
  • Not-animal derived (no infectious risk)
  • Lyophilizable

• **Limitations**
  • Cleared rapidly from bloodstream (~ 3-7h)
  • Bridging (field → hospital) role
  • Short-term (OR) needs
Abstract #0260

Hepcidin Protects Against Extracellular Infections by Eliminating Non-transferrin-bound Iron

Deborah Stefanova, Joao Arezes, Kathryn Michels, Barbara Dillon, Marcus Horwitz, Borna Mehrad, Yonca Bulut, Tomas Ganz, Elizabeth Nemeth
Background

Objective:
- elucidate the mechanism by which hepcidin mediates resistance to infection
Hepcidin and Innate Immunity

• Hepcidin: liver-derived hormone, central regulator of iron homeostasis, blocks iron absorption and iron release from recycling macrophages

• Hepcidin is induced during infection (primarily via IL-6), causing hypoferremia

• Hypothesis: hepcidin has a role in innate immunity to limit iron availability to pathogens, thus preventing their growth
Testing the role of hepcidin in infections

• Mouse models
  o *Wild-type* vs *hepcidin knockout* mice (naturally iron loaded)

• Infection models
  o *Siderophilic* ("iron-loving") gram-negative *Yersinia enterocolitica* and *Vibrio vulnificus*
    (increased virulence in iron-overloaded patients)
  o *Gram-negative* *Klebsiella pneumoniae*
  o *Gram-positive* *Staphylococcus aureus*
  o Primarily *intracellular* *Mycobacterium tuberculosis*
Hepcidin mediates host defense against Gram-negative bacteria

- Hepcidin KO mice had increased mortality in Gram-negative infections...

- ...but did not have increased susceptibility to *S. aureus* or *M. tuberculosis*.
Iron species in circulation

• Iron-transferrin
  • In healthy subjects, iron is bound to transferrin (Tf saturation is 20-50%)

• Non-transferrin-bound iron (NTBI)
  • Present when transferrin saturation exceeds ~70-80%
  • Easily accessible to microbes

• Does hepcidin control infection with Gram-negative pathogens by decreasing Fe-Tf or NTBI?
NTBI is essential to promote rapid growth of Gram-negative bacteria

- **Human plasma** supplemented with increasing concentration of iron (ferric ammonium citrate = FAC) to saturate transferrin and generate NTBI

- *Y. enterocolitica* growth on plasma agar plates
  - Low Tf saturation, no NTBI
  - High Tf saturation, NTBI present

- *V. vulnificus* growth in liquid plasma
  - NTBI present

- *K. pneumoniae* growth on plasma agar plates
  - Low Tf sat, no NTBI
  - High Tf sat, NTBI present

<table>
<thead>
<tr>
<th>Iron supplementation (µg/dL)</th>
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<tr>
<td>0</td>
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</table>
Hepcidin agonists effectively treat siderophilic infections

- Iron-overloaded hepcidin KO mice infected with *Y. enterocolitica*
- Injected daily for 7 days with solvent or hepcidin agonist minihepcidin (treatment started 2 days or 3 days after infection)
Conclusion

• Non-transferrin-bound iron (NTBI) triggers rapid growth of Gram-negative pathogens, overwhelming host defense mechanisms

• Hepcidin controls the growth of iron-sensitive pathogens by preventing the generation of NTBI in plasma

• Hepcidin agonists may be useful for the treatment of siderophilic infections that are particularly lethal in iron overload conditions