Innovative therapies for β-hemoglobinopathies

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Disclosures

**Consultant**
- Isis Pharmaceuticals
- Medgenics Pharmaceuticals
- Bayer Healthcare Pharmaceuticals
- Novartis Pharmaceuticals

**Collaboration**
- Acceleron Pharmaceuticals

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**Isis Pharmaceuticals**
- Merganser Biotech
- Bayer Healthcare Pharmaceuticals
- Medgenics LLC
- Meira GTX
Sickle Cell Anemia and β-Thalassemia

Consequences of Reduced β-Globin Chain Production

Ineffective Erythropoiesis
- Anemia
- Iron Overload
- Erythroid Marrow Expansion
- Splenomegaly
- Thrombosis

Consequences of Abnormal β-Globin Chain Structure

Vaso-Occlusion
- Pain
- Hemolysis
- Anemia
- Pulmonary hypertension
- Iron overload

Mutations that alter the structure
- (Glutamic acid to Valine at position 6)

Mutations that reduce the synthesis
- Alpha/Heme Aggregates or hemichromes

Sickling Hb and red cells
Erythropoiesis and iron absorption

- **Epo** (Erythropoietin)
- **EpoR** (Erythropoietin Receptor)
- **pJak2**
- **pStat5**
- **Cell Replication**
- **Protection from apoptosis**
- **β-Thalassemia:**
  - Apoptosis & ROS
- **GDF11**
  - Increased survival & decreased cell differentiation
  - Increased number of progenitor erythroid cells
- **“Erythroid factor(s)”**
  - Erythroferrone, ?
- **Hepcidin**
  - Increased iron absorption
Anaemia/IE

Erythroid cell replication
Erythroid cell differentiation

Hemichromes and ROS

Apo-Transferrin

Erythroid iron intake

Erythroferron (ERFE)

Activin receptor II trap ligands

SEMA inhibitors

Erythroid progenitor cells

ERFE inhibitors or antagonists

Minihepcidins and TMPRSS6 inhibitors

Iron absorption
Potential use of erythroid modulators for the treatment of ß-thalassemia
Potential use of JAK2 inhibitors for the treatment of β-thalassemia
Potential effect of JAK2 inhibitors on ineffective erythropoiesis

β-thalassemia → Ineffective erythropoiesis → High EPO levels → pJAK2:

Erythroid progenitors → JAK2 inhibitor

Spleen

Decreased differentiation of erythroid cells exacerbates ineffective erythropoiesis in β-thalassemia
Increased erythroid cell proliferation in spleens of human β-thalassaemic specimens

- **Normal**
- **Patient**

**Ki67**

**Ki67 +**

glycophorin-C

and spectrin

Potential use of Jak2 inhibitors for the treatment of ß-thalassemia

• In ß-thalassemia anemia is associated with increased EPO levels
• This leads to increased activation of the Jak2/Stat5 pathway
• One of the main consequences is increased proliferation and expansion of the pool of erythroid progenitors, leading to EMH and exacerbation of IE

Therefore, administration of Jak2 inhibitors might be helpful to reverse splenomegaly and ameliorate IE
Reduction of splenomegaly following administration of a Jak2 inhibitor in mice affected by β-thalassaemia intermedia is dose-mediated.
JAK2 inhibitor increases efficacy of transfusion in transfusion-dependent mice affected by \( \beta \)-thalassaemia major

Pedro Ramos, Carla Casu, Luca Melchiori, ASH 2011, San Diego
Use of JAK2i in β-thalassemia major

**TM: No Transfusion**

**TM + Transfusion**

**TM + Jak2i: Reduced Transfusion & iron overload**

Erythroid progenitors

RBC

Iron overload
Potential use of Hepcidin agonist or inducers for the treatment of \(\beta\)-thalassemia
Iron overload and anemia worsen over time in mice affected by β-thalassemia intermedia

**Anemia worsens with time in th3/+ mice**

<table>
<thead>
<tr>
<th>Months</th>
<th>Hemoglobin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>15.0</td>
</tr>
<tr>
<td>12.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

**Iron overload increases with time in th3/+ mice**

<table>
<thead>
<tr>
<th>Organ total iron content (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>+/+ 2M</td>
</tr>
<tr>
<td>+/+ 5M</td>
</tr>
<tr>
<td>+/+ 12M</td>
</tr>
</tbody>
</table>

Hepcidin is expressed at low levels in iron overloaded th3/+ mice

Hepcidin, the iron hormone regulator, acts on Ferroportin, the iron exporter
Hepcidin regulates dietary iron absorption and distribution by triggering the degradation of Fpn1.

Hepcidin is up-regulated by increased iron levels and inflammation.

Hepcidin is down-regulated by erythropoiesis, anemia, and hypoxia.
Hepcidin, a major player in hemochromatosis, iron overload and anemia of chronic disease

- Hemochromatosis
- Secondary Hemochromatosis (i.e. β-thalassemia, MDS)
- IRIDA
- Anemia of Inflammation
- Bacterial infections
- Cancer

**Too little:**
- Increased iron absorption
- Increased macrophage iron release
- Iron overload

**Too much:**
- Decreased iron absorption
- Macrophage iron retention
- Anemia
Hypothesis: Increased levels of Hepcidin in thalassemia intermedia are beneficial to prevent iron overload and ameliorate erythropoiesis

Hepcidin as a therapeutic tool to limit iron overload and improve anemia in β-thalassemic mice

The Journal of Clinical Investigation
Minihepcidins (MH): background

- Minihepcidins are short peptide mimetics (9 retro-inverso AA) of hepcidin (25 AA)
- Derived from the N-terminal amino acid sequence and modified for in vivo activity
- MH are effective in reducing iron overload in animal models of *HFE*- and *HAMP*-related hemochromatosis
Very high stability of the minihepcidin M004 in vivo

*Blood levels and effect on transferrin saturation following a single dose of 7.5 mg/kg of M004 in the rat*
Experimental Protocol

*th*3/+ animals treated by SC injection with M004 (52.5 µg twice weekly) for six weeks.

CBC assessment at four and six weeks. Serum and tissue iron assessment at six weeks.
Minihepcidin administration significantly reduces liver, spleen and kidney iron concentration in thalassemic mice

Carla Casu
Minihepcidin administration induces a mild functional iron deficiency

Transferrin Saturation

- Vehicle
- 52.5 ug
- wt

MCV

- Vehicle
- 52.5 ug
- wt

CHr

- Vehicle
- 52.5 ug
- wt
Minihepcidin administration significantly ameliorates hemoglobin levels

Carla Casu
Minihepcidin administration significantly ameliorates erythropoiesis and spleen weight in thalassemic mice

**RBC**
- Vehicle
- 52.5 ug
- wt

**RETIC**
- Vehicle
- 52.5 ug
- wt

**SPLEEN WEIGHT**
- Vehicle
- 52.5 ug
- wt

Carla Casu
Administration of minihepcidin is associated with reduced MCH in thalassemic mice

\[ \text{WT + MH} \quad \text{Th3/+ + MH} \]

<table>
<thead>
<tr>
<th></th>
<th>WT + MH</th>
<th>Th3/+ + MH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb</strong></td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td><strong>RBCs</strong></td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Tf sat</strong></td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td><strong>MCH</strong></td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

Carla Casu
Administration of minihepcidin is associated with reduced erythroid cell damage
Combination of MH and iron chelation

Using mice affected by ß-thalassemia intermedia, we evaluated if the simultaneous use of the iron chelator deferiprone (DFP) with MH can combine the positive effects of MH on erythropoiesis with the chelation benefit on organ iron content.
Combination of MH with iron chelation improves organ iron content and erythropoiesis

Carla Casu and Paraskevi Rea Oikonomidou
MH, but not Deferiprone, improves RBC morphology and reduces ROS in erythroid cells

Carla Casu and Paraskevi Rea Oikonomidou
Polycythemia vera and β-thalassemia

Polycythemia vera (PV) is caused by neoplastic proliferation and maturation of erythroid, megakaryocytic and granulocytic elements. In contrast to secondary polycythemias, PV is associated with a low serum level of the hormone erythropoietin (EPO).

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Mutation</th>
<th>Main features</th>
<th>Main treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobinopathies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-thalassemia</td>
<td>β-globin gene</td>
<td>IE, anemia</td>
<td>TX, iron chelation, BMT, Gene therapy (?)</td>
</tr>
<tr>
<td>Myeloproliferative disorder</td>
<td></td>
<td>Increased number of erythroid progenitor cells</td>
<td></td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>Mostly Jak2&lt;sup&gt;V617F&lt;/sup&gt;</td>
<td>Erythrocytosis</td>
<td>Phlebotomy, Jak2i</td>
</tr>
</tbody>
</table>
A mouse model to study Polycythemia vera

Physiological Jak2V617F Expression Causes a Lethal Myeloproliferative Neoplasm with Differential Effects on Hematopoietic Stem and Progenitor Cells

Ann Mullally,1,2,9 Steven W. Lane,2,4,9 Brian Ball,2 Christine Megerdichian,2 Rachel Okabe,2 Fatima Al-Shahrour,2,5 Mahnaz Pakdil,2 J. Erika Haydu,2 Elizabeth Housman,2 Allegra M. Lord,2 Gerlinde Wernig,2 Michael G. Kharas,2 Thomas Mercher,7 Jeffrey L. Kutok,7 D. Gary Gilliland,5,9 and Benjamin L. Ebert2,5,9
Administration of MH improves erythropoiesis in mice affected by Polycythemia vera
Administration of MH corrects Hb levels and HTC in mice affected by Polycythemia vera.
Deletion of *Tmprss6* improves iron overload and erythropoiesis in a mouse model of NTDT

Deletion of *TMPRSS6* attenuates the phenotype in a mouse model of β-thalassemia

Antonella Nai,1,2 Alessia Pagani,1,2 Giacomo Mandelli,3 Maria Rosa Lidonnici,1,3 Laura Silvestri,1,2 Giuliana Ferrari,1,3 and Clara Camaschella1,2
Antisense mechanism of action
*RNase H*-mediated degradation

Diagram showing the interaction between Antisense Strand and mRNA-Antisense Duplex, leading to RNA degradation by RNase H.
**Tmprss6-ASO treatment significantly improves anemia in thalassemic mice (th3/+)**

*Shuling Guo & Carla Casu*
TMPRSS6 ASO treatment improves thalassemic red cell parameters and morphology

Red Cell Distribution Width (RDW)

PBS  Tmprss6-ASO  PBS  Tmprss6-ASO
Males  Females

*** ***

Normal values

th3/+ PBS  th3/+ Tmprss6-ASO  WT
Use of Tmprss6 inhibitors improves iron overload in a mouse model of HFE-related hemochromatosis and erythropoiesis and organ iron content in NTDT

Reducing TMPRSS6 ameliorates hemochromatosis and β-thalassemia in mice

Shuling Guo, Carla Casu, Sara Gardenghi, Sheri Booten, Mariam Aghajan, Raechel Peralta, Andy Watt, Sue Freier, Brett P. Monia, and Stefano Rivella

An RNAi therapeutic targeting Tmprss6 decreases iron overload in Hfe−/− mice and ameliorates anemia and iron overload in murine β-thalassemia intermedia

Paul J. Schmidt, Iva Toudjarska, Anoop K. Sendamaral, Tim Racie, Stuart Milstein, Brian R. Bettencourt, Julia Hettinger, David Burncrot, and Mark D. Fleming
TMPRSS6 ASO treatment significantly reduces serum iron and transferrin saturation in non-human primates

- TMPRSS6-ASO candidates has been selected
  - Well tolerated in mice and in monkeys
  - Demonstrated excellent activity and pharmacology in both transgenic mice and non-human primates

Initiation of the Phase 1 clinical trial is expected in 2016

Shuling Guo, Isis P.
Potential effects of Hepcidin agonists or activators on iron absorption and erythropoiesis in β-thalassemia in presence or absence of iron chelation

<table>
<thead>
<tr>
<th>Normal Conditions</th>
<th>β-Thalassemia</th>
<th>β-Thalassemia +DFO</th>
<th>β-Thalassemia Hepcidin correct activity</th>
<th>β-Thalassemia Hepcidin correct activity +DFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin activity</td>
<td>Iron absorption</td>
<td>Iron absorption</td>
<td>Iron absorption</td>
<td>Iron absorption</td>
</tr>
<tr>
<td>Normal organ iron concentrations</td>
<td>Iron overload</td>
<td>Iron overload</td>
<td>Iron overload</td>
<td>Iron overload</td>
</tr>
<tr>
<td>α-chain/heme aggregates</td>
<td>Amelioration of erythropoiesis</td>
<td>Amelioration of erythropoiesis</td>
<td>Amelioration of erythropoiesis</td>
<td>Amelioration of erythropoiesis</td>
</tr>
</tbody>
</table>
Severe hemoglobinopathies:

Gene therapy approach
Gene Therapy Schematic Approach

- hematopoietic stem cells
- vector carrying the therapeutic gene or genetic modification of the HSC by genome editing
- reinfusion
- transduction or gene editing

Rivella S; Haematologica 2015
Human β-globin gene

Locus Control Region

Promoter/5' UTR

Ex1  IVS1  Ex2  IVS2  Ex3  3' UTR

91 110 222 849 128

1605
Therapeutic levels of Hb in mice affected by Cooley’s anemia

A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human β-globin gene transfer

Stefano Rivella, Chad May, Amy Chadburn, Isabelle Rivière, and Michel Sudelain
Beta thalassemia patients: $\beta^0/\beta^0$, $\beta^0/\beta^+$ and $\beta^+/{\beta}^+$

Based on the $\beta$-globin protein synthesis, all mutations can be classified as: $\beta^0$, where no $\beta$-globin protein is produced, or $\beta^+$, where some, but not sufficient $\beta$-globin chain is made

Therefore, all patients can be classified as $\beta^0/\beta^0$, $\beta^0/\beta^+$ or $\beta^+/{\beta}^+$, according to all possible combinations of these mutations
AnkT9W Increases significantly the HbA in β0/0 specimens

β0/β0 no-vector

β0/β0 + vector

Laura Breda
Patient Genotype Still Matters

Therapeutic Hb synthesis in $\beta^{+/+}$ and $\beta^{+/0}$ cells is reached at lower VCN compared to $\beta^{0/0}$ cells.

Breda L, Casu C; PloS One 2012
Median Hemoglobin Concentrations at 6 Months

- **Endogenous**
  - HbE/HbA (endogenous)
    - HbA^2
    - HbF
    - HbA^{T87Q}
  - HbA^2
  - HbF
  - HbA^{T87Q}
- **Transgenic**
  - Other Genotype
    - n=5
  - 4.9g/dL
- **Transfusion**
  - Other Genotype
    - n=4
  - 5.0g/dL

**Difference in transfusion independence between genotypes explained by endogenous non-HbA^{T87Q} hemoglobin production**
1. Elimination of the WPRE element. Modification of the vector backbone to preserve high titer viral production also in absence of WPRE.

2. Inclusion of the full second β-globin intron. It contains enhancers and Oct1 binding site that help with LCR looping globin synthesis.
ASL10 performs significantly better in $\beta^0\beta^0$ patient cells compared to AnkT9W

The goals are:

- Compare ALS10 to vectors already in clinical trial
- Generate GMP-quality vector and file and IND application
- Clinical trial for $\beta$-thalassemia and SCD
High level of curative hemoglobin in patient cells from sickle cell patients

CD34-derived ctrl

+ vector

\[ HbA=0\% \]

\[ HbA=60\% \]

\[ VCN = 1.3 \]
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Jeantherapy