Ten years of Highlights from EHA: Red cells and Iron

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Ten years of Highlights from EHA: Red cells and Iron

Summary:
Erythropoiesis: mechanisms
New Diagnostic Approach: new disease-causing genes
New Drugs for inherited anemias
New Treatment Approach: gene therapy and Crisp/Cas9
Molecular Events during Normal Erythropoiesis

Transcription factors, small RNAs, and DNA-binding factors:

Stem Cells → CFU-GEMM → BFU-Es → CFU-Es

Growth Factors:

Mean MCV
0 day 131 fL
7 day 99 fL
14 day 86 fL

3 days, 3-5 cell divisions
7-9 days, 9-16 cell divisions

Figure 1. An overview of erythropoiesis: regulation at multiple levels by multiple proteins and miRNAs. Formation of RBCs from HSCs is regulated by signaling through both external factors (blue), such as cytokines and fibronectin, as well as intracellular factors, such as transcription factors (black) and miRNAs (red). Below the differentiation network, a timeline and images are shown for in vitro methylcellulose colony formation of murine BFU-E and CFU-E.
Erythropoiesis block in Inherited Anemias (EIAs)

- **EIAs** are mendelian diseases affecting the normal differentiation-proliferation pathway of the erythroid lineage.

Erythroid hyperplasia with specific morphological alterations involving late erythroblasts

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[Images of erythrocytes and erythroblasts, showing stages of erythropoiesis]

**ERYTHROPOIETIN DEPENDENT**

- BFU-e
- CFU-e
- Proerythroblast
- Basophilic erythroblast
- Polychromatic erythroblast

**IRON DEPENDENT**

- DBA
- CDA
- Thal

*Common myeloid progenitor
*Multifactorial anemias
*XLTDA; CEP; XLTT
*FA; DKC

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*Williams Hematology, Ninth Edition, Chapter 39 by A. Iolascon - McGraw-Hill*
Mechanisms of systemic and intracellular iron homeostasis

(A) Intracellular iron metabolism

Low iron
- IRPs
- Ribosome
- 5'-UTR IRE
- CDS
- 3'
- mRNA stabilization

High iron
- IRPs
- Regulated genes
  - Ferritin H
  - Ferritin L
  - Ferroportin
- Functions
  - Iron storage
  - Iron export
- Translational repression
- Translational activation
- Endonuclease
- 5'-UTR IRE
- CDS
- 3'
- mRNA degradation

(B) Systemic iron metabolism

Hepatocyte
- Reserve
- Hepcidin
- Hepcidin

Serum
- Fpn
- Hepcidin
- Hepcidin

Enterocyte
- Dietary iron

Erythrocyte
- Macrophage
- Fpn

Divalent metal transporter 1
- Transferrin receptor 1
- Iron uptake
New Mechanisms regulating Iron Intracellular Metabolism

[Diagram showing various iron uptake, distribution, and utilization pathways, with key proteins and pathways such as Tf, Tfr1, PCBP2, and NCOA4 highlighted.]

Key: ○ Ferrous iron (Fe²⁺)  ● Ferric iron (Fe³⁺)
Physiopathology of iron overload

↑ Apoptosis

ACE-011
ACE-536

GDF11

ERFE

RBC precursor → Polychromatic erythroblast → Orthochromatic erythroblast → Reticulocyte → RBC

↓ Hepcidin

Anemia

↑ Hypoxia

↑ EPO

↑ Apoptosis

↑ EPO
New treatments in thalassemia syndromes: Targeting α/β chain imbalance

- Impaired α:β globin ratio
- Red cell pathology
- Ineffective erythropoiesis
- Hemolysis
- Iron overload
- Anemia
- Tissue oxygenation
- Erythroid marrow expansion
- Hypercoagulable state
- Gall stones

- HSCT
- Gene therapy
- Genome editing

- Diabetes mellitus
- Growth deficiency
- Hypothyroidism
- Hypoparathyroidism
- Hypogonadism
- Hepatic cancer
- Renal disease

- Leg ulcers
- Thrombotic events
- Pulmonary hypertension

- Bone deformities
- Osteoporosis

- Hepatosplenomegaly
- Extramedullary hematopoietic pseudotumors

New treatments in thalassemia syndromes: Targeting ineffective erythropoiesis

- Impaired $\alpha:\beta$ globin ratio
  - JAK2 inhibitors
    - Stotatercept
    - Luspatcept
- Red cell pathology
- Ineffective erythropoiesis
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  - Thrombotic events
  - Pulmonary hypertension
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  - Osteoporosis
- Hepatosplenomegaly
  - Extramedullary hematopoietic pseudotumors

Epo-EpoR-Jak2-STAT5 axis controls red cell production

Progenitor erythroid cells → Normal erythropoiesis → Red cell

Ineffective erythropoiesis in β-thalassemia: chronic stress erythropoiesis + apoptosis limit RBC production

JAK2 inhibitor

Ruxolitinib: a potent and selective oral JAK1 and JAK2 inhibitor
Phase 2: ruxolitinib in thalassemia

- **Primary end-point**
  - percent change in RBC transfusion requirement between weeks 6 and 30 compared with baseline

- **Secondary end-points**
  - change of spleen volume from baseline measured by MRI or CT
  - change of pre-transfusion Hb level from baseline at each post-baseline visit
  - pharmacokinetics
  - safety

CT, computerized tomography; MRI, magnetic resonance imaging.

NCT02049450.
Targeting ineffective erythropoiesis

- Impaired α:β globin ratio
  - JAK2 inhibitors
    - Stotatercept
    - Luspatercept

- Red cell pathology
  - Ineffective erythropoiesis

- Iron overload

- Hemolysis
  - Anemia

- Hypercoagulable state
  - Gall stones

- Tissue oxygenation
  - Erythroid marrow expansion

- Hypercoagulable state

- Diabetes mellitus
- Growth deficiency
- Hypothyroidism
- Hypoparathyroidism
- Hypogonadism
- Hepatic cancer
- Renal disease

- Leg ulcers
- Thrombotic events
- Pulmonary hypertension

- Bone deformities
- Osteoporosis

- Hepatosplenomegaly
- Extramedullary hematopoietic psuedotumors

ACE-011 and ACE-536: selective human ActRII receptor ligand TRAP

ACE-011
sotatercept

ActRIIA receptor
(inhibits activin A, B, GDF11)

Fc domain of human IgG\textsubscript{1} antibody

ACE-536
luspatercept

ActRIIB receptor
(only inhibition of GDF11)

Fc domain of human IgG\textsubscript{1} antibody

✓ ACE-011 (SOTATERCEPT) AND ACE-536 (LUSPATERCEPT) ARE HUMAN FUSION RECOMBINANT PROTEINS

✓ THEY ACT AS “LIGAND TRAPS” AGAINST TGF-β SUPERFAMILY MEMBER GDF11

✓ MURINE MODELS OF MDS AND β-THALASSEMIA SHOWED AN AMELIORATION OF HEMATOLOGIC PARAMETERS (RAP-011 and RAP-536)
Where Does Sotatercept Impact Erythropoiesis?

EPO Dependent Hemoglobin

How much EPO is required?

SOTATERCEPT
GDF11 INVOLVEMENT IN INEFFECTIVE ERYTHROPOIESIS

- DEFECTIVE ERYTHROID DIFFERENTIATION IN β-THALASSEMIA RESULTS IN AN ACCUMULATION OF GDF11-EXPRESSING ERYTHROID PROGENITORS

- GDF11 MANTAINS THE SURVIVAL OF THESE PROGENITORS AND INHIBITS FURTHER DIFFERENTIATION, AGGRAVATING THE INEFFECTIVE ERYTHROPOIESIS

Robert F Paulson et al. Nature Medicine, 2014
Phase 2 preliminary results: luspatercept* improves anemia in NTDT and TDT

Luspatercept increased Hb levels in NTDT patients, decreased transfusion requirement in TDT patients, and has a favorable safety profile

* s.c. injection once every 3 weeks for up to 5 doses with a 2-month follow-up.
24 patients: 20 NTDT; 4 TDT.

EVALUATION OF CYTOTOXICITY AFTER RAP-011 TREATMENT IN K562 sh-SEC23B CELLS

- CYTOTOXICITY ASSAY BY MTT
- TWO DRUG CONCENTRATIONS (50, 100 μM)
- TREATMENT WITH RAP-011 50 μM LEADS TO INCREASED SURVIVAL OF BOTH K562 sh-CTR and sh-SEC23B cells
Iron Overload in IEs: Case-control study

Table S1. Case-control study

<table>
<thead>
<tr>
<th></th>
<th>CDAI cases (n = 37)</th>
<th>BT-intermedia cases (n = 21)</th>
<th>HS cases (n = 13)</th>
<th>Control subjects (n = 29)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>19.1 ± 0.3 (13.0; 36)</td>
<td>42.1 ± 2.9 (37.0; 21)</td>
<td>21.3 ± 4.8 (15.0; 13)</td>
<td>22.3 ± 1.9 (20.0; 29)</td>
<td>0.39</td>
<td>&lt;0.0001</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Gender</strong> (Female/Male)</td>
<td>18 (48.6)/19 (51.4)</td>
<td>10 (47.6)/11 (52.4)</td>
<td>5 (38.5)/8 (61.5)</td>
<td>14 (48.3)/15 (51.7)</td>
<td>0.97</td>
<td>0.94</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>Complete blood count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC (10⁶/μL)</td>
<td>3.4 ± 0.1 (3.4; 37)</td>
<td>4.5 ± 0.2 (4.4; 21)</td>
<td>4.2 ± 0.1 (4.2; 13)</td>
<td>4.9 ± 0.1 (4.9; 27)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>10.0 ± 0.3 (10.0; 37)</td>
<td>9.3 ± 0.2 (9.2; 21)</td>
<td>10.8 ± 0.2 (9.9; 13)</td>
<td>14.3 ± 0.2 (14.2; 27)</td>
<td>&lt;0.0001</td>
<td>0.19</td>
<td>0.23</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>29.8 ± 1.0 (29.6; 37)</td>
<td>32.3 ± 0.8 (33.5; 21)</td>
<td>35.2 ± 1.7 (36.9; 12)</td>
<td>43.3 ± 0.6 (42.0; 27)</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.008</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>88.0 ± 1.4 (87.1; 37)</td>
<td>75.8 ± 3.2 (75.0; 21)</td>
<td>82.3 ± 2.4 (85.3; 13)</td>
<td>87.6 ± 0.9 (87.0; 27)</td>
<td>0.81</td>
<td>0.0002</td>
<td>0.04</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>29.8 ± 0.6 (28.8; 35)</td>
<td>22.2 ± 1.2 (22.5; 21)</td>
<td>28.9 ± 0.6 (29.6; 12)</td>
<td>29.0 ± 0.3 (29.4; 27)</td>
<td>0.31</td>
<td>&lt;0.0001</td>
<td>0.44</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.6 ± 0.3 (33.5; 35)</td>
<td>29.0 ± 0.5 (29.5; 21)</td>
<td>35.0 ± 0.7 (34.9; 12)</td>
<td>33.2 ± 0.2 (33.1; 27)</td>
<td>0.26</td>
<td>&lt;0.0001</td>
<td>0.03</td>
</tr>
<tr>
<td>PLT (10⁹/μL)</td>
<td>417.4 ± 40.9 (357.0; 33)</td>
<td>514.5 ± 70.0 (605.0; 21)</td>
<td>234.5 ± 24.7 (227.0; 13)</td>
<td>245.9 ± 10.6 (246.0; 27)</td>
<td>0.0005</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>21.0 ± 1.3 (20.7; 27)</td>
<td>-</td>
<td>18.8 ± 1.2 (19.1; 11)</td>
<td>12.4 ± 0.2 (12.5; 27)</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>0.32</td>
</tr>
<tr>
<td>Retics abs cont (x10³/μL)</td>
<td>76.3 ± 9.3 (60.0; 36)</td>
<td>44.0 ± 6.7 (51.7; 17)</td>
<td>278.6 ± 57.3 (244.0; 11)</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMRI</td>
<td>54.1 ± 6.5 (47.2; 36)</td>
<td>29.1 ± 4.5 (32.2; 17)</td>
<td>208.7 ± 41.6 (196.1; 11)</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Laboratory data**

| Total bilirubin (mg/dL) | 3.0 ± 0.5 (2.3; 35) | 2.4 ± 0.2 (2.4; 21) | 4.3 ± 1.4 (2.1; 13) | 0.5 ± 0.1 (0.5; 12) | <0.0001 | 0.29       | 0.27       |
| Unconjugated bilirubin (mg/dL) | 2.6 ± 0.5 (1.8; 29) | 1.8 ± 0.2 (1.7; 21) | 4.0 ± 1.4 (1.6; 12) | -                     | -     | 0.24       | 0.21       |
| Serum iron (µg/dL)      | 159.8 ± 11.5 (163.0; 31) | 151.5 ± 13.4 (151.0; 21) | 74.7 ± 8.4 (73.0; 11) | 71.2 ± 7.9 (59.0; 17) | <0.0001 | 0.64       | 0.0001     |
| Ferritin (ng/mL)        | 275.9 ± 61.0 (148.5; 358) | 398.1 ± 67.1 (338.0; 21) | 262.0 ± 86.6 (161.0; 10) | -                     | -     | 0.20       | 0.91       |
| Ferritin level/dosage age⁵ | 29.6 ± 9.7 (13.9; 35) | 10.2 ± 2.0 (7.3; 20) | 12.9 ± 3.0 (11.6; 10) | -                     | -     | 0.14       | 0.37       |
| Transferrin saturation (%) | 71.4 ± 5.5 (70.4; 25) | 62.9 ± 5.5 (65.0; 21) | 26.0 ± 4.3 (23.6; 9) | -                     | -     | 0.28       | <0.0001    |
| sTfR (mg/L)             | 4.1 ± 0.4 (4.0; 17)  | 9.4 ± 0.8 (9.5; 20)        | -                   | -                     | <0.0001 | -          | -          |

Data are not available for all subjects. For quantitative variables data are presented as average ± SEM (median; n). For qualitative variables data are presented as n (%)/n (%). Student t test for quantitative unpaired data; chi square test for categorical data. P¹ CDAI cases vs control subjects; P² CDAI vs BT-intermedia cases; P³ CDAI vs HS. ⁵ Normalization of ferritin by means of “Ferritin level/dosage age ratio”, as described by Iolascon et al, Haematologica 2010, 95(5)
Increased levels of ERFE-encoding FAM132B in patients with Congenital Dyserythropoietic Anemia type II

- CDAII patients exhibit over-expression of ERFE at both gene and protein level
- When we analyzed ERFE expression in β-thalassemia (BT)-intermedia patients, exhibiting iron overload likewise for CDAII patients, we observed similar results compared to CDAII. Conversely, only a slight increase of ERFE expression was observed in patients with mild well-compensated anemia, such as hereditary spherocytosis (HS)

These data suggested that the marked increased ERFE expression observed in both CDAII and BT-intermedia patients is mainly due to the ineffective erythropoiesis
Correlation analysis

High-FAM132B patients:
- Reduced Hb
- Reduced Ht
- Increased EPO
- Increased sTfR
- Reduced Hepcidin
- Reduced Hepcidin/ferritin
- Increased Transferrin saturation

However ...

The iron balance data do not differ significantly between the two CDA II sub-groups

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Table 1. FAM132B expression and clinical correlations in CDAII patients

<table>
<thead>
<tr>
<th></th>
<th>Low FAM132B (n = 20)</th>
<th>High FAM132B (n = 17)</th>
<th>p^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.3 ± 4.9 (16.0; 19)</td>
<td>12.1 ± 2.5 (10.0; 17)</td>
<td>0.03</td>
</tr>
<tr>
<td>Onset symptoms (years)</td>
<td>7.5 ± 2.5 (5.0; 16)</td>
<td>3.2 ± 1.3 (1.3; 16)</td>
<td>0.14</td>
</tr>
<tr>
<td>Gender (Female/Male)</td>
<td>9 (45.0)/11 (55.0)</td>
<td>9 (52.9)/8 (47.1)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

**Complete blood count**

<table>
<thead>
<tr>
<th></th>
<th>Low FAM132B (n = 20)</th>
<th>High FAM132B (n = 17)</th>
<th>p^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^6/μL)</td>
<td>3.6 ± 0.2 (3.5; 20)</td>
<td>3.2 ± 0.1 (3.3; 17)</td>
<td>0.05</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>10.7 ± 0.5 (10.4; 20)</td>
<td>9.2 ± 0.4 (9.5; 17)</td>
<td>0.02</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>31.7 ± 1.4 (30.6; 20)</td>
<td>27.5 ± 1.2 (28.0; 17)</td>
<td>0.03</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>89.7 ± 1.8 (90.2; 20)</td>
<td>86.0 ± 2.2 (84.7; 17)</td>
<td>0.20</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>30.6 ± 0.7 (31.0; 18)</td>
<td>28.9 ± 0.9 (27.9; 17)</td>
<td>0.12</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.8 ± 0.4 (33.5; 19)</td>
<td>33.3 ± 0.3 (33.1; 16)</td>
<td>0.32</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>19.9 ± 2.5 (18.9; 12)</td>
<td>21.8 ± 1.2 (22.0; 15)</td>
<td>0.48</td>
</tr>
<tr>
<td>PLT (10^3/μL)</td>
<td>373.0 ± 41.1 (290.0; 17)</td>
<td>459.2 ± 69.2 (390.0; 17)</td>
<td>0.30</td>
</tr>
<tr>
<td>Retics abs count (10^3/μL)</td>
<td>67.4 ± 9.2 (59.2; 20)</td>
<td>87.3 ± 17.5 (79.7; 16)</td>
<td>0.30</td>
</tr>
<tr>
<td>Retics (%)</td>
<td>2.0 ± 0.3 (1.5; 20)</td>
<td>2.7 ± 0.6 (2.2; 16)</td>
<td>0.25</td>
</tr>
<tr>
<td>Reticulocyte Index</td>
<td>1.3 ± 0.2 (1.2; 20)</td>
<td>1.7 ± 0.3 (1.5; 16)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**Iron balance**

<table>
<thead>
<tr>
<th></th>
<th>Low FAM132B (n = 20)</th>
<th>High FAM132B (n = 17)</th>
<th>p^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin/ferritin</td>
<td>0.04 ± 0.01 (0.02; 16)</td>
<td>0.01 ± 0.003 (0.006; 16)</td>
<td>0.01</td>
</tr>
<tr>
<td>Haptoglobin (nM)</td>
<td>5.8 ± 1.9 (2.7; 17)</td>
<td>1.0 ± 0.3 (0.6; 16)</td>
<td>0.02</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>372.1 ± 107.7 (200.0; 19)</td>
<td>168.5 ± 36.0 (99.8; 17)</td>
<td>0.10</td>
</tr>
<tr>
<td>Ferritin level/dosage age</td>
<td>32.9 ± 17.2 (14.9; 18)</td>
<td>26.1 ± 8.6 (12.7; 17)</td>
<td>0.73</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>67.7 ± 6.8 (62.5; 19)</td>
<td>81.8 ± 7.8 (86.0; 8)</td>
<td>0.23</td>
</tr>
<tr>
<td>Serum iron (μg/dL)</td>
<td>157.8 ± 13.6 (159.5; 18)</td>
<td>162.7 ± 20.4 (172.0; 13)</td>
<td>0.84</td>
</tr>
<tr>
<td>sTfR (mg/L)</td>
<td>3.7 ± 0.4 (3.7; 12)</td>
<td>5.1 ± 0.5 (5.7; 8)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Laboratory data and transfusion regimen**

<table>
<thead>
<tr>
<th></th>
<th>Low FAM132B (n = 20)</th>
<th>High FAM132B (n = 17)</th>
<th>p^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPO (mIU/mL)</td>
<td>82.5 ± 19.1 (61.9; 14)</td>
<td>154.3 ± 14.5 (170.1; 13)</td>
<td>0.01</td>
</tr>
<tr>
<td>GDF15 (pg/mL)</td>
<td>814.9 ± 251.1 (503.5; 13)</td>
<td>781.9 ± 140.6 (304.0; 9)</td>
<td>0.92</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>3.7 ± 0.8 (2.5; 19)</td>
<td>2.3 ± 0.3 (2.1; 16)</td>
<td>0.15</td>
</tr>
<tr>
<td>Unconjugated bilirubin (mg/dL)</td>
<td>3.1 ± 0.8 (2.2; 17)</td>
<td>1.9 ± 0.3 (1.5; 12)</td>
<td>0.22</td>
</tr>
<tr>
<td>Transfusion need (Yes/No)</td>
<td>7 (46.7)/8 (53.3)</td>
<td>10 (58.8)/7 (41.2)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Data are not available for all patients. For quantitative variables data are presented as average ± SE (median; n). For qualitative variables data are presented as n (%)/n (%)^2 Student t test for quantitative unpaired data; chi square test for categorical data
^3 Normalization of ferritin by means of “Ferritin level/dosage age ratio”, as described by Iolascon et al., Haematologica 2010; 95(6)

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

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
Table 1. Major reported clinical studies that have attempted to improve the outcome of patients with class 3 thalassemia major.

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>Median Age (yrs) / (range)</th>
<th>Proportion in Class 3 (%)</th>
<th>Proportion in Class 3HR (%)</th>
<th>Major defining feature of change in protocol</th>
<th>Treatment related mortality (%)</th>
<th>Graft rejection (%)</th>
<th>EFS (%)</th>
<th>OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>115</td>
<td>11 (3-16)</td>
<td>100</td>
<td>NA</td>
<td>Bu / Cy based regimen with reduction in Cy total dose from 200 mg/kg to 160 mg/kg</td>
<td>24</td>
<td>35</td>
<td>49</td>
<td>74</td>
</tr>
<tr>
<td>2004</td>
<td>33</td>
<td>11 (5-16)</td>
<td>100</td>
<td>NA</td>
<td>Reduction in Cy dose to ≤160 mg/kg with addition of Flu. Additional therapy from day -45 with immunosuppression with Azt and suppression of erythropoiesis with HU</td>
<td>6</td>
<td>6</td>
<td>85</td>
<td>93</td>
</tr>
<tr>
<td>2010</td>
<td>71</td>
<td>9 (1.6-27)</td>
<td>57.3</td>
<td>NA</td>
<td>Intravenous Bu, dose adjustments with therapeutic drug monitoring</td>
<td>7</td>
<td>5</td>
<td>87</td>
<td>91</td>
</tr>
<tr>
<td>2010</td>
<td>53</td>
<td>8 (1-17)</td>
<td>47</td>
<td>NA</td>
<td>Intravenous Bu, dose adjustments with therapeutic drug monitoring</td>
<td>4</td>
<td>15</td>
<td>79</td>
<td>96</td>
</tr>
<tr>
<td>2010</td>
<td>25</td>
<td>NA</td>
<td>100</td>
<td>NA</td>
<td>Intravenous Bu, dose adjustments with therapeutic drug monitoring</td>
<td>4</td>
<td>34</td>
<td>66</td>
<td>96</td>
</tr>
<tr>
<td>2012</td>
<td>60</td>
<td>7 (1-37)</td>
<td>27^</td>
<td>NA</td>
<td>Treo based conditioning regimen</td>
<td>7</td>
<td>9</td>
<td>84</td>
<td>93</td>
</tr>
<tr>
<td>2012</td>
<td>82</td>
<td>6 (0.5-15)</td>
<td>NA</td>
<td>NA</td>
<td>Conditioning with age adjusted PK based IV Bu, Cy (110mg/kg), high-dose Flu (200mg/kg), Thio. Additional therapy from day -45 with immunosuppression with Azt and suppression of erythropoiesis with HU</td>
<td>8.5^</td>
<td>4^</td>
<td>88^</td>
<td>91^</td>
</tr>
<tr>
<td>2013</td>
<td>28</td>
<td>9.6 (2-18)</td>
<td>75</td>
<td>39</td>
<td>Treo based conditioning regimen</td>
<td>21</td>
<td>7</td>
<td>71</td>
<td>79</td>
</tr>
<tr>
<td>2013</td>
<td>18</td>
<td>14 (10-18)</td>
<td>100</td>
<td>NA</td>
<td>Conditioning regimen of Flu &amp; IV Bu Pre-conditioning immunosuppression therapy with Flu and Dexe for 1-2 months.</td>
<td>5</td>
<td>0</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>2013</td>
<td>50</td>
<td>11 (2-21)</td>
<td>100</td>
<td>48</td>
<td>Treo based conditioning regimen with PBSC graft in 74%</td>
<td>12</td>
<td>8</td>
<td>79</td>
<td>87</td>
</tr>
<tr>
<td>2013</td>
<td>24</td>
<td>12 (3-21)</td>
<td>100</td>
<td>100</td>
<td>Treo based conditioning regimen with PBSC graft in 74%</td>
<td>13</td>
<td>8</td>
<td>78</td>
<td>87</td>
</tr>
<tr>
<td>2016</td>
<td>37</td>
<td>10 (5-17)</td>
<td>100</td>
<td>NA</td>
<td>As in Sodani et al. but with higher dose of Flu (150 mg/kg) and addition of Thio(10 mg/kg)</td>
<td>8</td>
<td>0</td>
<td>92</td>
<td>92</td>
</tr>
</tbody>
</table>

*Adapted from Mathews et al.®; Only patients <17 years included in this table; Subset of high-risk cases from same paper; V includes all adult cases as well (assumed to be Class 3); ▲ includes low-risk patients also; ▲ subset of high-risk cases from same paper. Cy: Cyclophosphamide; Flu: Fludarabine; Dexe: Dexamethasone; Bu: Busulfan; Treo: Treosulfan; Azt: Azathioprine; HU: Hydroxyurea; Thio: Thiopeta. HR: high-risk; EFS: event-free survival; OS: overall survival; NA: not applicable; PK: pharmacokinetics; PBSC: peripheral blood stem cell.
Figure 1. Evolution of chimerism after hematopoietic stem cell transplantation (HSCT). Early mixed chimerism is associated with higher risk of rejection while late chimerism often persists with a stable graft. RHCS: residual host cells. MC: mixed chimerism.
Patients with major hemoglobin disorder

Gene corrected HSCs → Autologous HSC transplantation → Autologous HSC transplantation → Gene corrected HSCs

HSCs collected from BM or PB

Purified HSCs (if needed)

Gene editing to modify BCLL11A gene expression, correct β globin mutation or replace the gene

Viral vectors

Viral vector based introduction of a normal β/γ globin gene or modification of BCLL11A gene expression

Figure 3. Overview of current approaches to gene therapy for the major hemoglobin disorders. Gene modifications may be through viral vectors or genome editing technologies to achieve the desired therapeutic effect. HSC: Hematopoietic stem cell; BM: Bone marrow; PB: Peripheral blood; ZFN: zinc finger nucleases; TALEN: transcription activator-like effectors with FokI nuclease; CRISPR: clustered regularly interspaced short palindromic repeats.
Figure 2. Strategies for gene therapy for SCD: schematic overview of various approaches for correcting the sickle phenotype via gene therapy. Gene correction: targeted genome engineering leads to correction of the sickle mutation such that \( \beta^s \) is repaired as \( \beta^a \). HbF induction: multiple strategies for induction of \( \gamma \)-globin expression include shRNA-mediated knockdown of BCL11A, targeted disruption of the +58 DNase I HS site in the BCL11A erythroid-specific enhancer, and forced chromatin looping to promote association of the \( \beta \)-globin LCR with the \( \gamma \)-globin genes. Gene addition: integrating lentiviral vector carrying a \( \beta \)-globin, \( \gamma \)-globin, or antisickling \( \beta \)-globin cassette. Ldb1, transcription factor; ZF/SA, zinc-finger self-association domain.
Genetic Era: From DNA structure to Complete Sequence

1953 - Discovery of double helix (Watson and Crick)

1975 - Southern blotting

1978 - First Molecular Diagnosis

1985 - Description of PCR technology

1990 - Start of Human Genome Project

1990 - First experiment of Gene Therapy: ADA deficiency correction

2000 - First announcement of decodification of entire human genome
RedPlex: a targeted next generation sequencing-based diagnosis for patients with hereditary hemolytic anemias

- **RedPlex**
  - **43** HHA patients from 34 unrelated families
  - Panel of **34 causative/candidate genes** of HAMDs and CDAs
  - **ROIs**: coding regions, UTRs, regulatory regions, 100 bp flanking splice junctions
  - HaloPlex target enrichment; Illumina NextSeq 500; SureCall software

- **HS/HE/HPP**: 11; 26%
- **HST**: 11; 26%
- **CDAs**: 14; 32%
- **Undefined phenotype**: 3; 7%
- **Overlapping phenotype**: 4; 9%

- ✔ Target regions (ROIs): 538
- ✔ Total Amplicons: 8874
- ✔ Total Target Bases Analyzable: 239.59 kbp
- ✔ Target Coverage: 99.9 %
RedPlex: a targeted next generation sequencing-based diagnosis for patients with hereditary hemolytic anemias

RedPlex data study on 29 HHA patients with conclusive diagnosis

- Dark gray squares highlight prioritized variants
- Light gray squares indicate clinically related or modifier variants.
Polygenic contribute in monogenic disease

Hereditary Spherocytosis
- SPTA1 mutations
- αLELY variant

Dyserythropoietic phenotype
- SEC23B mutation
- GATA1 modifier variant

SPTA1 LOCUS
WT
- c.5029G>A, p.Gly1677Arg
Alpha Laly
- c.2319C>A, p.Cys773Ter

SEC23B LOCUS
WT
- c.1254 T>G, p.Ile418Met

GATA1 LOCUS
WT
- g.4909G>A

GATA1

Fold change on HCs

HC  g.4909 G>A
SEC23B  86.5 KDa
GATA 1  42.7 KDa
GAPDH  37 KDa

ECULIZUMAB AND PNH: EFFECTS ON SURVIVAL

Long term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival

Richard J Kelly, Anita Hill, Louise M Arnold, Gemma L Brooksbank, Stephen J Richards, Matthew Cullen, Lindsay D Mitchell, Dena R Cohen, Walter M Gregory and Peter Hillmen

Untreated vs Ecu-treated PNH

Treated PNH vs normal population

Hill et al, Blood 2011
Whole exome sequencing and targeted deep sequencing in 60 PNH patients

- Additional somatic mutations in 10 out of 12 patients studied by WES
- In total, 21 mutations in 21 genes (including some also found in MDS, such as TET2, ASXL1U2AF1, SUZ12, BCOR)
- Clonal architecture re-created by single-colony sequencing
Deep sequencing reveals stepwise mutation acquisition in paroxysmal nocturnal hemoglobinuria

Wenyi Shen,1,2 Michael J. Clemente,1 Naoko Hosono,1 Kenichi Yoshida,2 Bartlomiej Przychodzen,1 Tetsuichi Yoshizato,3 Yuichi Shiraishi,4 Satoru Miyano,4,5 Seishi Ogawa,3 Jaroslaw P. Maciejewski,1 and Hideki Makishima1

Is PNH a cancer???
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Medical Genetics Unit