Improving Outcomes in Sickle Cell Disease: From Targeting Adhesion and Inflammation to Gene Therapy

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Overview

• Background on sickle cell disease
• Management of complications of sickle cell disease
  – Hydroxyurea in pulmonary complications
  – Targeting adhesion and inflammation in vaso-occlusive crisis (VOC)
• Gene therapy/editing
Sickle Cell Disease

Shankaran VG et al, Nature Medicine 2015
Sickle Cell Disease

DNA Point mutation

Deoxy conditions

Peripheral smear
Pathophysiology and Clinical Manifestations

- Activation of vascular endothelium
- Increased adhesiveness of leukocytes, erythrocytes, platelets, and plasma proteins
- Formation of multicellular aggregates
- Oxidant production leads to a pro-inflammatory state

- Vaso-occlusive crisis
- Acute chest syndrome
- Stroke
- Pulmonary hypertension
- Chronic Renal dysfunction

Prognosis in Sickle Cell Disease

Risk factors for early death included history of ACS, renal failure, seizures, and increased WBC

Hydroxyurea for Sickle Cell Disease

- Double-blind RCT - 299 patients
- Median time to first (3m vs. 1.5m) and second (8.8m vs. 4.6m) vaso-occlusive crisis were statistically significantly decreased in patients who received hydroxyurea
- FDA approval in 1998 for SCD

Causes of Death in Sickle Cell Disease

Table 2. Cause of Death According to Original Treatment Assignment in Patients in the MSH

<table>
<thead>
<tr>
<th>Causes of Death*</th>
<th>Hydroxyurea, No. (%)</th>
<th>Placebo, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 152)</td>
<td>(n = 147)</td>
</tr>
<tr>
<td>Total deaths</td>
<td>36 (23.7)</td>
<td>39 (26.5)</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>7 (4.6)</td>
<td>14 (9.5)</td>
</tr>
<tr>
<td>Death during crisis</td>
<td>4 (2.6)</td>
<td>5 (3.4)</td>
</tr>
<tr>
<td>Sepsis/infection</td>
<td>2 (1.3)</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>1 (0.7)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>6 (3.9)</td>
<td>0</td>
</tr>
<tr>
<td>Hepatic disease</td>
<td>2 (1.3)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>2 (1.3)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>1 (0.7)</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td>Unintentional injury/homicide</td>
<td>3 (2.0)</td>
<td>4 (2.7)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>4 (2.6)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Not yet classified</td>
<td>4 (2.6)</td>
<td>4 (2.7)</td>
</tr>
</tbody>
</table>

Abbreviation: MSH, Multicenter Study of Hydroxyurea in Sickle Cell Anemia.
*The single death from malignancy in the MSH Patients’ Follow-up occurred after data files were closed for this analysis and is not included in this table.
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Mechanisms of Action for Hydroxyurea

1. Hydroxyurea enters the bone marrow and affects erythroid progenitor cells.
2. Erythroid progenitor cells differentiate into mature erythrocytes.
3. Hydroxyurea inhibits the production of neutrophils.
4. Hydroxyurea stimulates the production of nitric oxide in endothelial cells.
5. Nitric oxide acts as a vasodilator, reducing blood vessel tone.

Ware RE, Blood 2010
Pulmonary Complications in SCD

- Acute chest syndrome (ACS): development of a new pulmonary infiltrate on CXR accompanied by fever and/or respiratory symptoms
- 16.4% in hydroxyurea group developed ACS vs. 34.7% patients in the placebo group, P < 0.001
- Long-term follow-up of MSH study showed patients with ACS (during the trial) had increased mortality (32% vs. 18%)

Steinberg MH, JAMA 2003
Pulmonary Hypertension

• **PH**: mean pulmonary artery systolic pressure > 25mmHg at rest on right heart catheterization

• **TTE** with calculation of the tricuspid regurgitant jet velocity (TRV) is often used as the initial imaging study to evaluate for pulmonary hypertension

• **Right heart catheterization is necessary** to confirm diagnosis, identify mechanism, and perform vasoreactivity testing to guide therapy

Nef et al. Heart 2010
Pathophysiology of PH in SCD: Hemolysis associated?

- Impaired vasodilation
- Increased vaso-constriction
- Hyperplasia/Proliferation
- Increased platelet activation
- Increased adhesion molecules
- Uncoupled NOS

Pulmonary Hypertension in SCD

- Previous studies had suggested a prevalence of PH of 30% based on echocardiographic findings of an elevated TRV\(^1\)
- Prevalence based on right heart catheterization was 6%
- Positive Predictive Value 25%
- 13 patients had post-capillary PH and 11 had pre-capillary PH, suggesting that PH in the sickle disease population is multifactorial
- No difference in baseline hydroxyurea use between groups (\(P = 0.31\))

\(^1\)Gladwin et al. N Engl J Med 2004
Does an elevated TRV represent a preclinical phase of pulmonary hypertension?

Pulmonary Complications in SCD

Follow-up of MSH trial (17.5 yrs) showed that 87% of the deaths associated with pulmonary complications occurred in patients treated with hydroxyurea for < 5 years.

Steinberg MH et al. Am J Hematol 2010
Underutilization of HU in Community Practice

<table>
<thead>
<tr>
<th>Percent of SCD patients</th>
<th>No. of physicians</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 10%</td>
<td>75</td>
<td>45</td>
</tr>
<tr>
<td>10–30%</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>31–60%</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>61–90%</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Over 90%</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>166</td>
<td>100</td>
</tr>
</tbody>
</table>

Zumberg MS et al. Am J Hematol 2005

<table>
<thead>
<tr>
<th>Reason for not prescribing HU</th>
<th>“Not Important”</th>
<th>“Important”</th>
<th>“Very Important”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compliance</td>
<td>9</td>
<td>28</td>
<td>62</td>
</tr>
<tr>
<td>Contraception, pregnancy issues</td>
<td>20</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>Patients’ anticipation of side effects</td>
<td>28</td>
<td>56</td>
<td>16</td>
</tr>
<tr>
<td>Patient’s age</td>
<td>49</td>
<td>36</td>
<td>14</td>
</tr>
<tr>
<td>Cost</td>
<td>41</td>
<td>45</td>
<td>14</td>
</tr>
<tr>
<td>Concern about carcinogenic potential</td>
<td>60</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td>Doubt effectiveness</td>
<td>59</td>
<td>36</td>
<td>4</td>
</tr>
</tbody>
</table>
Pulmonary Effects of VOC

Hydroxyurea in Sickle Cell Disease

Summary

• Decreases overall mortality
• Decreases number of acute chest syndrome events
• Decreases frequency of vaso-occlusive episodes
• May prevent or improve outcomes in pulmonary hypertension
• Underutilized and sub-optimally dosed in clinical practice
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Leukocyte Adhesion in VOC

- Intravital microscopy (IVM) was used to monitor the cremasteric microcirculation of leukocytes and RBCs and their interactions with the endothelial cell wall
- IVM1 = monitoring for 75 minutes after isolation of tissue
- IVM2 = exposed to TNF-α for 90 minutes, then monitored for an additional 90 minutes
Leukocyte Adhesion in VOC

- P- and E-selectin KO mice had fewer rolling and adherent leukocytes at both time points
- Mean number of SS RBC interactions per adherent leukocyte were also significantly lower in the selectin KO mice (P =0.002)
- Percentage of adherent leukocytes that interacted with SS RBCs were also significantly different (4% vs. 18%, P=0.002)
Targeting Adhesion in VOC

- In preclinical studies, a pan-selectin inhibitor, GMI-1070 (rivipansel) blocked predominately E-selectin mediated adhesion and blocked RBC-leukocyte interactions with improved blood flow and survival\(^1\)

- Phase II – 76 patients enrolled, primary endpoint of time to resolution of VOC (1.5 point decrease in pain and transition to po analgesics, documenting that pt is ready for discharge, or written discharge order)

- SelG1, mAb against P-selectin, being evaluated in prevention of VOC (NCT01895361)

\(^1\)Chang J, et al. Blood 2010
Inflammation in VOC

• Ischemia-reperfusion injury secondary to ongoing, intermittent microvascular occlusions promotes a chronic pro-inflammatory state

• High-dose methylprednisolone has been shown to decrease duration of severe pain and hospitalization in children and adolescents, but high readmission rates\(^1\)

• Unfortunately, a subsequent study evaluating tapering oral dexamethasone in patients with ACS closed because of poor accrual (11 patients)\(^2\)

\(^1\)Griffin TC et al. N Engl J Med 1994
\(^2\)Quinn CT et al. Br J Haematol 2011
iNKT cells induce inflammation in SCD

- Invariant NKT (iNKT) cells have a restricted T-cell receptor that is activated by CD1d found on APCs leading to cytokine release.
- NY1DD mice have increased IFN-γ inducible chemokines (CXCL9 and CXCL10) and elevated numbers of lymphocytes expressing CXCR3 receptors.
- Treatment with anti-CD1d Ab reversed inflammation-induced pulmonary dysfunction and decreased pulmonary levels of IFN-γ and CXCR3.

Wallace KL et al. Blood 2009
iNKT cells induce inflammation in SCD

When crossed with lymphocyte-deficient mice, there is decreased pulmonary dysfunction which is counteracted by adoptive transfer of 1 million NKT cells

Wallace KL et al. Blood 2009
iNKT inhibition via adenosine A\textsubscript{2A} receptor activation

- Adenosine A\textsubscript{2A} receptor activation inhibits CD1d-dependent iNKT cell activation
- Phase I study of A\textsubscript{2A} R agonist, regadenoson in adults with SCD
  - Stage I: MTD dose level during 12 hour infusion (1.44ug/kg/hr)
  - Stage II: 6 patients treated at steady state for 24 hours at this dose
  - Stage III: 6 patients treated during VOC for 24 hours at this dose

Wallace KL et al. Blood 2010
iNKT inhibition via adenosine $A_{2A}$ receptor activation

iNKT inhibition via adenosine $A_2A$ receptor activation

Targeting Adhesion and Inflammation Summary

• Selectin and iNKT cell targeted treatments shows promise in vaso-occlusive crisis

• Preventing vaso-occlusive crisis may be a superior approach with these interventions

• Both of these targets play a role in immunity and chronic inhibition may predispose patients to infection
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Gene Therapy

• Gene therapy is the modification of the expression of an individual’s gene or correction of an abnormal gene.

• Viral vectors of the Retroviridae family have been primarily used for stable gene transfer to hematopoietic stem cells: γ-retroviral vectors and lentiviral vectors (derived from HIV-1).

• These viruses can be turned into gene delivery vectors by removing the genetic elements needed for pathogenicity and replication and replacing them with the human transgene of interest.
Gene Therapy

Shankaran VG et al, Nature Medicine 2015
# Gene Therapy in Hemoglobinopathies

<table>
<thead>
<tr>
<th></th>
<th>Gamma RV Vectors</th>
<th>LV Vectors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viral genome</strong></td>
<td>Retroviridae</td>
<td>Retroviridae</td>
</tr>
<tr>
<td><strong>Pathogenicity</strong></td>
<td>Derived from pathogenic murine leukemia virus</td>
<td>Derived from pathogenic HIV virus</td>
</tr>
<tr>
<td><strong>Cell division requirement</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Packaging limitation</strong></td>
<td>Up to 5 Kb, unstable with large transgene cassettes</td>
<td>Up to 7 Kb</td>
</tr>
<tr>
<td><strong>Transduction efficiency</strong></td>
<td>High in dividing cells</td>
<td>High in dividing and nondividing cells</td>
</tr>
<tr>
<td><strong>Integration site</strong></td>
<td>Preferentially near transcription start sites (60%–70%)</td>
<td>Preferentially within transcribed genes (60%–70%)</td>
</tr>
<tr>
<td><strong>Safety in clinical trials</strong></td>
<td>Leukemia in X-SCID and WAS trials MDS/monosomy 7 in CGD trial</td>
<td>No overt malignancy seen after 2–6 years Clonal expansion of HMGA2 in one thalassemia patient, that has now decreased</td>
</tr>
<tr>
<td><strong>Experimental genotoxicity data</strong></td>
<td>High risk for insertion oncogenesis</td>
<td>Safer than RV. Safety high with self-inactivating design and use of cellular promoters and enhancers</td>
</tr>
<tr>
<td><strong>Main advantages</strong></td>
<td>Longest human data and experience SIN RV vector design may address safety concerns</td>
<td>Better safety profile than RV vectors Can stably carry larger cargo Efficiently transduce nondividing HSC</td>
</tr>
<tr>
<td><strong>Main disadvantage</strong></td>
<td>Clinically significant risk of insertion oncogenesis Lower capacity Does not transduce non-dividing cells</td>
<td>Might induce dysregulation/disruption of cellular genes, albeit at low frequency</td>
</tr>
</tbody>
</table>

Gene Therapy in Hemoglobinopathies

- Locus control region (LCR) is required for physiologic β-globin gene expression
- Incorporation of small elements spanning HS2, HS3, and HS4 led to low level expression and transcriptional inactivation
- Larger HS2, HS3, and HS4 elements led to improved β-globin transcript levels

May et al., Nature 2000
Gene Therapy in Hemoglobinopathies

May et al., Nature 2000
Gene Therapy in Hemoglobinopathies

- 18yo transfusion-dependent $\beta^E\beta^0$ since early childhood
  - Transfused at least monthly
  - Splenectomy at age 6
  - HU ineffective
  - Iron chelation with parental deferoxamine 5x/week
  - No HLA-matched donor
- Conditioned with 4 days of busulfan (3.2 mg/kg/day) before auto-transplantation of $3.9 \times 10^6$ CD34+ cells/kg with SIN LV-based $\beta^{T87Q}$ vector
- Gene-marked cells ranged from 10-20% in bone marrow and peripheral blood

Cavazzan-Calvo et al., Nature 2010
Gene Therapy in Hemoglobinopathies

- Insertion site analysis showed clonal expansion over time at the HMGA2 locus
- Overexpression of HMGA2 is mostly associated with benign lesions (ie lipomas), however still concern for leukemic transformation

Cavazzan-Calvo et al., Nature 2010
Gene Editing

Zinc finger nucleases (ZFNs)
Transcription activator–like effector nucleases (TALENs)
RNA-guided nuclease - CRISPR/Cas system

Defective β gene locus

DSB

XXXXXX XXXXXX
Repair donor DNA-e.g Plasmid

Repair

Corrected β gene locus

Gene Editing

**CRISPR Cuts**
Clustered regularly interspaced short palindromic repeats (CRISPR) use RNA to guide precise cuts in the genome.

1. A section of RNA is engineered to target a specific region of DNA.

2. The RNA sequence, linked to a Cas9 cleaving enzyme, finds the right spot on the genome, then unzips the double helix and snips the gene.

3. Before the DNA is repaired, small amounts of engineered DNA can be inserted to alter the gene.

**ZFNs**
Zinc finger nucleases (ZFNs) are used in pairs to cut either side of the double-stranded DNA.

1. Zinc fingers (blue) recognize specific DNA sequences — typically three base-pairs per finger — and must match precisely.

2. The fingers bind to matching DNA. The Fok1 enzymes bind to each other and snip out the offending gene.

3. As the cells repair themselves, they incorporate a healthy version of the gene (red).

**TALENs**
Transcription activator-like effector nucleases (TALENs) work like ZFNs. Using one long matching sequence allows gene recognition to be precise.

1. TALENs are also used in pairs, one for each DNA strand. They consist of amino-acid sequences (coloured dots), each of which binds to a single nucleotide.

2. When the long sequences match up on either side, the Fok1 enzymes bind to each other and snip out the unwanted gene.

Gammon A, Nature 2014
Gene Editing

- Efficient targeted cleavage at the β-globin locus with minimal off-target modification
- Co-delivering a homologous donor template led to high levels of gene modification in CD34+ HSPCs

Hoban MD et al, Blood 2015
Gene Editing

ZFN + IDLV modified cells maintained ability to engraft and produce cells from multiple lineages in immune-deficient NSG mice

Hoban MD et al, Blood 2015
ZFN-driven gene correction in CD34+ cells from the bone marrow of patients with SCD resulted in the production of wild-type tetramers.
Gene Therapy/Gene Editing Summary

• Gene therapy presents an alternative to stem cell transplantation to cure patients with hemoglobinopathies

• Gene editing technology can potentially decrease the risk for insertional oncogenesis
Acknowledgments

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