Sickle Cell Disease: A CRISPR way to a Cure

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Disclosures

- GLG, Consultant
- Karius, Scientific Advisory Board
Outline

- Review sickle cell disease and therapies
- Review bone marrow transplant and gene therapy
- Discuss CRISPR technology
- Show preclinical data
- Hurdles to overcome for FDA approval
Sickle cell disease (SCD)

- Most common genetic disorder amongst African Americans
- Caused by a single base pair mutation in the beta-hemoglobin chain

<table>
<thead>
<tr>
<th>Partial DNA Sequence of Beta Globin Gene:</th>
<th>CCT GAG GAG</th>
<th>CCT GTG GAG</th>
<th>CCT AAG GAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial RNA Sequence:</td>
<td>GGA CTC CTC</td>
<td>GGA CAC CTC</td>
<td>GGA TTC CTC</td>
</tr>
<tr>
<td>Partial Amino Acid Sequence for Beta Globin:</td>
<td>Pro-Glu-Glu</td>
<td>Pro-Val-Glu</td>
<td>Pro-Lys-Glu</td>
</tr>
<tr>
<td>Hemoglobin Molecule:</td>
<td>β α</td>
<td>β α</td>
<td>β α</td>
</tr>
</tbody>
</table>

Red Blood Cell:

- Hgb A
- Hgb S
- Hgb C
Pathophysiology in SCD

Endothelial injury
Decrease NO at vessel wall
Activation of adhesion molecules
Sickle cell carriers have some protective advantage from malaria
Epidemiology and statistics

- The average life expectancy: 42 years for males and 48 years for females
- Approximately 1 in 600 African American births results in sickle cell disease
- About 100,000 Americans have sickle cell disease, 2000 new births annually
- Nigeria, 90,000 born with SCD annually
- This is a WORLD WIDE problem
- 1000 children with SCD in Alabama
- Newborn screening 50-60 new cases/yr in Alabama
Initial complications of SCD

- Vasocclusive crises = pain crises
  - Sudden onset, begin early life
  - Bone infarctions
  - Extremities, sternum/rib
  - Dactylitis

- Management
  - Fluids
  - Analgesia
    - Oral vs IV
    - Algorithms
Early complications of SCD

- **Splenic sequestration**
  - Rapid increase in size of spleen from outflow obstruction
  - Anemia, reticulocytosis, thrombocytopenia
  - Hypovolemia
  - More prevalent in HbSC
  - Recurrent
  - Treatment
    - Transfusion
    - Fluids
    - Splenectomy for severe or recurrent events

- **Aplastic crisis**
  - Anemia with lack of RBC production
  - Often caused by parvovirus
  - Anemia without reticulocytosis
  - Treatment
    - Transfusion, slowly
    - Fluids
Acute chest syndrome
- Clinical definition
  - CXR finding
  - Hypoxia/dyspnea
  - Fever
- Cause
  - Fat embolism
  - Infection
  - Infarction
  - Unknown
- Leading cause of death
- Treatment
  - Transfusion
  - Oxygen
  - Fluids
  - Incentive spirometry
  - Empiric antibiotics
Common complications of SCD

- Avascular necrosis
  - Femoral and humeral heads common
  - Limited collateral circulation
  - Up to 30-50% of adults

- Biliary stone formation
  - Chronic hemolysis
  - Frequent cause of abdominal pain and jaundice
  - 30-50% of patients develop by adulthood
  - Treat with cholecystectomy via laparoscopic procedure
Infectious complications of SCD

- Increased risk due to splenic dysfunction
- Encapsulated bacteria susceptibility
- Pneumococcus is leading cause of sepsis in SCD patients
- PCN prophylaxis in newborn till 5 yo
  - Study by Gaston et al. NEJM 1986
    - 13 of 110 positive infections on placebo arm
    - 2 of 105 positive infections on penicillin arm
- Prevnar vaccination (13 valent)

Table 1. Rates of invasive pneumococcal disease (IPD) among individuals with sickle cell disease (SCD).

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>No. of patients with IPD and SCD</th>
<th>Rates of IPD, cases per 100,000 person-years</th>
<th>Comparison of pre- and post-PCV periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-PCV era</td>
<td>Transition year</td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>All</td>
<td>21</td>
<td>2044</td>
</tr>
<tr>
<td></td>
<td>&lt;2 years</td>
<td>16</td>
<td>3630</td>
</tr>
<tr>
<td></td>
<td>≥5 years</td>
<td>16</td>
<td>161</td>
</tr>
</tbody>
</table>

NOTE. The pre-pneumococcal conjugate vaccine (PCV) era was defined as the period 1995–1999, the transition year was 2000, and the post-PCV era was defined as the period 2001–2004.
Neurologic complications of SCD

- **Overt stroke**
  - 5-10% of HbSS patients prior to 18yo
  - Thrombotic or infarctive event of large intracranial arteries
  - Presents with weakness, aphasia, seizures, LOC
  - Permanent neurological damage and long-term disability

- **Silent stroke**
  - Most common neurologic injury
  - 22% of children b/w 6-19 yo
  - No overt clinical features
  - Increased T2 signal abnormalities on MRI without corresponding deficits.
Neurologic complications of SCD

- **Treatment-acute**
  - Exchange transfusion (preferred)
  - If Hb < 10, could start with simple transfusion if time issue
  - Oxygen to keep sat > 95%

- **Long term management**
  - SIT trial (DeBaun NEJM 2014)
    - Randomized SCD 5-15 yo with silent stroke to receive regular blood transfusions or standard care (observation group).
    - Transfusion 1/99 vs stroke Observation 7/96. Concluded transfusion better than observation for secondary prevention
  - STOP trial-
    - For patients with abnormal TCD, transfusion is superior to no therapy
  - TWITCH-
    - Multicenter phase 3 trial showed HU was as good as transfusion to prevent stroke in children with abnormal TCD
Neurologic complications of SCD

- Prevention of strokes
  - Transfusion

- Trans cranial Doppler (TCD)
  - Flow velocity of cerebral artery

Abnormal TCD pts

STOP Trial - Adams NEJM 1998

Pegelow JPeds 1995
Hydroxyurea therapy in SCD

- **Mechanism of action**
  - Increases Hgb F, lowers white blood cell count
  - Makes blood less “viscous” and white cells less sticky

- **BABY HUG Trial**
  - Randomized placebo control trial 2003-9 (~100 pts each group)
  - HgbSS age 1-2, received HU 20 mg/kg/d
  - Primary endpoint improvement in splenic or renal function

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**Table 6: Adverse events**

<table>
<thead>
<tr>
<th>Event</th>
<th>Hydroxyurea (N=50)</th>
<th>Placebo (N=50)</th>
<th>Hazard ratio (95% CI)*</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>157/62</td>
<td>155/75</td>
<td>0.59 (0.42-0.83)</td>
<td>0.002</td>
</tr>
<tr>
<td>Pain alone</td>
<td>63/37</td>
<td>121/55</td>
<td>0.54 (0.36-0.83)</td>
<td>0.004</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>8/7</td>
<td>27/18</td>
<td>0.36 (0.15-0.87)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hospitalization (for any cause)</td>
<td>232/69</td>
<td>324/84</td>
<td>0.73 (0.52-1.00)</td>
<td>0.05</td>
</tr>
<tr>
<td>Transfusion</td>
<td>35/20</td>
<td>63/33</td>
<td>0.55 (0.32-0.95)</td>
<td>0.03</td>
</tr>
<tr>
<td>Dactylitis</td>
<td>24/14</td>
<td>123/42</td>
<td>0.27 (0.15-0.50)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stroke</td>
<td>6/0</td>
<td>1/1</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Priapism</td>
<td>4/3</td>
<td>2/2</td>
<td>1.48 (0.25-8.84)</td>
<td>0.67</td>
</tr>
<tr>
<td>Septic or bacteraemia</td>
<td>3/2</td>
<td>6/5</td>
<td>0.40 (0.08-2.00)</td>
<td>0.20</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>137/37</td>
<td>188/36</td>
<td>0.87 (0.54-1.40)</td>
<td>0.56</td>
</tr>
<tr>
<td>Splenic sequestration</td>
<td>12/8</td>
<td>12/9</td>
<td>0.88 (0.34-2.27)</td>
<td>0.75</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>26/38</td>
<td>70/41</td>
<td>0.35 (0.29-0.60)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Death</td>
<td>0/0</td>
<td>0/0</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

* Hazard ratios and 95% CIs are generated from a Cox model. †p values are generated from the log-rank test comparing the time to first event between the two treatment groups.

Wang et al, Lancet 2011
Experimental therapies in SCD

- **Anti-sickling agents**
  - Voxelotor (GBT440)

- **Selectin inhibitors**
  - Rivipansel (GMI 1070)

- **Hgb F upregulators/inducers**
  - Pomalidomide, HQK-1001

- **NK T cell antibodies**
  - IKTT

- **Leukotriene antagonists**
  - montelukast

- **Anti-coagulants and anti-platelet agents**
  - Heparin like molecules, NAC, aspirin
Curative options in SCD

- Hematopoietic Stem Cell Transplantation
  - Allogeneic
    - Long standing track record
    - Various donors
    - Different conditioning
  - Autologous
    - Experimental
    - Gene correction/gene addition approaches
      - Active clinical trials for lentiviral Hgb b
      - Active clinical trial for knocking down Bcl11a, leading to increases in Hgb F
Allogeneic HSCT

Bone marrow/PBSC harvested from donor

Baby with malignancy or “genetic disease”

Myeloablative chemotherapy or serotherapy

Day -8 to -1

Stem cell infusion

Day 0

Prevention of GvHD and infection, immune system development

Day 1 to 120

Cord blood donor from NMDP registry
Source of hematopoietic stem cells for transplant

Bone Marrow

Peripheral blood stem cells

CD34+

Cord Blood
Allogeneic HSCT

Baby with malignancy or “genetic disease”

Myeloablative chemotherapy or serootherapy

Day -8 to -1

Stem cell infusion

Day 0

Bone marrow/PBSC harvested from donor

Prevention of GvHD and infection, immune system development

Cord blood donor from NMDP registry

Day 1 to 120
Transplant preparative regimens

- **Myeloablative conditioning (MIC)**
  - Bu 16mg/kg + Cytoxan 200 mg/kg
  - Cytoxan 120 mg/kg + 1200 TBI
  - Blood counts drop out completely

- **Reduced intensity conditioning (RIC)**
  - Flu 150 mg/m² + Melphalan 140 mg/m² + CAMPATH
  - Counts drop but less toxicity

- **Non myeloablative conditioning (NIC)**
  - Flu 150 mg/m² + 200 TBI
  - Counts may not drop, goal may only be to achieve partial chimerism
Transplant phases and complications

- **Pre/early engraftment**
  - Hematopoietic recovery
    - Transfusion dependent!!
  - Infections
    - Prophylaxis and treatment strategies
  - Severe mucositis
    - Prevention and treatment strategies (KGF, pain medications)
  - Early tissue repair requires PMNs

- **Post engraftment (1-12 month)**
  - Graft vs host disease
  - Viral reactivation
  - Immune reconstitution

![Graph showing hematopoietic recovery over time]

Days (post BMT) vs. % normal (Hgb, ANC, Plts)
Infection risks based on transplant phase

- **Phase I: Pre-engraftment**
  - Neutropenia, barrier breakdown (mucositis, central venous access devices)
  - Graft-versus-host-disease: Acute

- **Phase II: Post-engraftment**
  - Impaired cellular and humoral immunity: NK cells recover first, CD8 T cell numbers increasing but restricted T cell repertoire
  - Gastrointestinal *Streptococci* species

- **Phase III: Late phase**
  - Impaired cellular and humoral immunity: B cell & CD4 T cell numbers recover slowly and repertoire diversifies
  - Encapsulated bacteria

**Bacterial**
- Gram negative bacilli
- Gram positive organisms
- Gastrointestinal *Streptococci* species

**Viral**
- Herpes Simplex virus
- Respiratory and enteric viruses (Seasonal/intermittent)
- Other viruses eg. HHV
- EBV PTLD
- Varicella Zoster virus

**Fungal**
- *Aspergillus* species
- *Candida* species
- *Pneumocystis*

Day 0 - Day 15-45 - Day 100 - Day 365 and beyond

Mackall et al, BMT, 2009
Transplant specific complication

- **Acute graft vs host disease**
  - Manifest by rash, diarrhea, increase LFTs
  - Occurs within first 100 days

- **Chronic graft vs host disease**
  - Occurs after 100 days
  - Skin-dyspigmentation, leathery
  - Mouth-leukoplakia
  - Eyes-dry, itchy
  - Joints- decreased ROM
  - Liver- elevated Bili
BMT for sickle cell disease

- Donor identification is a problem
  - 15% will have matched-sibling donor
  - 50% will have HLA-matched unrelated donor (MUD)
  - >35% have no suitable MUD
  - 100% have haplo-identical family member!

- Due to toxicities, BMT is reserved for those with severe disease
  - Some debate for those with matched sibling donors!!
Matched sib BMT for SCD

<table>
<thead>
<tr>
<th>Center</th>
<th>Preparative Regimen</th>
<th>GVHD Prophylaxis</th>
<th>n Age Range (yr)</th>
<th>Published Outcomes Follow-Up (yr)</th>
<th>Death (mo)</th>
<th>Complications</th>
<th>Latest Follow-Up IST</th>
<th>GVHD</th>
<th>Donor Chimerism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rome</td>
<td>BU 14 mg/kg, CY 200 mg/kg, rATG 10 mg/kg, ±Flu 150 mg/m²</td>
<td>CsA, MTX, Pred</td>
<td>40 2-17</td>
<td>1-10</td>
<td>3 (2.5, 6, 15)</td>
<td>3 deaths from GVHD</td>
<td>1 of 40</td>
<td>17.5% aGVHD, 5% cGVHD</td>
<td>25-100%</td>
</tr>
<tr>
<td>(Lucarelli, 2014) [15]</td>
<td></td>
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</tr>
<tr>
<td>Brussels</td>
<td>BU 13-18 mg/kg, CY 200 mg/kg, ±rATG (10-20 mg/kg), ±HU</td>
<td>CsA, MTX or MMF (UCB)</td>
<td>50 1.7-15.3 .4-21.3</td>
<td>2 (6, 78)</td>
<td>4, sepsis, 1 IMI, seizures 21%, 6, PRES</td>
<td>1 of 50</td>
<td>20.5% aGVHD, 20% cGVHD</td>
<td>15-100%</td>
<td></td>
</tr>
<tr>
<td>(Dedeken, 2014) [16]</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>New York</td>
<td>BU 12.8-16 mg/kg, Flu 180 mg/m², Alem 54 mg/m²</td>
<td>Tacrolimus, MMF</td>
<td>18 2.3-20.2 .4-7.5</td>
<td>None</td>
<td>ICH in 1, PRES in 1, CMV react in 4</td>
<td>1 of 10</td>
<td>40% aGVHD, 10% cGVHD</td>
<td>15-100%</td>
<td></td>
</tr>
<tr>
<td>(Bhatia, 2014) [17]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mississippi</td>
<td>BU 14 mg/kg, CY 200 mg/kg, ATG 90 mg/kg</td>
<td>CsA, Pred</td>
<td>10 2.8-16.3 .2.9-9.9</td>
<td>1</td>
<td>1 death from sepsis, 1 AIHA</td>
<td>None</td>
<td>17% aGVHD, 11% cGVHD</td>
<td>Mean 88% at 1 yr</td>
<td></td>
</tr>
<tr>
<td>(Majumdar, 2010) [18]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlanta</td>
<td>BU 14 mg/kg, CY 200 mg/kg, ATG 90 mg/kg</td>
<td>CsA, MTX</td>
<td>27 3.3-17.4 .1-10</td>
<td>1 (3)</td>
<td>8 VOD, 16% seizures, 2 ICH</td>
<td>None</td>
<td>12% aGVHD, 1 death from cGVHD</td>
<td>62-100%</td>
<td></td>
</tr>
<tr>
<td>(McPherson, 2011) [19]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pavia</td>
<td>BU 16 mg/kg, Flu 160 mg/m² or Treo 14 gm/m², TT 10 mg/kg, Flu 160 mg/m², ATG</td>
<td>CsA, MTX or MMF</td>
<td>30 1.7-18.8 .5-14</td>
<td>None</td>
<td>Stomatitis (43%), GI toxicity (17%); no VOD</td>
<td>None</td>
<td>7% Grades I-II aGVHD, 50% (BU) and 36% cGVHD in BU group, none in Treo group</td>
<td>29-100%</td>
<td></td>
</tr>
<tr>
<td>(Strocchio, 2015) [20]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>Alem 48 mg, Flu 140-150 mg/m², melohalan 140 mg/m²</td>
<td>CsA or tacrolimus</td>
<td>43 3-20.3 .75-11.83</td>
<td>3 (11, 18, 21)</td>
<td>3 deaths from GVHD</td>
<td>19% by 1 yr; 23% aGVHD, 9% by 2 yr</td>
<td>13.4% cGVHD</td>
<td>29-100%</td>
<td></td>
</tr>
</tbody>
</table>

Walter et al, BBMT, 2016
## MUD and cord transplant for SCD

<table>
<thead>
<tr>
<th>Type/year</th>
<th>Transplant regimen</th>
<th># pts age</th>
<th>Alive without SCD</th>
<th>GVHD Acute/chronic</th>
<th>Death (cause)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cord-related 2016</strong></td>
<td>Bu/CY/ATG</td>
<td>44 (2-20)</td>
<td>38</td>
<td>3/0</td>
<td>4 MOF, hem</td>
</tr>
<tr>
<td><strong>Cord-MUD 2014</strong></td>
<td>Flu/Mel/Cam Bu/Flu/Cam</td>
<td>43 (1-22)</td>
<td>20</td>
<td>13/4</td>
<td>14% MOF, infxn</td>
</tr>
<tr>
<td><strong>MUD 2015</strong></td>
<td>TT/TreoFlu/ATG//CsA-Mtx</td>
<td>6 (27-48)</td>
<td>5</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td><strong>MUD 2016</strong></td>
<td>Flu/Mel/Campath//CsA-Mtx-Pred</td>
<td>29 (6-19)</td>
<td>19</td>
<td>8/11</td>
<td>8 (GVHD)</td>
</tr>
</tbody>
</table>
**Haplo transplant for SCD**

- **Good data to date**
- **Everyone has a donor**
- **Short term follow-up**

<table>
<thead>
<tr>
<th>Reference year</th>
<th>Transplant regimen</th>
<th># pts age</th>
<th>Alive w/o SCD</th>
<th>GVHD Acute/chronic</th>
<th>Death (cause)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H Bolanos-2012</td>
<td>Cy/Flu/TBI/ATG ptCy/CsA/MMF</td>
<td>14 (15-52)</td>
<td>8</td>
<td>0/0</td>
<td>0</td>
</tr>
<tr>
<td>H Dallas 2013</td>
<td>Flu/Cy,TT,Bu/ATG/CD34+PBSC</td>
<td>8 (4-17)</td>
<td>5</td>
<td>4/3</td>
<td>2 (GVHD)</td>
</tr>
<tr>
<td>H Saraf 2018</td>
<td>ATG/Cy/Flu/TBI PBSC</td>
<td>8</td>
<td>7</td>
<td>2/1</td>
<td>1 (?)</td>
</tr>
<tr>
<td>summary</td>
<td>multiple</td>
<td>82 (3-51)</td>
<td>60 (73%)</td>
<td>13/8</td>
<td>9 (multiple)</td>
</tr>
</tbody>
</table>
Haplo protocol open at Children's of Alabama
CTN 1507

Eligibility

- Children aged 5-15
  - History of overt stroke ischemia based on neuroimaging, or clinical evidence of permanent neurological injury lasting for 24 hours
- Adolescents and adults aged 15-46 with
  - History of two or more episodes of acute chest syndrome
  - Transfusion dependent to prevent VOC complications
  - History of three or more severe VOC pain crises per year in the 2-year period

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>HU</td>
<td>-70 thru -10</td>
</tr>
<tr>
<td>ATG</td>
<td>-9 thru -7</td>
</tr>
<tr>
<td>TT</td>
<td>(10 mg/kg) -7</td>
</tr>
<tr>
<td>Cy</td>
<td>(14.5 mg/kg) -6 thru -5</td>
</tr>
<tr>
<td>Flu</td>
<td>(30 mg/m2) -6 thru -2</td>
</tr>
<tr>
<td>TBI</td>
<td>(2 Gy) -1</td>
</tr>
<tr>
<td>BM</td>
<td>infusion</td>
</tr>
<tr>
<td>Cy</td>
<td>(50 mg/kg) +4, +5</td>
</tr>
<tr>
<td>Siro</td>
<td>+5</td>
</tr>
<tr>
<td>MMF</td>
<td>+5 thru +35</td>
</tr>
</tbody>
</table>

Gene therapy

• Introduction of new genetic material into the cells of an organism for therapeutic purposes
  • Abnormal gene and normal functioning gene identified and cloned
  • Cells responsible for disease are identified and accessible for manipulation
  • A means of introducing and expressing genetic material (viral vector/gene editing)
  • Over 2300 clinical gene therapy trials from 1989-2016 (per Wikipedia)
  • 250 CAR T cell trials
Steps in ex-vivo gene-addition therapy

1. Produce virus with therapeutic payload
   Produce Lentiviral vector carrying a functional gene sequence.

2. Isolate target cells from patient
   Mobilize, extract and isolate patient’s HSCs or T cells.

3. Transduce target cells ex vivo
   Insert target gene sequence into the patient’s HSCs or T cells.

4. Test & re-infuse gene modified cells
   Prepare patient & re-infuse patient’s correct HSCs or T cells.
Gene therapy for inherited disorders of the hematopoietic system

- **Severe immune deficiencies**
  - Common γ chain ~50 pts
  - ADA deficiency ~40 pts
  - CGD ~ 5 pts
  - WAS ~ 10 pts

- **Marrow failure disorders**
  - Fanconi’s anemia ~7 pts

- **Hemoglobinopathies**
  - Sickle cell disease ~ 10 pts
  - Thalassemia ~25 pts

- **Metabolic storage disorders**
  - Adrenoleukodystrophy ~ 30 pts
  - MLD ~ 9 pts
Sustained Correction of X-Linked Severe Combined Immunodeficiency by ex Vivo Gene Therapy

- 12 males with X-linked SCIDS
- Bone marrow derived CD34+ HSC
- Ex vivo expanded
- Transduced using retroviral vector containing common $\gamma$ chain

\[ \text{LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1} \]

Hacein-Bey-Abina et al, NEJM 2002

Hacein-Bey-Abina et al, Science 2003
BlueBird Bio
Phase 1/2 study HGB-206 for SCD:

- Eligibility - Severe SCD, age 18-35
  - 9 enrolled thus far
- CD34+ cells collected from patient
  - BM harvest (poor yields), now plerixafor mobilized PBSC
- Transduction with lentiglobin BB305 lentiviral vector and anti-sickling HgbA\textsuperscript{T87Q}
  - Poor efficiency and low vector copy number
- Myeloablation with full dose Busulfan
  - Expected toxicities of fever, infection, cytopenia
BlueBird schema

HSC collection

CD34 sort
Lentivirus transduction
Expansion
Quality control

Hospitalization

Busulfan

Hgb S quantification
Transfusion needs

myeloablative

Table 1. DP Characteristics and Key Outcomes

<table>
<thead>
<tr>
<th>Patient</th>
<th>DP cell dose (x10^8 CD34+ cells/kg)</th>
<th>CD34+ cells transduced (%)</th>
<th>DP VCN (copies/diploid genome)</th>
<th>Peripheral VCN (at last visit)</th>
<th>HbA1c (%)</th>
<th>% “Anti-sickling” Hb (HbA + HbA2 + HbF) l at last visit</th>
<th>Last study visit (month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1301</td>
<td>2.6</td>
<td>13%, 8%</td>
<td>0.5, 0.6</td>
<td>0.1</td>
<td>0.4</td>
<td>11.4%</td>
<td>21</td>
</tr>
<tr>
<td>1303</td>
<td>2.8</td>
<td>29%</td>
<td>1.3</td>
<td>0.1</td>
<td>0.8</td>
<td>27.2%</td>
<td>21</td>
</tr>
<tr>
<td>1304</td>
<td>1.6</td>
<td>17%, 24%, N/A</td>
<td>0.5, 0.5, 0.3</td>
<td>0.1</td>
<td>0.6</td>
<td>30.1%</td>
<td>15</td>
</tr>
<tr>
<td>1306</td>
<td>2.1</td>
<td>30%</td>
<td>0.6</td>
<td>0.1</td>
<td>1.2</td>
<td>16.0%</td>
<td>15</td>
</tr>
<tr>
<td>1308</td>
<td>1.9</td>
<td>18%, 41%</td>
<td>0.5, 0.8</td>
<td>0.1</td>
<td>0.9</td>
<td>30.2%</td>
<td>15</td>
</tr>
<tr>
<td>1309</td>
<td>1.8</td>
<td>42%</td>
<td>0.9</td>
<td>0.2</td>
<td>2.4</td>
<td>27.5%</td>
<td>12</td>
</tr>
<tr>
<td>1310</td>
<td>5.1</td>
<td>29%, 25%</td>
<td>0.9, 0.4</td>
<td>0.1</td>
<td>0.9</td>
<td>20.7%</td>
<td>15</td>
</tr>
<tr>
<td>1312</td>
<td>3.2</td>
<td>95%, 90%</td>
<td>5.0*, 2.9*</td>
<td>2.6</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1313</td>
<td>2.2</td>
<td>46%, 83%</td>
<td>1.4, 3.3*</td>
<td>0.5</td>
<td>1.5</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*Not yet available; *Lentiglobin DP manufactured using refined process

Kanter et al, ASH 2017
Issues with gene addition approach

- Failure to control integration sites
  - Random gene insertion in or near proto oncogenes
    - Common gamma chain SCIDs 6/20
    - WAS trial 7/10
    - CGD 3/3

- Inefficiency/inconsistency of transduction
  - Variable numbers of gene modified cells

- Not under control of endogenous regulators

- VERY EXPENSIVE ($1,000,000/pt)
Our approach vs others

1. Patient population- who should we transplant?
   A. Older age, adolescents/adults
   B. Infants where disease-specific damage is minimal

2. Stem cell collection- how should we do this?
   A. Bone marrow harvest or plerixafor-mobilized HSC
   B. Cord blood

3. What is the best method for gene delivery with regards to safety, efficiency, cost?
   A. Gene addition, with Lentivirus
   B. Gene editing with CRISPR/Cas9

4. How to safely prepare the patient with minimal toxicities and preservation of fertility?
   A. Full dose Busulfan myeloablation
   B. Mini-dose Busulfan
UAB IRB approved protocol to collect HSCs from SCD patients

1. Bone marrow-SCD adult patients consent to bone marrow aspirate
2. Cord blood-sickle carrier moms at UAB OB agree to donate cord blood
3. PB HSC- SCD pediatric patients undergoing exchange transfusion, collect discarded blood anonymously
CRISPR-Cas System
(Clustered Regularly Interspersed Palindromic Repeats)

Provides bacteria with innate immunity to defend against invading viruses

The CRISPR/Cas immune response proceeds in three stages: fragments of viral DNA are inserted into CRISPR regions during adaptation, CRISPR RNAs are produced and processed during biogenesis, and complementary viral DNA sequences are targeted and destroyed during interference. Credit: Asako Miyakawa and Sam Sternberg
Gene correction via CRISPR/Cas

- The CRISPR/Cas system has been used for gene editing (adding, disrupting or changing the sequence of specific genes)
- By delivering the Cas9 protein/enzyme and appropriate guide RNAs into a cell, the genome can be cut/repaired at any desired location
CRISPR gene editing

- **Critical components**
  - guide RNA
  - modified recombinant Cas9
  - single stranded oligodeoxynucleotide
    - Template for HR, 100 bp surrounds HgbA gene sequencing encompassing sickle mutation

- **Nucleofection (electric shock)**
  - Optimal settings to minimize cell death

- **Cells need to be in “correct state”**
  - S phase
  - Influence of culture conditions and cytokines used
Differentiation in vitro of CRISPR/Cas modified sickle CD34+ bone marrow stem cells

- Purify CD34+ cells on immunomagnetic beads
- Nucleoporate with RNP/ssODN complex
- Plated onto methocult
- Red blood cell precursor colonies (single cell assay) are picked and DNA is sequenced
Mouse model of sickle cell disease

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RBC $10^6$/$\mu$L</th>
<th>Hb g/dL</th>
<th>MCV fl</th>
<th>WBC $10^3$/$\mu$L</th>
<th>NE $10^3$/$\mu$L</th>
<th>LY $10^3$/$\mu$L</th>
<th>PLT $10^3$/$\mu$L</th>
<th>Retics %</th>
<th>Urine osmolality mOsm</th>
<th>Spleen to body weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbAA</td>
<td>11±0.5</td>
<td>11.6±1.1</td>
<td>36.1±0.5</td>
<td>8.9±3.7</td>
<td>1.3±0.7</td>
<td>6.5±3.1</td>
<td>988±244</td>
<td>5.9±1.1</td>
<td>2150±142</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>HbAS</td>
<td>11.9±0.5</td>
<td>13.8±0.9</td>
<td>37±1.2</td>
<td>13.7±5.7</td>
<td>2.1±1.1</td>
<td>9.7±3.9</td>
<td>921±191</td>
<td>12.7±3.3</td>
<td>2077±157</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>HbSS</td>
<td>6.4±0.9**</td>
<td>9.1±1.1*</td>
<td>51.4±3.4**</td>
<td>47±8.9**</td>
<td>10.6±2.6**</td>
<td>31.3±6.3**</td>
<td>924±618</td>
<td>66.2±4.8**</td>
<td>1083±206**</td>
<td>5.9±0.9**</td>
</tr>
</tbody>
</table>

Treosulfan conditioning permits engraftment and rescues the SCD phenotype

Devadasan et al, BBMT, 2018
Our challenges for a phase 1 study

- HSC collection from sickle cell patients
  - Plerixafor mobilization of children?
  - Coordinating cord blood collection of at risk infants

- Preparative therapy
  - Determining optimal Busulfan dose in children/infants

- Utilization of a GMP facility
  - Production scaled up

- Demonstrate efficacy and safety in murine and NSG mouse model for FDA approval
  - Transplant mice with “therapeutic dose”
  - Off target effects?

- Patient enrollment
  - Barriers to participation
The first attempts to use a groundbreaking gene-editing technology in people will likely target patients with sickle cell disease, a crippling inherited disorder that in the U.S. predominantly strikes African-Americans. That should be welcome news, after decades of sickle cell patients being neglected by the health care system, scientists, and drug companies. But the long and ugly history of unethical experimentation and mistreatment of black patients could make recruiting volunteers to try largely untested CRISPR therapies a tough sell.

“You can’t expect this population is just going to stick out their arm for an IV,” said Mary Brown, who heads the Sickle Cell Disease Foundation of California and has worked with patients for four decades. “There’s a lot of education that needs to be done. I don’t want to say hand-holding, but that’s what it is.” A 2016 analysis of thousands of genomic studies showed what researchers called “persistent bias:” 81 percent of participants had European ancestry, while people with African, Latin, or indigenous ancestry totaled less than 4 percent.
### Comparison of transplant options

<table>
<thead>
<tr>
<th></th>
<th>Allogeneic MUD HSCT</th>
<th>Allogeneic Haplo HSCT</th>
<th>Allogeneic MRD HSCT</th>
<th>Current Lenti HSCT</th>
<th>Proposed CRISPR HSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor available</td>
<td>40%</td>
<td>100%</td>
<td>15%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>GVHD</td>
<td>+++</td>
<td>+/-+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infection</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-/+</td>
</tr>
<tr>
<td>Reject risk</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>?</td>
</tr>
<tr>
<td># done</td>
<td>~100</td>
<td>~100</td>
<td>~500</td>
<td>~10</td>
<td>0</td>
</tr>
<tr>
<td>Cost</td>
<td>~ 600k</td>
<td>~ 600k</td>
<td>~ 400k</td>
<td></td>
<td>low</td>
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<tr>
<td>Fertility</td>
<td>Unlikely</td>
<td>Unlikely</td>
<td>Unlikely</td>
<td>Unlikely</td>
<td>probable</td>
</tr>
<tr>
<td>Chemo</td>
<td>Hi dose</td>
<td>Mid dose</td>
<td>Mid dose</td>
<td>Hi dose</td>
<td>Lo dose</td>
</tr>
</tbody>
</table>
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  - GMP facility
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  - Hematology health care providers

- **All of our sickle cell patients and their families**