Application of HSCT in SCD

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Hematopoietic stem cells as vehicles for therapeutic gene delivery

Allogeneic stem cell transplantation

- Transplantation using allogeneic stem cells from a normal donor
  - HLA-matched sibling

Autologous stem cell gene transfer

- Transplantation using autologous stem cells which have been corrected by transfer of a normal or therapeutic gene
  - Retroviral vectors
Hematopoietic stem cells as vehicles for therapeutic gene delivery

Allogeneic stem cell transplantation

- 22 children <16 years old transplanted
- Conditioning with Bu/Cy/ATG
- OS 91%
- DFS 73%
- Rejection 18%
- Myeloablative transplantation established as curative in children with sickle cell disease
Hematopoietic stem cells as vehicles for therapeutic gene delivery

Allogeneic stem cell transplantation

- 87 consecutive patients age 2-22 yrs
- Sibling donor marrow or cord blood
- Conditioning with Bu/Cy, ATG added
- OS 93.1%
- EFS 86%
- Rejection 22.6% to 3% after ATG
- Results improved significantly with time
  - EFS 95.3% after January 2000

“These results indicate that HLA-identical sibling HSCT after myeloablative conditioning with ATG should be considered as a standard of care for SCD children who are at high risk for stroke”

Bernaudin et al, Blood, 1 October 2007, Volume 110, Number 7
Stable Mixed Chimerism Can Be Curative in Patients with SCD

Allogeneic stem cell transplantation

• 59 patients age 3-15 years received HLA-identical sibling marrow allografts
  • OS 93%, EFS 84%
  • Stable mixed chimerism sufficient
    • 13/50 patients 11-99% donor chimerism
    • No further sickle cell related events
    • No GvHD in mixed chimeric patients
  • Toxic conditioning and GVHD limit application of myeloablative conditioning to children and young adults
    • Engraftment without ablation?

Nonmyeloablative conditioning sufficient for reliable allogeneic PBSC engraftment in adults

- Cytoxan/fludarabine based immune ablative conditioning piloted in patients with metastatic cancer

- Extended to high-risk patients ineligible for conventional myeloablative conditioning
Application to sickle cell disease?

- NIH experience overall (n>100)
  - Engraftment through donor alloimmune response
  - GVHD common
    - T cell alloreactivity not necessary in nonmalignant disorders
  - Treatment related mortality 21%
    - GVHD principal cause
    - Prohibitive in nonmalignant disorders

Days Post Transplant

TRM in all patients

21% (±5)
Cumulative non-ablative BMT experience in sickle cell disease

A non-ablative protocol for adults with severe sickle cell disease is needed

Chakrabarti S et al, BBMT 2004
A Murine Model of Nonmyeloablative Stem Cell Transplantation for the Treatment of Sickle Cell Disease

- Develop regimen that:
  - Promotes tolerance without need for long term immunosuppression
  - Allow for stable mixed chimerism
- F1-Hybrid donor mice
  - Myeloid-flow cytometry
  - Erythroid-Hb electrophoresis
- Donors mobilized with G-CSF
- Mobilized cells collected day 6
- Recipient mice conditioned with 300 cGy and a 30d course of either
  - Cyclosporine (CSA)
  - Rapamycin (RAPA)
Why Rapamycin (sirolimus)?

Powell, J, Fitzhugh, C. et al., Transplantation, 80(11):1541-5, 2005
Sickle Hemoglobin is Replaced by Donor Hemoglobin in Chimeric Homozygote Mice

Powell, J, Fitzhugh, C. et al., Transplantation, 80(11):1541-5, 2005
Eligibility: Adults with Hb SS, SC, or Sb<sup>0</sup>-thal

Severe end-organ damage
- Stroke
- TRV ≥2.5 m/s
- Renal damage
- Liver damage

- Or modifiable complication(s), not ameliorated by hydroxyurea
  - > 2 hospital admissions per year for pain crises
  - previous acute chest syndromes
  - red cell alloimmunization
  - osteonecrosis of multiple joints
Screening and Accrual of Initial Cohort

HLA typing performed in 169 siblings and 112 patients

88 Patients did not have matched related donors

24 Patients were eligible

4 Were excluded for major ABO incompatibility

1 Died before HSCT

8 Are receiving optimizing medical therapy

1 Is in pre-HSCT evaluation

10 Underwent transplantation
### Characteristics of 10 Initial Patients Undergoing Nonmyeloablative Hematopoietic Stem-Cell Transplantation (HSCT)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at HSCT (yr)</th>
<th>Sex</th>
<th>Type of Sickle Hemoglobin</th>
<th>Coexisting Conditions and Indications for HSCT</th>
<th>Medical Management before HSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>F</td>
<td>SS</td>
<td>Recurrent TIA and stroke, elevated TRV</td>
<td>Simple and exchange red-cell transfusions</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>M</td>
<td>SS</td>
<td>Frequent VOC, priapism, proteinuria (1.7 g/24 hr)</td>
<td>Hydroxyurea, simple and exchange red-cell transfusions</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>F</td>
<td>SS</td>
<td>TIA, frequent VOC, acute chest syndrome</td>
<td>Hydroxyurea, exchange red-cell transfusions</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>M</td>
<td>SS</td>
<td>Frequent VOC, acute chest syndrome, narrow CNS arteries on MRA</td>
<td>Hydroxyurea, exchange red-cell transfusions</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>M</td>
<td>SS</td>
<td>Frequent VOC, acute chest syndrome</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>M</td>
<td>SC</td>
<td>Frequent VOC, priapism, narrow CNS arteries on MRA, lacunar infarcts</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>F</td>
<td>SS</td>
<td>Frequent VOC, elevated TRV</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>F</td>
<td>SS</td>
<td>Frequent VOC, elevated TRV</td>
<td>Hydroxyurea and simple red-cell transfusions</td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>F</td>
<td>SS</td>
<td>Sickle-cell–related FSGS (baseline creatinine, 2.5–2.7 mg/dl [221–239 µmol/liter], elevated TRV, acute chest syndrome, frequent VOC, red-cell alloimmunization, hepatitis C</td>
<td>Hydroxyurea, simple and exchange red-cell transfusions, darbepoetin</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>M</td>
<td>SS</td>
<td>Sickle-cell–related nephrotic syndrome, elevated TRV, acute chest syndrome</td>
<td>Hydroxyurea, simple red-cell transfusions, prednisone</td>
</tr>
</tbody>
</table>
Conditioning regimen

- Hydroxyurea maximized and continued through day -8
- Red cell exchange performed to lower HbS ~ 30%
- Platelet transfusion threshold of 50k/uL to prevent CNS bleeding
- Immunosuppression taper planned at 1 year if CD3 donor chimerism is >50%

![Diagram showing the conditioning regimen with key dates and treatments]
Transplant course

• All patients tolerated conditioning without serious adverse events
  – No need for nutritional support
  – No acute or chronic GVHD
  – No sickle cell anemia related events
• Nine of 10 with stable mixed chimerism and disease reversion
Cohort expanded to 30 patients ranging in age from 16 to 65 years

<table>
<thead>
<tr>
<th>Indications for HSCT</th>
<th>Number of individuals</th>
<th>Co-morbid conditions</th>
<th>Number of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaso-occlusive crisis</td>
<td>23</td>
<td>DLCO &lt;50%</td>
<td>3</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>7</td>
<td>Serum ferritin &gt;1000 ng/L</td>
<td>15</td>
</tr>
<tr>
<td>Stroke or abnormal brain vessels</td>
<td>8</td>
<td>Hepatopathy: cirrhosis, hepatitis C, direct bilirubin &gt;20 mg/dL</td>
<td>5</td>
</tr>
<tr>
<td>Tricuspid regurgitant velocity, 2.6 m/s or higher by echo</td>
<td>12</td>
<td>Proteinuria</td>
<td>4</td>
</tr>
<tr>
<td>Sickle nephropathy</td>
<td>5</td>
<td>Others: PE/DVT, rheumatoid arthritis</td>
<td>2</td>
</tr>
</tbody>
</table>
Patients tolerated conditioning well
- No acute sickle cell-related events
- No VOD, seizures, or interstitial pneumonitis
- Brief duration of neutropenia (ANC <500)
  - Median 16 days (6-21)
  - Median neutrophil nadir 0.12
- First line IV antibiotics support in most
  - No sepsis
  - 6 patients required no IV antibiotics
- Preemptive CMV treatment in 2 patients, resolved after 1 week
- Low usage of blood products
- No acute or chronic GvHD

### Transplant outcome: expanded cohort

<table>
<thead>
<tr>
<th>Red cell units (exchange)</th>
<th>Median (range)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 (1-9)</td>
<td>5.88</td>
</tr>
<tr>
<td>Red cell units (day -7 to +45)</td>
<td>6 (0-15)</td>
<td>6.2</td>
</tr>
<tr>
<td>Platelet units (day -7 to +45)</td>
<td>4 (0-19)</td>
<td>4.4</td>
</tr>
</tbody>
</table>
Transplant outcome: expanded cohort

Overall survival, 100%

Disease free survival, 87%
Transplant outcome: expanded cohort

![Graph showing % Donor over Months after HSCT for CD3+ and CD14/15+ cells.](Image)
Transplant outcome: expanded cohort

Total Hemoglobin

- male
- female

G/dL

1 mo pre, exch, 6 mo, 1 yr, > 1 yr
Improvement in tricuspid regurgitant jet velocity among 10 recipients with >2.5 m/s pre-transplant
Replacement by donor derived red cells allows tapering of narcotic analgesics
Stable mixed chimerism persists after withdrawal of sirolimus
Interim conclusions

- Allogeneic PBSC transplantation after low dose TBI, campath, sirolimus conditioning and resulting mixed hematopoietic chimerism sufficient to revert the sickle phenotype
- Low toxicity allows application in adults with severe disease
- ‘Split’ or mixed chimerism and absence of acute or chronic GvHD suggests operational tolerance
  - 11 patients off immunosuppression with stable mixed chimerism
- Longer follow-up and further accrual necessary
- Alternative stem cell sources need exploration
Barriers to therapeutic gene delivery using HSCs: HSC source

- Of 112 patients HLA typed, 88 had no HLA matched family donor

Ten patient’s high resolution DNA HLA typing entered into Translink

**POTENTIAL MARROW DONORS**

- **BMDW**
  - Number of potential 6/6 allele-matched donors reported
  - Donors who were not potential 10/10 allele matches were excluded

- **NMDP**
  - Number of potential 6/6 allele-matched donors reported
  - Donors who were not potential 10/10 allele matches were excluded
  - Number that were same ethnicity and had the best likelihood of being a 6/6 allele match according to HapLogic reported

**CORD DONORS**

- Search limited to NMDP Network cord blood banks
  - “Cord list criteria” function within Translink used to narrow the available cord list
  - TNC ≥ 1.5 x 10⁷/kg
  - Number ABO-matched with pt reported

  - TNC ≥ 2.5 x 10⁷/kg
  - Number ABO-matched with pt reported
Barriers to therapeutic gene delivery using HSCs: HSC source/MUD bone marrow

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMDW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential 6/6 allele match</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>32</td>
<td>42</td>
</tr>
<tr>
<td>Potential 10/10 allele match</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td><strong>NMDP (duplicated in BMDW report)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential 6/6 allele match</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Potential 10/10 allele match</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Potential 10/10 allele match of African descent</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HapLogic: highest probability of being 6/6 allele match</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>50%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Seven of 10 with potential 6/6 donor

Using Haplogic, only 1/7 with >1% chance of having a 6/6 donor
Barriers to therapeutic gene delivery using HSCs: HSC source/Cord blood

<table>
<thead>
<tr>
<th>HLA match</th>
<th># of pts matched</th>
<th>TNC ≥ 1.5 x 10⁷/kg # of pts with matched UCB (# with ABO matched)</th>
<th>TNC ≥ 2.5 x10⁷/kg # of pts with matched UCB (# with ABO matched)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥4/6 HLA match</td>
<td>9 of 10</td>
<td>9 (9)</td>
<td>8 (7)</td>
</tr>
<tr>
<td>≥5/6 HLA match</td>
<td>6 of 10</td>
<td>5 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>6/6 HLA match</td>
<td>1 of 10</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Median # of units available per pt (range)

<table>
<thead>
<tr>
<th>HLA match</th>
<th>Median # of units available per pt (range)</th>
<th>Median UCB with TNC ≥1.5 x 10⁷/kg (# with ABO matched)</th>
<th>Median UCB with TNC ≥2.5 x10⁷/kg (# with ABO matched)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥4/6 HLA match</td>
<td>103.5 (20-875)</td>
<td>29 (8) [1.5-7.04]</td>
<td>3 (1) [2.51-7.04]</td>
</tr>
<tr>
<td>≥5/6 HLA match</td>
<td>2.5 (1-114)</td>
<td>0.5 (0) [1.51-4.39]</td>
<td>0 (0) [2.55-4.39]</td>
</tr>
<tr>
<td>6/6 HLA match</td>
<td>0 (0)</td>
<td>0 (0) [0]</td>
<td>0 (0) [0]</td>
</tr>
</tbody>
</table>

Nine of 10 have at least one 4/6 cord blood match identified
Higher degree of matching, higher cell dose, and ABO compatibility limits applicability
Barriers to therapeutic gene delivery using HSCs: Haploidentical grafts?

- Haploidentical donors
  - Most accessible
  - Large cell doses feasible
  - Repeat collections feasible
- Immunologic barrier greater
  - Higher degree of immunosuppression
- Post-graft cyclophosphamide
  - Reduce graft rejection/GvHD
  - Targets proliferating lymphocytes
  - Early success in ongoing clinical trials

HLA haploidentical bone marrow transplantation with post transplant cyclophosphamide

14 haploidentical SCD recipients 15-33 years old

- Conditioning with Flu/Cy/ATG/low dose TBI and PTCy with Tacro or Siro/MMF
- OS 100%
- DFS ~50%
- Rejection ~50%

- Expands access to potentially curative transplantation to nearly all patients

Bolaños-Meade, et al, Blood, November 2012, Volume 120, Number 22
Mismatched Mouse Model to Evaluate Post Transplant Cyclophosphamide

Donor BalbC (Kd)

6 days G-CSF 200 mcg/kg

Recipient C57Bl6 (Kb)

Harvest Bone Marrow

22-25 x 10^6 Bone Marrow Cells

100 x 10^6 Splenocytes

Harvest Splenocytes
Will Sirolimus and Cyclophosphamide Synergize on Engraftment?

Sirolimus (Sir, 3mg/kg IP) days -1 or +4 for 30 days
200cGy TBI, bone marrow cells infused on day 0
Cyclophosphamide (Cy, 200mg/kg IP) day +2

% Donor Chimerism

Month Post Transplant
Post-transplant Cy does not improve engraftment in the setting of lymphocyte depletion.

Day -7 thru -4
Thy 1.2 monoclonal ab (1mg IP)
Day -1
Sirolimus (3mg/kg)
Day 0
200 or 400cGy TBI
Day +2
Cytoxan IP

0mg/kg
50mg/kg
100mg/kg (5 mice/group)
200mg/kg

% Donor Chimerism

Month Post BMT
Cyclosporine Insufficient for Induction of Stable Mixed Chimerism

Sirolimus (Sir, 3mg/kg IP) or Cyclosporine (CSA, 20mg/kg IP) day -1 for 14 days
200cGy TBI, bone marrow day 0
Cyclophosphamide (Cy, 200mg/kg IP) day +2

Can CsA Also Provide for Synergy With Post Transplant Cy?
Observations

- Sirolimus and post-transplant cyclophosphamide are synergistic in a mismatched mouse model.
- Post-transplant cyclophosphamide does not improve engraftment in the setting of profound lymphocyte depletion.
- Cyclosporine is insufficient for the induction of stable mixed chimerism in our model.
Nonmyeloablative Haploidentical PBSC Transplantation for Adults with Severe Congenital Anemias

**Eligibility:** Adults with Hb SS, SC, or Sb\(^0\)-thal

- Severe end-organ damage
  - Stroke
  - Elevated TRV $\geq 2.5$ m/s
  - Sickle cell nephropathy
  - Sickle hepatopathy
- Or modifiable complication(s), not ameliorated by hydroxyurea
  - $>$ 2 hospital admissions per year for pain crises (VOC)
  - Previous acute chest syndromes (ACS)
Conditioning regimen

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Cy dose</th>
<th>Day Post Tx</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>NA</td>
<td>3/3 rejected</td>
</tr>
<tr>
<td>2</td>
<td>50 mg/kg</td>
<td>+3</td>
<td>4/8 engrafted</td>
</tr>
<tr>
<td>3</td>
<td>50 mg/kg</td>
<td>+3 and +4</td>
<td>Accruing</td>
</tr>
</tbody>
</table>
Choice of approach to patients with SCD

- **Child or young adult**
  - HLA-matched sibling available
    - Myeloablative BMT or CBT
      - ~95% DFS
    - Nonmyeloablative PBSCT
      - ~90% DFS

- **Adult with organ dysfunction**
  - HLA-matched sibling NOT available
    - Nonmyeloablative Haplo PBSCT or BMT
      - ~50% DFS
Crew

• Tisdale lab
  – Naoya Uchida
  – Jun Hayakawa
  – Courtney Fitzhugh
  – O.J. Phang
  – Coen Lap
  – Kareem Washington
  – Matt Hsieh

• Department of Transfusion Medicine
  – Charley Carter
  – Susan Leitman
  – Dave Stoncek

• Roger Kurlander

• Elizabeth Kang

• Jonathan Powell

• 5 Research Court
  – Mark Metzger
  – Allen Krouse
  – Barrington Thompson
  – Bob Donahue

• Cindy Dunbar
  – Stephanie Sellers
  – Tong Wu

• Derek Persons

• Michel Sadelain

• Terri Wakefield
• Beth Link
• Karen Kendrick
• Griffin Rodgers