CURE IS PRACTICAL BUT ONLY AVAILABLE TO A FEW
# Cure Is Practical

## Table 1. Hematopoietic cell transplantation

<table>
<thead>
<tr>
<th>Location</th>
<th>Belgium</th>
<th>Pesario</th>
<th>France</th>
<th>Multicenter</th>
<th>Other US/Europe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>BU,CY (30), BU,CY,TLI (6), BU,CY,ATG (14)</td>
<td>BU,CY (17), BU,CY,TLI (1), BU,CY,ATG (21)</td>
<td>BU,CY (12), BU,CY,TLI (1), BU,CY,ATG (21)</td>
<td>BU,CY,ATG</td>
<td>BU,CY,ATG (13), CY/TBI (3)</td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>36 (14)</td>
<td>19 (19)</td>
<td>34 (59)</td>
<td>59 (16)</td>
<td>16 (1)</td>
<td>175 (1)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>8.6 (1.7–23)</td>
<td>7 (4–38)</td>
<td>8.6 (2.3–17.2)</td>
<td>9.9 (3.3–15.9)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0 (0)</td>
<td>3 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stroke/CNS</td>
<td>6 (0)</td>
<td>1 (0)</td>
<td>16 (1)</td>
<td>31 (2)</td>
<td>2 (0)</td>
<td>2 (0)</td>
</tr>
<tr>
<td>ACS</td>
<td>20 (0)</td>
<td>15 (1)</td>
<td>20 (0)</td>
<td>18 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>VOC</td>
<td>36 (0)</td>
<td>15 (0)</td>
<td>8 (0)</td>
<td>11 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MM donor</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11 (9)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>34 (94)</td>
<td>14 (100)</td>
<td>14 (74)</td>
<td>31 (91)</td>
<td>55 (94)</td>
<td>13 (81)</td>
</tr>
<tr>
<td>Deaths</td>
<td>2 (0)</td>
<td>0 (0)</td>
<td>5 (0)</td>
<td>3 (0)</td>
<td>4 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Graft rejection/ recurrent SCD (%)</td>
<td>4 (0)</td>
<td>1 (0)</td>
<td>4 (0)</td>
<td>5 (0)</td>
<td>1 (0)</td>
<td>16 (9)</td>
</tr>
<tr>
<td>Stable mixed chimerism</td>
<td>6 (0)</td>
<td>–</td>
<td>5 (0)</td>
<td>10 (0)</td>
<td>1 (0)</td>
<td>11 (0)</td>
</tr>
<tr>
<td>Disease-free survival (%)</td>
<td>30 (83)</td>
<td>13 (93)</td>
<td>13 (68)</td>
<td>27 (79)**</td>
<td>50 (85)</td>
<td>12 (80)**</td>
</tr>
<tr>
<td>aGVHD</td>
<td>15**</td>
<td>5 (0)</td>
<td>4 (0)</td>
<td>6 (grade II)</td>
<td>11 (grade I–III)</td>
<td>2 (0)</td>
</tr>
<tr>
<td>cGVHD</td>
<td>8 (2)</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>5 (1)</td>
<td>1 (12%)</td>
</tr>
<tr>
<td>Seizures</td>
<td>18 (1)</td>
<td>1 (0)</td>
<td>7/26 (0)</td>
<td>13 (0)</td>
<td>1 (0)</td>
<td>25%</td>
</tr>
</tbody>
</table>
# Non-Myeloablative BMT in Adults

**Table 1. Characteristics of 10 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>

Non-Myeloablative BMT in Adults


| Patient No. | Composition of Infused Graft | Months after Transplantation | Duration of ANC <0.50×10^9/liter | Duration of ALC <0.75×10^9/liter | Donor CD3+ Cells | Donor CD4+15+ Cells | Hemoglobin | Hemoglobin S Donor | Hemoglobin S Recipient |
|-------------|-------------------------------|-----------------------------|----------------------------------|----------------------------------|------------------|---------------------|------------|===================|=======================|
| 1           | 5.72 ×10^6†                   | 3.21 ×10^6†                 | 54                               | 21                               | 7                | 48                  | 12.0       | 0                  | 0                     |
| 2‡          | 7.56 ×10^6†                   | 2.27 ×10^6†                 | 36                               | 18                               | 2.5              | 63                  | 11.1       | 40.5               | 51.6                  |
| 3           | 10.0 ×10^6†                   | 3.42 ×10^6†                 | 42                               | 12                               | 6                | 61                  | 14.8       | 35.2               | 35.2                  |
| 4‡          | 8.3 ×10^6†                    | 5.35 ×10^6†                 | 33                               | 29                               | 6                | 0                   | 0          | 11.4               | 45.9§                  |
| 5           | 5.51 ×10^6†                   | 3.71 ×10^6†                 | 30                               | 10                               | 4                | 72                  | 14.3       | 0                  | 0                     |
| 6           | 23.8 ×10^6†                   | 2.81 ×10^6†                 | 32                               | 10                               | 6                | 35                  | 14.7       | 38.2               | 37.0                  |
| 7           | 18.8 ×10^6†                   | 3.32 ×10^6†                 | 29                               | 19                               | 8                | 62                  | 12.2       | 36.6               | 35.4                  |
| 8           | 20.1 ×10^6†                   | 3.04 ×10^6†                 | 30                               | 11                               | 1.5              | 63                  | 100        | 12.1               | 0                     |
| 9           | 16.6 ×10^6†                   | 3.7 ×10^6†                  | 16                               | 15                               | 3.5              | 23                  | 97         | 11.7¶              | 0                     |
| 10          | 15.1 ×10^6†                   | 3.64 ×10^6†                 | 15                               | 18                               | 4                | 75                  | 100        | 10.5¶              | 35                    | 34.6                  |

* Results are from the most recent follow-up assessment. ALC denotes absolute lymphocyte count, and ANC absolute neutrophil count.
† Values are per kilogram of the recipient's body weight.
‡ The results shown are from a second transplantation.
§ The patient had received an exchange transfusion within the previous 2 months.
¶ The patient was receiving supportive treatment with erythropoietin owing to renal insufficiency.
APPROACH TO TREATMENT

- Complications precipitated by extra erythrocytic factors, not directly related to hemoglobin
  - Increased cell/cell and endothelial adherence
  - Activation of thrombosis
  - Decrease vasoconstriction
SICKLE CELLS ARE STICKY
VASO – OCCLUSION
Wick et al

"Vicious Cycle" (Ham & Castle Trans Am Assoc Phys 55:1940;127)


Kinetic Hypothesis (Eaton, et al., Blood 47:1976;621)
MULTIPLE SPECIFIC PATHWAYS

Wick et al and Others

Sickle Erythrocyte (Reticulocyte)

α₄β₁ (VLA-4)
CD36
α₅β₁ (VLA-5)
α₅β₃ (VnR)
α₉β₃ (VnR)
α₁₁β₃ (GPIIb/IIIa)
GPIb (?)
GPIb (?)
TSP
Fibrinogen
Fibrinogen
VWF
CD36 (MEC)
ICAM-1
Endothelial Cell
RGD Peptides Inhibit Plasma Dependent SRBC Endothelial Adherence

Adherent RBC/mm³

6Z (0.1 uM) 8X (1.0 uM)

SFM 30% Autologous Plasma Peptide on MEC Peptide on RBC
SICKLE CELL DISEASE:  
A Hypercoagulable State?

- Increased platelet count
- Decreased platelet survival
- Drop in count during crisis
- Increased F VIII & vWF
FLOW CYTOMETRIC ANALYSIS OF PLATELET ACTIVATION

- FIBRINOGEN
- Receptor Induced Binding Site
- Fibrinogen Binding Site
- Ligand Induced Binding Site
- PLATELET
- GPIIb/IIIa
- Ca++
- Activation
- anti-RIBS1
- anti-LIBS
- FITC
- PAC1
- FITC
PLATELET ACTIVATION

Flow Cytometric analysis of Surface Markers

Log Immunofluorescence

NC
BL
Post

PAC1
anti-RIBS
GA6
Annexin V
anti-Factor V
THROMBOSIS and FIBRINOLYSIS

PROTHROMBIN

PROTHROMBIN F1.2

FIBRINOGEN

THROMBIN

TAT

PLASMIN

ANTITHROMBIN

PLASMINOGEN

PAP

<2 ANTIPLASMIN

D-DIMERS

FIBRIN MONOMER

FIBRIN POLYMER

FIBRIN CLOT
THROMBOGENIC ACTIVITY

Circulating Plasma Markers

- TAT (Thrombin-Antithrombin)
- F1.2 (Fibrinopeptide)
- D-dimers
- PAP

IU/ml
Correlation Between Plasma Level of D-dimers Fragments and Frequency of Pain Episodes in SCD

$r = 0.74$
$p < 0.01$
INUIT NATIVE AMERICANS

N 3 FATTY ACIDS
n-3 FATTY ACIDS

- Attenuate platelet reactivity
- Increase blood fluidity
  - Increase RBC deformability
  - Decrease blood viscosity
- Decrease VLDL
- Decrease release of proinflammatory mediators LTB 4 and IL 1β
- Cause vasodilation
- Decrease re-infarction rate in CAD
## Frequency of Pain Crisis

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>n-3FA</th>
<th>placebo</th>
<th>(P^{**})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients:</strong></td>
<td>9</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pain episodes / year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre treatment</td>
<td>7.8</td>
<td>7.6</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>on treatment</td>
<td>3.8</td>
<td>7.1</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

\(P^{*}\) < 0.01 > 0.05

* for comparison between pre treatment v treatment

** for comparison between n-3fas v placebo
<table>
<thead>
<tr>
<th></th>
<th>Study group N=127 Mean ± SEM</th>
<th>Control group N=126 Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Days in severe pain</strong></td>
<td>1.28 ± 0.06*</td>
<td>1.72 ± 0.06</td>
</tr>
<tr>
<td><strong>Duration of crisis</strong></td>
<td>2.57 ± 0.12*</td>
<td>4.35 ± 0.11</td>
</tr>
<tr>
<td><strong>Duration of Hospitalization</strong></td>
<td>7.08 ± 0.36*</td>
<td>12.06 ± 0.76</td>
</tr>
</tbody>
</table>

*P< 0.05  Qari et al Blood 2005;106:2340a
Why is there simultaneous onset of pain in so many parts of the body?
VASO - OCCLUSION
Poiseuille’s Formula

\[ F = \Delta P \times \frac{\Pi r^4}{8 \eta L} \]
Blood vessel NO concentration

Blood vessel

SICKLE PAIN EPISODES

- Increased hemolysis
- Release of arginase reduces NO production and free hemoglobin scavenging of nitric oxide
- Diffuse vasoconstriction with ischemia
- More sickling and hemolysis
- Positive feedback loop intensifying vasoconstriction and diffuse ischemia
- Diffuse Pain
Molecular Genetic Therapy

Zou et al Blood 211;118:4599-4608

Zou et al Cell Research 2012;22:491-494