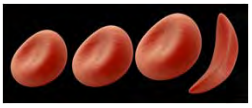
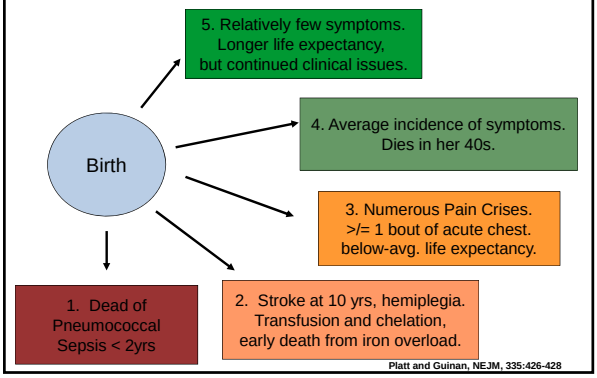


Emerging Therapies in Sickle Cell Disease: Bone Marrow Transplantation As A Curative Therapy

Leslie S. Kean MD, PhD
Director, Pediatric Blood and Marrow Transplant Program
Aflac Cancer Center and Blood Disorders Service
Children's Healthcare of Atlanta
Emory University School of Medicine

The Clinical Course of Sickle Cell Disease is Highly Variable and Unpredictable.



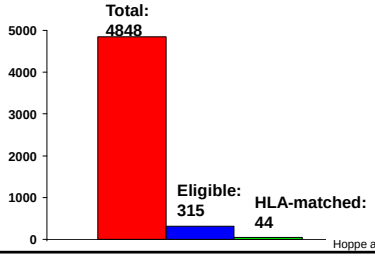
Platt and Guinan, NEJM, 335:426-428

Currently the only curative therapy for sickle cell disease is allogeneic bone marrow transplant.

But significant barriers exist…

Toxicities of Allogeneic BMT and the Lack of HLA-matched Donors Represent Significant Barriers to Transplant for SCD

The two major barriers are
1. Toxicity associated with myeloablation, Graft rejection after allo-BMT, and GvHD.
2. Lack of HLA-identical donors.



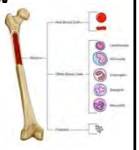
Hoppe and Walters 2001.

Can we Design a Strategy for Non-toxic BMT That Would Still Cure Sickle Cell Disease?

The answer may lie in mixed chimerism using both allogenic and autologous BMT Approaches…

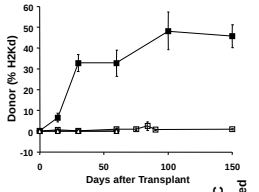
Key Terms in Transplant for SCD

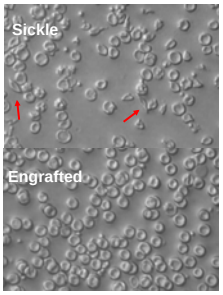
- **Allogeneic Transplant: Using Bone Marrow from a Donor to Cure SCD**
  - ❖ **HLA Matched Sibling**
  - ❖ **Unrelated Donor Transplant**
  - ❖ **HLA Mismatched Family Member**

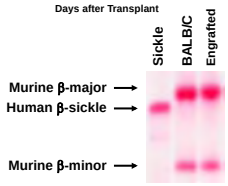


- **Gene-Corrected Autologous Transplant**
- **Mixed Chimerism: The Phenomenon by which both sickle and normal stem cells co-exist after transplant.**

Mixed WBC Chimerism Results in Full Replacement With Normal RBC and Cure of Murine SCD

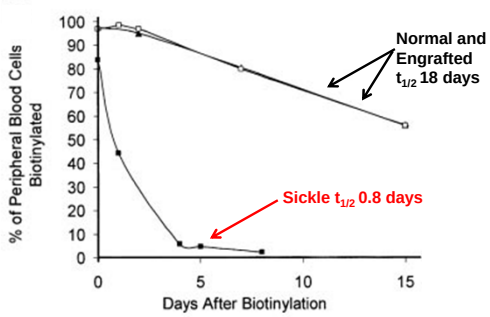






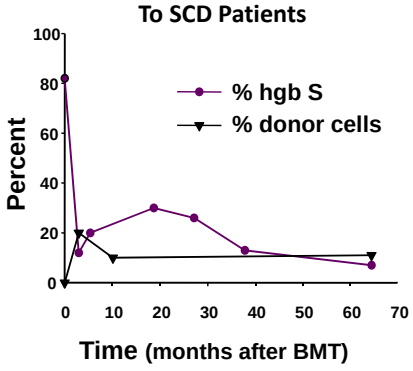
Kean et al., Blood 2002

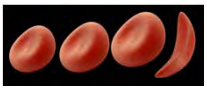
Normal RBCs Demonstrate a Significant Survival Advantage Compared to Sickle RBCs



Kean et al., Blood 2002

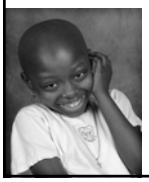
Sustained Mixed Chimerism Is Beneficial To SCD Patients





Can We Translate The Results in SCD Animal Models to Our Patients?

- Safer Allogeneic HSCT
- Gene Therapy Linked with Autologous HSCT



The Standard

Busulfan
Cytoxan
ATG

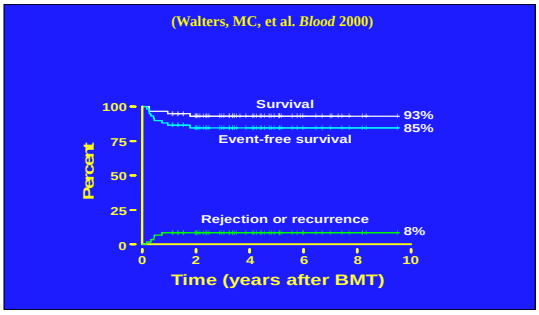
- Severe Sickle Cell Disease
  - Stroke
  - Recurrent Acute Chest Syndrome
  - Frequent Pain
  - Chronic Complications
    - AVN, Retinopathy, lung disease and nephropathy
- <16 years
- HLA matched related donor

Cyclosporine and Methotrexate

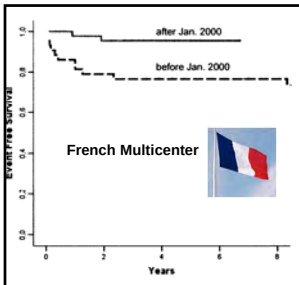
-10 0 100 1000

Days

Results With Matched Sibling Transplants In Children Have Been Very Encouraging

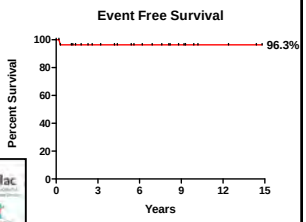


And For Children, Are Improving…



French Multicenter

Bernaudin, F. et al. Blood 2007;110:2749-2756



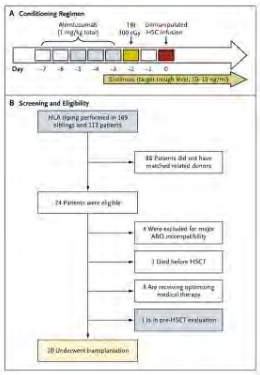
Children's Healthcare of Atlanta

The New Frontier for Adults with SCD: Non-Myeloablative Transplantation

The NEW ENGLAND JOURNAL of MEDICINE

Allogeneic Hematopoietic Stem-Cell Transplantation for Sickle Cell Disease
Hsieh MM, et al.
N Engl J Med 2009; 361:2309-2317

- **Adults**
- **TBI (300 cGy), Alemtuzumab**
- **PBSC**
- **Sirolimus (indefinite)**



Sustained Engraftment in 9 of 10 Patients

Table 2. Hematopoietic-Graft Composition and Outcome after Hematopoietic Stem-Cell Transplantation (HSCT).*

Patient No.	Composition of Infused Graft		Months after Transplantation	Duration of ANC <0.50×10⁹/liter	Duration of ALC <0.75×10⁹/liter	Donor CD3+ Cells	Donor CD14+15+ Cells	Hemoglobin	Hemoglobin S	
	CD34+ Cells ×10⁶/kg†	CD3+ Cells ×10⁶/kg†		days	months	%		g/dl	Donor %	Recipient %
1	3.72	3.21	54	31	3.9	7	48	12.0	0	0
2‡	7.56	2.37	36	18	2.5	63	19	11.1	40.5	51.6
3	10.0	3.42	42	12	6	61	100	14.8	33.2	35.2
4§	8.3	5.35	33	29	6	0	0	11.4	0	45.9¶
5	5.53	3.71	30	10	4	72	100	14.3	0	0
6	23.8	2.81	32	10	6	35	97	14.7	38.2	37.0
7	18.8	5.32	29	19	8	62	100	12.2	36.6	35.4
8	20.1	3.04	30	11	1.3	63	100	12.1	0	0
9	16.6	3.7	16	15	3.3	23	97	11.7¶	0	0
10	15.1	3.64	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.

Other Recent Successful Attempts at Reduced Intensity Conditioning for Patients with Hemoglobinopathies

- Krishnamurti L, et al. *Curr Probl Pediatr Adolesc Health Care.* Jan 2008;38(1):6-18
- Resnick IB, et al. *Bone Marrow Transplant.* Nov 2007;40(10):957-964

❖ **Results suggest that reduced intensity conditioning is feasible.**

❖ **But, that the rate of graft rejection may be as high as 15-20% in the absence of chronic immune suppression**.

Despite Successes, Patients Continue to Face Significant Toxicities of HSCT:

- **Rejection—SCD patients are at increased risk.**
- **Graft-versus-host disease**
- **Neurologic Complications: Intracranial hemorrhage and encephalopathy with seizures.**
  - **Implementation of specific supportive measures, has reduced the incidence of hemorrhage.**
  - **Seizures are an ongoing risk**
- **Most common complication: hypogonadism and infertility**

For Children, An Opportune Time to Develop a Safer, Yet Still Highly Effect Approach to BMT

- **An approach that….**
- **Maintains a Sustained Engraftment Rate of at least 95%.**
- **Decreases the rate of Neurological Complications to less than 10%.**
- **Decreases the rate of infertility to less than 25%.**

The Safe BMT for SCD Trial:

PI: John Horan, Children's Healthcare of Atlanta

Primary Objective:

- Determine the feasibility of reducing

Cytoxan: From 200 mg/kg → to 90 mg/kg

Busulfan: From 12.8 mg/kg → 9.6 mg/kg

By incorporating Fludarabine:

A non-vasculopathic, non-gonadotoxic, yet potent T cell depleting agent into the BMT.

The Safe BMT for SCD Trial:

PI: John Horan, Children's Healthcare of Atlanta

•Multicenter Trial:

South Carolina, Mississippi, Louisiana, Texas, Boston, North Carolina, Birmingham, Detroit, St. Petersburg FL, Columbus, NYC, Atlanta

•Open to Patients with SCD who have a matched sibling donor

•For US Residents Funds Available at Children's Healthcare of Atlanta to Facilitate Transplant Without Regard to Insurance Status.

For the SCD Patient Without an HLA Matched Sibling: The Outlook is More Complicated

•BMT is More High-Risk

- **Rejection Rates are Higher**
- **Risk of Graft-versus-host disease increases**
- **Immune Dysfunction Post-transplant Increases**

Cord Blood Transplant Trial Closed Due to High Rates of Rejection

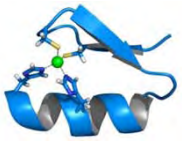
•Several Novel Approaches are Now Being Designed For Adults and Children With HLA-mismatched Related Donors.

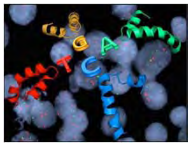
•Significant Efforts Underway to Understand The Immune Effects of Sickle Cell Disease So That Targeted Therapies Can Be Designed.

•Remains a Critical Area of Unmet Need For Our Patients.

On The Horizon: Autologous BMT: Gene Correction of A Patient's Own Stem Cells To Cure Their SCD:

Curing SCD without the need for a matched-sibling donor or Allogeneic BMT

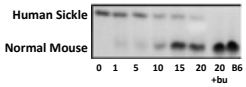




Question: What Level of Gene Correction Will Cure Sickle Cell Disease? Or Would This Approach Really Be A Feasible Cure?

Strategy:
•Without myeloablation, transplant increasing amounts of normal, healthy stem cells into Sickle Cell Disease Mouse

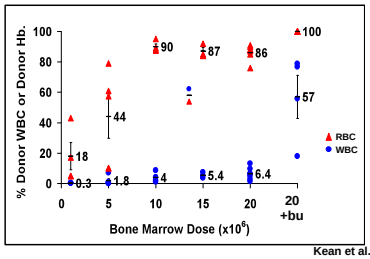
Goal:
•Create low level Stem Cell Correction and Determine the Degree of RBC Replacement with Low Levels of Bone Marrow Chimerism



Dose of Normal Bone Marrow $x10^6$

Kean et al., Blood 2003

WBC Chimerism As Low a 1-5% Leads to High Levels of RBC Chimerism and Correction of SCD Pathophysiology



Kean et al., Blood 2003

•These results suggest that gene correction of 1-5% could cure Sickle Cell Disease.

•Provides the rationale for the gene correction strategy currently being pursued by multiple research groups.

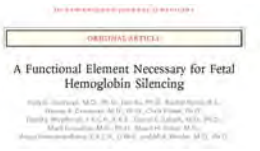
Progress is Being Made….

STEM CELLS

Regenerative Medicine
In Situ Genetic Correction of the Sickle Cell Anemia Mutation in Human Induced Pluripotent Stem Cells Using Engineered Zinc Finger Nucleases†‡ §

Vittorio Sebastiano1,2, Morgan L. Maeder4,5, James F. Angstman4, Bahareh Haddad1, Cyd Khayter4, Dana T. Yeo1, Mathew J. Goodwin4, John S. Hawkins1, Cherie L. Ramirez4,5, Luis F. Z. Batista3, Steven E. Artandi3, Marius Wernig1,¶,*, J.Keith Joung4,5,6,ll,*

Exciting Experimental Alternative: Inducing a Functional Cure of SCD Through Increasing Fetal Hemoglobin Through Gene Targeting Techniques


ORIGINAL ARTICLE

A Functional Element Necessary for Fetal Hemoglobin Silencing

Science AAAS

Science. 2011 Nov 18;334(6058):993-6. Epub 2011 Oct 13.
Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing.
Xu J, Peng C, Sankaran VG, Shao Z, Esrick EB, Chong BG, Ippolito GC, Fujiwara Y, Ebert BL, Tucker PW, Orkin SH.
Source

Division of Hematology/Oncology, Children's Hospital Boston and Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.

Emerging Therapies for SCD: Conclusions:

- Bone Marrow Transplantation Is Currently the Only Curative Therapy for Sickle Cell Disease.
- Transplants, especially from matched-sibling donors, are getting safer, and new data supports reduced intensity transplants and cure with mixed-chimerism is feasible.
- Several trials ongoing, with mechanisms for financial aid for eligible patients, with matched sibling donors.
- Intense efforts to apply HSCT to patients without matched siblings are underway.
- The survival advantage of normal RBC compared to Sickle RBC means that even low levels of stem cell correction can cure disease.
- This has inspired several new initiatives designed to manipulate the genes that result in SCD and cure SCD by gene therapy and autologous transplant.

Acknowledgements:

The Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta:

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Katherine Spencer, PNP
Elyse Bryson, PA

Ify Osunkwo, MD, MPh
Peter Lane, MD
Clark Brown, MD

Aflac Cancer Center & Blood Disorders Service
Children's Healthcare of Atlanta

EMORY UNIVERSITY SCHOOL OF MEDICINE

Survival isn't everything

- Neurologic:
  - 93% stroke-free survival
  - 1 pt had second stroke as S fraction rose after rejection, 1 had ICH
  - Many MRI's stable to improved, including most silent stroke patients
  - CNS events and vascular disease progression are arrested after BMT for SCD in this series
  - Efficacy of stroke prevention favorable compared to chronic transfusion

Key issues in BMT for non-malignant disease

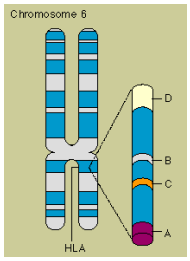
- Relative risks and benefits to weigh
- Stable mixed chimerism an acceptable goal
- Graft-versus-host not helpful

The DONOR: Who?

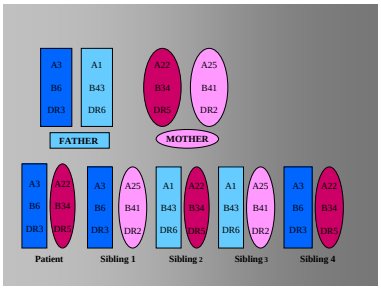
- HLA testing and matching
- Healthy matched sibling
  - Can have sickle trait
  - Gender doesn't matter
  - Blood type doesn't matter
  - Age generally doesn't matter
- "Alternate" donors
  - Partially mismatched family member
  - Unrelated donors from registries

Human Leukocyte Antigen

- Determines self vs non-self
- Inherited on short arm of chromosome 6
- Class I
  - HLA-A, HLA-B, HLA-C
- Class II
  - HLA-DR, HLA-DQ, HLA-DP
- Haplotypes
  - inherit one from each parent



Human Leukocyte Antigen



Extended family typing usually not helpful unless there is consanguinity

Difficulty finding African American donors

- Few otherwise eligible SCD patients have suitable matched sibling donor
- NMDP currently has over 5 million donors
  - Only about 400,000 (8%) are African American
- NMDP minority recruitment efforts
  - www.marrow.org or 1-800-MARROW
  - No-fee donor registration

Waiting for engraftment

For every BMT patient

- Isolation
- Low blood counts
- Transfusions
- Infection prevention/treatment
- GVHD prevention
- Controlling symptoms
- Coping with acute illness
- Staying busy, staying active
- Treatment of complications
- Individualized psychosocial support

Special for SCD

- Keep platelets >50,000
- Keep hgb goal 9-11 g/dl
- Suspicion for pneumococcus
- More aggressive GVHD prevention
- More vigilant control of hypertension
- Prolonged seizure prophylaxis
- Maintain serum Mg >2

Outpatient recovery

For every BMT patient

- Polypharmacy
- Transfusions
- Tests
- Regaining strength
- Coping with body changes
- Avoiding infection
- Treatment of complications
- BMT clinic visits
- Re-hospitalization
- Psychosocial support

Special for SCD

- Keep platelets >50,000
- Keep hgb goal 9-11 g/dl
- Prophylaxis for pneumococcus
- Slower CSA taper
- Vigilant control of hypertension
- Prolonged seizure prophylaxis
- Maintain serum magnesium >2
- Looking for SCD complications

Did it work?

- Engraftment studies
  - The counts
  - Chimerism
- Hemoglobin electrophoresis
- Arrest of clinical sickle events

Dosing

- Fludarabine 35 mg/m2/day for 3 doses (-4 to- 2)
- ATG 30 mg/kg/day for 3 doses (-4 to- 2)
- Busulfan and Cyclophosphamide
- Dose level 1:
  - Busulfan 12.8 mg/kg IV (16 doses)
  - Cyclophosphamide 135 mg/kg (3 doses)
- Dose level 2
  - Busulfan 12.8 mg/kg IV (16 doses)
  - Cyclophosphamide 90 mg/kg (2 doses)
- Dose level 3
  - Busulfan 9.6. mg/kg IV (12 doses)
  - Cyclophosphamide 90 mg/kg (2 doses)

What can be done about sickle cell?

Supportive

- Diagnose early (newborn)
- Education
- Prophylactic PCN
- Recognize complications
- Treatment of pain
- Empiric antibiotics

Interventions

- Drugs to increase HbF
- Chronic transfusions and chelation
- Erythrocytapheresis

Cure

- Gene therapy
- Transplant
  - Matched sib
  - Alternate donor

New outlook
New risks

Who has had BMT for sickle cell?

- First BMT in 1984: leukemia patient with sickle c
- Since then
  - Over 200 patients worldwide
    - Pesaro n=19
    - France n=60
    - Multicenter n=59
    - Belgium n=50
    - Other Europe/US n=10
  - Overall survival is 91% (n=201)
  - Rejection 8%
  - Stable mixed chimerism 11%
  - Chronic GVHD 12%

NIH/NHLBI Workshop, 2003

What are the results?
Myeloablative Matched Sibling SCD BMT

	Wordwide	Walters et al	Atlanta
Total	n = 175	n = 55	n = 20
OS 93%		95%	100%
DFS 85%		85%	100%
Rejection	12%	9%	0%
aGVHD	10%	15%	0%
cGVHD	12%	11%	10%

Survival isn't everything

- Median 4 year follow-up
- Of the 50 engrafted patients
  - No VOC, ACS, sequestration or RBC transfusion
  - QOL appears good, though no formal study
- Growth: grew better after an initial lag
- Endo: Most girls had primary amenorrhea

A Year After Transplant: The "new" normal

- Elimination/decrease of sickle complications.
- Dealing with transplant-specific issues
- Stable grafts
- The Cured Patient: "Ex-sickle"!