Umbilical Cord Blood Transplantation
Current Results

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Donor Choices

Unrelated Marrow/PBSC

Results in SCID
- 50-77% survival
- complete immune reconstitution
- limited by donor availability
- potential for late effects (CGVHD, infertility, growth)

Unrelated UCB

Haploidentical Relative
Unrelated Donor Bone Marrow Transplantation
Limitations

- Adverse effect of HLA mismatch restricting access
- Prolonged interval between search initiation and donor acquisition
- High risk of acute and chronic GVHD
- High risk of opportunistic infection
Haploidentical Related Donor Bone Marrow Transplantation

Limitations

• Prolonged immune reconstitution
• High risk of opportunistic infection
• KIR mismatching requirement?
• Impact of TCD on relapse?
UCB as an alternative stem cell source

Immune Properties
- ↓ cytotoxicity
- ↑ suppressor cell activity
- altered T cell cytokine production profile
- tolerance to NIMA
- Less HLA restriction
- No requirement for TCD
- Reduced GVHD

HSC Properties
- ↑ repopulating capacity
- high frequency of LTC-IC, ML-IC and SRC
- amenable to ex vivo expansion? transduction?
- Engraftment despite low cell dose
- Reduced GVHD
Hypothesis

UCB will extend the donor pool allowing greater access to HSCT.

• Target collections
• Tolerability of HLA disparity
Question

Will transplantation of UCB reduce TRM and improve survival?

• General overview
• Registry data on outcomes in SCID/WAS
**Patient Eligibility**
- Age 0-55
- No available 5-6/6 HLA matched related donor

**Donor Eligibility**
- $< 2$ HLA ag mm
- HLA typing
  - HLA A and B (serological level)
  - HLA DRB1 (high resolution)
Rapid Availability

Formal Search Time

BM
- 19 days (1-257)
- 30 days (10-101)
- 50 days (32-293) (N = 58)

UCB
- 13.5 days (2-387) (N = 50)

Donor identified
Donor available
Unrelated Donor UCB Transplantation
Cell dose rather than HLA restricts UCB Use

2003-2004
- 122 UCB searches
- UCB donors identified for 120 patients

1999-2001

% Patient Referrals

HLA  |  Cell Dose

Children  |  Adult

University of Minnesota
Search Outcome

UCB donors can be identified for nearly all patients

HLA match and cell dose are not limiting

Donor identification is rapid
Neutrophil Recovery

- Median NC = $3.1 \times 10^7$/kg (0.7-58)
- Median CD34 = $2.8 \times 10^5$/kg (0.4-39)
- Median CD3 = $8.0 \times 10^6$/kg (0.003-1002)

88% (81-95%)

Median (range): 23 (9-54)
Neutrophil Recovery by HLA Disparity

Days

0 7 14 21 28 35 42

HLA 1 antigen match

HLA 2 antigen mm

p = NS

Incidence

0.0 0.2 0.4 0.6 0.8 1.0

Days

0 7 14 21 28 35 42
Neutrophil Recovery by Nucleated Cell Dose (x10^7/kg)

p = 0.06

[Graph showing neutrophil recovery over days with different dose ranges indicated]
Rate of neutrophil recovery is cell dose dependent
Engraftment is cell dose dependent

- Incidence vs. Days
- Engraftment rates:
  - < 1.7
  - 1.7-2.7
  - 2.8-5.4
  - ≥ 5.5
- Significance: p < 0.01
CD34+ Cell Dose ($x10^5$/kg) defines the critical threshold

Units with a CD34 dose $<1.7 \times 10^5$/kg (NC dose $<2.0 \times 10^7$/kg) are NOT acceptable for routine use.
# Neutrophil Recovery
## Multiple Regression Analysis

<table>
<thead>
<tr>
<th>HLA Match</th>
<th>Relative Risk</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/6*</td>
<td>1.0</td>
<td>NS</td>
</tr>
<tr>
<td>4/6</td>
<td>0.9 (0.6-1.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CD34 Dose (x10^5/kg)</th>
<th>Relative Risk</th>
<th>P-Value</th>
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</thead>
<tbody>
<tr>
<td>&lt;1.7</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1.7-2.7</td>
<td>1.7 (0.8-3.6)</td>
<td>0.14</td>
</tr>
<tr>
<td>2.8-5.4</td>
<td>2.6 (1.3-5.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&gt;5.5</td>
<td>4.7 (2.2-9.8)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Engraftment Outcome

Engraftment is high in patients with malignancy

Rate of recovery is cell dose dependent

Cell dose $<1.7 \times 10^5$ CD34/kg is unacceptable
Acute GvHD

Incidence vs Days for Grade II-IV and Grade III-IV

HLA Disparity:
- 0=14
- 1=44
- 2=42
- 3=2
Grade II-IV Acute GvHD by HLA Disparity

p = NS

HLA 0 ag mm
HLA 1 ag mm
HLA 2 ag mm

Days

Incidence
# Acute GVHD

## Multiple Regression Analysis

<table>
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<th>HLA Match (graft vector)</th>
<th>Relative Risk</th>
<th>P-Value</th>
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<tbody>
<tr>
<td>6/6 and 5/6§</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>4/6</td>
<td>1.2 (0.5-2.7)</td>
<td>NS</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>CD3 Dose (x10⁶/kg)</th>
<th>Relative Risk</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>≥8</td>
<td>0.7 (0.2-1.9)</td>
<td>NS</td>
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<table>
<thead>
<tr>
<th>Age (decade)</th>
<th>Relative Risk</th>
<th>P-Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1.0 (0.98-1.03)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Chronic GvHD

Incidence

Months

0.0
0.2
0.4
0.6
0.8
1.0

0 2 4 6 8 10 12

10% (4-16%)
Chronic GVHD by Prior AGVHD

Incidence vs Months

prior AGVHD

no prior AGVHD

p = NS
GVHD Outcome

GVHD risk is low despite HLA mismatch
Transplant Related Mortality by CD34+ Cell Dose (x10^5/kg)

- p < 0.01
- Incidence
- Months
Treatment Related Mortality by Age

p < 0.01

Incidence

Months

Treatment Related Mortality by Age

p < 0.01

Incidence

Months
Incidence of TRM is low if the cell dose is $>1.7 \times 10^5$ CD34/kg
Survival by CD34+ Cell Dose (x10^5/kg)

- < 1.7
- 1.7 - 2.7
- 2.8 - 5.4
- > 5.4

p < 0.01
Survival is cell dose and HLA match dependent

Survival by CD34+ Cell Dose (x10^5/kg)
HLA 1 ag mm Recipients

Survival by CD34+ Cell Dose (x10^5/kg)
HLA 2 ag mm Recipients

p = 0.03
# Survival
## Multiple Regression Analysis

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</tr>
<tr>
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<td>0.3 (0.1-0.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Grade II-IV Acute GVHD (time-dependent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No*</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.5 (1.5-7.9)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
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Negative effect of HLA mismatch can be partially overcome by increasing cell dose.
Survival Outcome

Survival is impacted by cell dose and HLA match

Impact of HLA mismatch is partially overcome by higher cell dose
UCBT in the Treatment of Immunodeficiency Disorders

Critical issues:

• Most patients are young
• Many patients come into transplant with infections
• Most do not have HLA identical sibling donors
UCBT for Severe Immune Combined Immunodeficiency

• 57 transplanted February 1995-Northern 2004
• 52 with outcome data reported.
# Patient Characteristics (n=52)

**Age:** Range: 1 month - 4 years 11 months  
Median: **11 months**

**Sex:** 60% male

**Ethnicity:** 59% Non-Caucasian

**Transplant Center:** 23% non-US

**Prior Transplant:** n=5

**HLA Match:**
- 6/6 n=6
- 5/6 n=18
- 4/6 n=24
- 3/6 n=4

**TNC Dose (x10⁷/kg):**
- ≥ 10 (n=30)
- 5.0-9.9 (n=19)
- 2.5-4.9 (n=3)
- Median 11.3 x 10⁷/kg
Conditioning Regimen

- ATG, Busulfan, Cyclophosphamide: 23 patients
- ATG ± Other: 15 patients
- Other: 5 patients
- Unknown: 9 patients
Myeloid Engraftment

% with ANC 500

Days Post-Transplant

(Kaplan-Meier)

(Cumulative Incidence)

81%

75%

NYBC NCBP
Grade II-IV Acute GvHD

% with Acute GvHD

Days Post-Transplant

(Kaplan-Meier) 29%

(Cumulative Incidence) 24%
5 Year Survival in Children with SCID

% Surviving (K-M)

0 1 2 3 4 5

Years Post-Transplant

57%
UCBT for the Treatment of Wiskott-Aldrich Syndrome

- 38 transplanted March 1996-December 2004
- 33 with outcome data reported
WAS Patient Characteristics (n=33)

**Age:** Range: 2 month - 7 years 10 months  
Median: 19 months

**Sex:** 100% male

**Ethnicity:** 59% Non-Caucasian

**Transplant Center:** 30% non-US

**Prior Transplant:** n=0

**HLA Match:**
- 6/6  n=3
- 5/6  n=15
- 4/6  n=12
- 3/6  n=3

**TNC Dose (x10^7/kg):**
- ≥ 10  n=10
- 5.0-9.9  n=19
- 2.5-4.9  n=4

Median 8.0 x 10^7/kg
Conditioning Regimen

ATG, Busulfan, Cyclophosphamide: 22 patients
ATG ± Other: 6 patients
Other: 1 patient
Unknown: 4 patients
% with ANC 500

(Kaplan-Meier)

Myeloid Engraftment

Days Post-Transplant

Cumulative Incidence

NYBC NCBP

3/05
Grade II-IV Acute GvHD

% with Acute GvHD

(Kaplan-Meier) 27%

(Cumulative Incidence) 26%

Days Post-Transplant

NYBC NCBP 3/05
5 Year Survival in Children with WAS

% Surviving (K-M)

Years Post-Transplant

67%
Conclusions:

- Engraftment has been suboptimal for patients with SCID despite high cell dose.
- No data on immune reconstitution.
- Survival is comparable to results with HLA matched unrelated donor marrow.
Unrelated Donor UCB Transplantation
Next Generation

**Improve**
**Engraftment**
**Reduce TRM**
**Reduce Late Effects**
**Improve Survival**

Enhance Host Immune Suppression
Augment the Number of Hematopoietic Stem and Progenitor Cells
Eliminate Pre and Post Transplant Myelotoxic Drugs

Improve Engraftment Reduce TRM Reduce Late Effects Improve Survival

Enhance Host Immune Suppression
Augment the Number of Hematopoietic Stem and Progenitor Cells
Eliminate Pre and Post Transplant Myelotoxic Drugs
Effect of Enhanced Immune Suppression

- TBI: 165 cGy/fraction
- CY: 60 mg/kg/dose
- FLU: 25 mg/kg/dose
- MMF: 1 g q12 hr d0-30
- CSA (maintain level 200-400)
- G-CSF: 5 mg/kg/day
Improved Engraftment in Patients Aged ≥ 16 Years: due to addition of Fludarabine to the Preparative Regimen

Weeks Post-Transplant
% with ANC 500
(Kaplan-Meier)

Cox Regression
RR
P
Minnesota (n = 28)  1.69  0.017
Other US (n = 245) Reference
Non-US (n = 49)  0.64  0.038
Minnesota (n = 28)  1.69  0.017
Other US (n = 245) Reference

P = 0.002
Development of a new clinical model

Advantages
- Infusion on day 0
- Genetic marker

Cytoreductive Therapy

Ex vivo culture

HLA ≤ 2 ag mm
CD34 >0.85 x 10^5/kg

UCB 2

HLA ≤ 2 ag mm
CD34 >1.7 x 10^5/kg

UCB 1

Speed of PMN Recovery

TIME

BMBx

BMBx
Patient Eligibility

- Age 18-55 years (myeloablative therapy)
- High risk and/or advanced hematological malignancy
- No available 5-6/6 HLA matched related donor

Donor Eligibility

- Unit 1
  - $\geq 1.5 \times 10^7$ NC/kg combined
  - $\leq 2$ HLA ag mm

- Unit 2
  - $\leq 2$ HLA ag mm
Neutrophil Recovery
CY-FLU-TBI 1320

ANC ≥ 0.5 @ 23 days (15-41)

100%

N=37 adults

Cumulative Incidence

Days

0.0 0.2 0.4 0.6 0.8 1.0

0 7 14 21 28 35 42
Disease-Free Survival

For Patients Transplant in CR
72% (95% CI: 49-95)

N=27 adults

University of Minnesota
The Second Unit is Additive Based on Mathematical Model

Mean diff = -0.83 days (1.32)

Observed Days to Engraftment for Double UCBT

Predicted Days to Engraftment using Infused CD34+ Dose of both units

Predicted Days to Engraftment using Infused CD34+ Dose of Winning Unit
Is engraftment after double UCBT superior to single UCBT?

Days

Cumulative Proportion

Double UCBT

Single UCBT

n=37

p<0.01
Hypothesis

A non myelablative therapy will reduce the period of neutropenia by allowing host hematopoiesis to continue until donor HSC recovery and reduce TRM and potentially late effects (sterility).
Non-Myeloablative Regimen

- Cytoxan 50 mg/kg
- Fludarabine 200 mg/m²
- sTBI 200 cGy
- Mycophenolate - 3 to + 30
- G-CSF until ANC >2500/uL
- CSA - 3 to + 100
- Fludarabine 200 mg/m²
- Cytoxan 50 mg/kg
- CSA - 3 to + 100
- Mycophenolate - 3 to + 30
- G-CSF until ANC >2500/uL

Eligibility:
- < 70 yrs
- Heme malignancy
- High risk for TRM
  - age ≥ 45
  - extensive prior Rx
  - poor fitness

N=97 (30 single; 67 double)
High Incidence of Neutrophil Recovery and Sustained Chimerism

Cumulative Incidence

Days

0.0 0.2 0.4 0.6 0.8 1.0

ANC ≥ 0.5 @ 8 days
Range 5-32

90% (95% CI: 82-98%)
Unrelated Donor UCB Transplantation

Measures of success

• uUCBT surpasses uBMT/PBSC in children in U.S., Europe and Japan!

• uUCBT > uBMT in children and adults in Japan as reported at the JSH December 19, 2003. (18 UCBT in 1997 to 562 UCBT in 2003; total 1750)
The Next Generation Research

Cells to facilitate engraftment

- MSC
- Allogeneic NK cells
- CD4+/CD25+ T regulatory cells

IBMI

Ex vivo expansion culture

Up regulation of homing receptors
Assisted Reproduction in patients with Genetic Disorders
**Ovarian Hyperstimulation**

**Goal 10-20 Oocytes**

- **Gonadotropin stimulation**
- **Daily estradiol monitoring**
- **Daily transvaginal U/S**

Oocyte retrieval occurs when follicular size is 18-22 mm; estradiol levels are >3000 pg/dL
Embryonic Development

**Prezygote**
- Polar body
- Pronuclei
- Nucleoli
- Zona pellucida
- x 200

**18 hours**
- Prezygote

**2 days**
- 2 cell embryo
- 8 cell embryo

**3 days**
- Blastocyst

**5 days**
Embryo Biopsy
Reproductive Biology Associates, Atlanta, Georgia
Trisomy 13
Identification of HLA Matched Embryo Using Allele Specific Amplification of HLA-DQβ and HLA-A

Embryos 2 and 4 are HLA-A and DQ matched with patient
Identification of IVS4+4A→T mutation

Embryos 3 and 9 have FA

Embryo 10 is normal and not a carrier

Embryos 1, 2, 4 and 6 are normal but are carriers
Hatched Blastocyst

- Trophoectoderm
- Extrusion of blastocelic cavity
- Inner cell mass
U/S Guided Embryo Transfer

PGD Center
- Diagnosis
- Identification of embryos to transfer

IVF Center
- Embryo transfer
- Verification of pregnancy thru day 35
- Transfer of care

Limited survival ex vivo

Tip of catheter

Cervix

Uterus
Logistics

- Referring Physician
- PGD Center
- IVF Center
- Obstetrical Unit
- Transplant Center
- Shipment of UCB unit
- HLA matched UCB
Assisted Reproduction for Patients with Immunodeficiency Disorders

- High risk couples need to be informed of the technology
- May be an option for patients with WAS and some with SCID
Decision Algorithm

SCID Patient

Genotypic Identical Donor → BMT

Haploidentical BMT vs Unrelated Donor BMT

Gene Transfer (subpopulations)

IVF/PGD (subpopulations)
The Second Generation

The first generation demonstrated the ‘proof of principal’ that UCB is an acceptable alternative source of HSC.

The second generation will define the full potential of UCB in the treatment of children and adults with malignant and non malignant disorders

Stem Cells  Effector Cells