Pathophysiological Basis for Cord Blood Transplantation
There have now been over 20,000 cord blood transplants done to treat a wide variety of malignant and non-malignant disorders with hematopoietic stem cells.

Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival.

Wagner, JE et al

Blood. 100(5):1611-18, 2002

Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies.

Kurtzberg, J. et al

Blood. 112(10):4318-27, 2008

Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia.

Rocha, V. et al

Advantages of Cord Blood

• Readily available source of hematopoietic stem and progenitor cells

• Able to be used to treat all malignant and non-malignant disorders currently treated by bone marrow transplantation

• Lowered levels of GVHD when used as a single minimally manipulated unit, compared to bone marrow

  ➢ more flexibility in sibling or unrelated donors using partially HLA-mismatched cord blood than bone marrow for transplantation
Disadvantages of Cord Blood

• Slower time to neutrophil and platelet engraftment compared to bone marrow (BM) and mobilized peripheral blood (mPB)

• More limiting numbers of hematopoietic stem and progenitor cells compared to BM and mPB
  - May be more problematic for single unit cord blood transplantation for adult and higher weight pediatric recipients
  - “Enhanced graft failure”
Human Umbilical Cord Blood As A Potential Source Of Transplantable Hematopoietic Stem/Progenitor Cells


*PNAS USA 86:3828, 1989*
Key Points

Broxmeyer et al PNAS 86:3828, 1989

- Numbers of unseparated hematopoietic progenitor cells (CFU-GM, BFU-E, CFU-GEMM) in single collections of cord blood fell within the range reported for successful engraftment by bone marrow cells.
- In its own plasma in unmanipulated form these progenitor cells remained viable in terms of extensive proliferative capacity after being left at room temperature for a number of days and could be shipped by overnight express mail from a distant obstetrical unit.
- Progenitor cells could be cryopreserved, stored frozen for prolonged periods of time, and thawed with high efficiency recovery of viable cells.
Hematopoietic Reconstitution In A Patient With Fanconi Anemia By Means Of Umbilical Cord Blood From An HLA-Identical Sibling


20th anniversary first cord blood transplant (2008)

Eliane gluckman, md
Hopital st. louis, paris

Matthew farrow, recipient
First cord blood transplant

Hal broxmeyer, phd
Indiana university school of medicine
Possible reasons for success of cord blood as a source of transplantable cells even though volume collected is small compared to that of a bone marrow harvest.

1) Enough stem/progenitor available – perhaps enhanced frequency of stem/progenitors per volume of cord blood compared to bone marrow.

2) Enhanced proliferation and/or self-renewal of cord blood vs. bone marrow stem/progenitor cells.

3) Active engrafting cells – as assessed in immune-deficient mice with NOD/SCID genotype.
Enrichment, Characterization and Responsiveness of Single Primitive CD34+++ Human Umbilical Cord Blood Hematopoietic Progenitors with High Proliferative and Replating Potential

Lu, Xiao, Shen, Grigsby, Broxmeyer

Blood 81: 41-48, 1993

Carow, Hangoc, Broxmeyer

*Blood 81: 942-949, 1993*
Immature Human Cord Blood Progenitors Engraft and Proliferate to High Levels in Immune-Deficient SCID Mice

J. Vormoor, T. Lapidot, F. Pflumio, G. Risdon, B. Patterson, H.E. Broxmeyer, and J. Dick

*Blood* 83:2489, 1994
Improved Engraftment of Human Hematopoietic Cells in Severe Combined Immunodeficient (SCID) Mice Carrying Human Cytokine Transgenes

T.A. Bock, D. Orlic, C.E. Dunbar, H.E. Broxmeyer, and D.M. Bodine


• Non Obese Diabetic (NOD) SCID mice highly engrafted with cord blood cells
Immunohistochemistry Represents a Useful Tool to Study Human Cell Engraftment in SCID Mice Transplantation Models

Orazi, Braun, Broxmeyer

*Blood Cells 20: 323-330, 1994*

Staining of cells that engrafted mouse bone using human Ki67, a marker of cell proliferation.
Means to Enhance Engraftment of Living Numbers of Hematopoietic Stem and Progenitor Cells

- **Double cord blood transplantation (Wagner, Barker, Brunstein)**
  - One unit wins out and does not greatly, if at all, accelerate time to engraftment
  - No definitive proof, as of yet, that two cord blood units are better than one
  - More GVHD than single unit.

- **Ex vivo expansion (Delaney et al, Nature Medicine)**
  - No rigorous evidence that human hematopoietic stem cells with long-term marrow repopulating capacity have been expanded, although perhaps there is some expansion of short term repopulating cells in a clinical setting
Means to Enhance Engraftment of Living Numbers of Hematopoietic Stem and Progenitor Cells (con’t)

- **Intrabone transplant** (Frasoni et al Lancet Oncol 9:831, 2008)
  - Some beginning studies in this area but may not greatly accelerate time to engraftment

- **Fucosylation** (Xia et al Blood 104:3091, 2004)
  - Interesting preclinical data, but no clinical trial data yet.
To enhance effectiveness of HSC transplantation, focus on:

- CD26/DPPIV influence on SDF-1/CXCL12-CXCR4 and hematopoietically active cytokines
- Rapamycin m-TOR sensitive pathway

Overall guiding principle for translation of lab bench basic science and preclinical studies to clinical utility: the simpler the better
CD26/DPPIV (dipeptidylpeptidase IV) cleaves dipeptides from the N-terminus after a proline or an alanine.
Diprotin A

- Diprotin A = Ile-Pro-Ile
- Inhibit CD26/DPPIV peptidase activity

Other inhibitors of CD26 include Val-Pyr
Modulation of Hematopoietic Stem Cell Homing and Engraftment by CD26

K.W. Christophersen, Giao Hangoc, Charlie Mantel and Hal E. Broxmeyer

Science 30:1000-1003, 2004
Follow-up papers demonstrating enhancement of homing/engraftment of murine bone marrow HSC by Inhibition of CD26 (Dipeptidylpeptidase IV)

- Inhibition of CD26 Peptidase Activity Significantly Improves Engraftment of Retrovirally Transduced Hematopoietic Progenitors
  C Tian, J. Bagley, D. Forman, J. Iacomini

- CD26 Inhibition Enhances Allogeneic Donor Cell Homing and Engraftment After In Utero Bone Marrow Transplantation
  W.H. Peranteau, M. Endo, O.O. Adibe, A. Merchant, P. Zoltick, and A.W. Flake
  Blood 108:4268-4274, 2006

- Enhanced Homing and Engraftment of Fresh but Not Ex Vivo Cultured Murine Marrow Cells in Submyeloablated Hosts Following CD26 Inhibition by Diprotin A
Enhancement of Homing/Engraftment of Human CD34+ Cells

**Cord Blood**

Inhibition of CD26 in Human Cord Blood CD34+ Cells Enhances Their Engraftment of NOD/SCID Mice.

T.B. Campbell, G. Hangoc, Y. Liu, K. Pollok, and H.E. Broxmeyer

*Stem Cells and Development, 16: 347-354, 2007*

CD26 Inhibition on CD34+ or Lineage- Human Umbilical Cord Blood Donor HSC/HPC Improves Long-Term Engraftment into NOD/SCID/Beta2null Immunodeficient Mice.

K.W. Christopherson, L. Paganessi, S. Napier, and N.K. Porecha

*Stem Cells and Development, 16: 355-360, 2007*

**G-CSF Mobilized Peripheral Blood**

Diprotin A Infusion Into NOD/SCID Mice Markedly Enhances Engraftment of Human Mobilized CD34+ Peripheral Blood Cells

T. Kawai, U. Choi, P-C Lui, N.L. Whiting-Theobold, G.F. Linton, and H.L. Malech

*Stem Cells and Development, 16: 361-370, 2007*
Inhibition of CD26 in Human Cord Blood CD34+ Cells Enhances Their Engraftment of NOD/SCID Mice

Timothy B. Campbell, Giao Hangoc, Ying Liu, Karen Pollok, and Hal E. Broxmeyer

SDF-1/CXCL12 – CXCR4 Axis

Involved in:

• Enhancing survival and decreasing apoptosis of HSC/HPC
  
  e.g.  Lee et al, Blood 99:4307, 2002

• Enhancing ex-vivo expansion of human cord blood hematopoietic progenitor cells
CD26/Dipeptidylpeptidase IV Regulates Potency of Selected Hematopoietic Growth Factors Through Truncation, and Recovery In Vivo After Cytotoxic Stress

H.E. Broxmeyer, J. Hoggatt, S. Cooper, G. Hangoc, S. Farag, L.M. Pelus, T.B. Campbell
In addition to SDF-1/CXCL12 and a number of other chemokines, there are other cytokines that have putative CD26 truncation sites.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Murine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>IL-3</td>
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<tr>
<td>M-CSF</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Epo</td>
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</tr>
<tr>
<td>LIF</td>
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<tr>
<td>Flt3-L</td>
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<tr>
<td>SCF</td>
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<td>No</td>
</tr>
<tr>
<td>TPO</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Clinical Trial

Use of CD26/DPPIV inhibition to enhance engraftment of single cord blood unit transplantation in adults with malignancy

Sherif Farag, MD, PhD
Results are shown for 3-5 1\textsuperscript{0} recipients each and 5 recipients for 2\textsuperscript{0} transplants using pooled 1\textsuperscript{0} BM cells. Significance compared to control for that number of transplanted cells.
Cord blood banking is crucial to the success of cord blood transplantation

Therefore, information on the length of time that cord blood can be stored in a cryopreserved form, and subsequently thawed with efficient recovery of hematopoietic and, perhaps other sources of stem and progenitor cells is important.
Hematopoietic stem/Progenitor Cells, Generation of Induced Pluripotent Stem Cells, and Isolation of Endothelial Progenitors from 21-23.5 Year Cryopreserved Cord Blood

H.E. Broxmeyer, M-R. Lee, G. Hangoc, S. Cooper, N. Prasain, Y-J. Kim, C. Mallett, Z. Ye, S. Witting, K. Cornetta, L. Cheng, and M.C. Yoder

Blood (Brief Report) in press (2011)
<table>
<thead>
<tr>
<th></th>
<th>Percent Recovery compared to T=0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleated</strong></td>
<td></td>
</tr>
<tr>
<td>Cellularity</td>
<td></td>
</tr>
<tr>
<td>10 years</td>
<td>10 (n=10)</td>
</tr>
<tr>
<td>15 years</td>
<td>9 (n=9)</td>
</tr>
<tr>
<td>21/23.5 years</td>
<td>23 (n=23)</td>
</tr>
<tr>
<td>(n= same 9 as at 15 yrs)</td>
<td></td>
</tr>
<tr>
<td><strong>CFU-GM</strong></td>
<td></td>
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<tr>
<td>10 years</td>
<td>10 (n=10)</td>
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<tr>
<td>15 years</td>
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<tr>
<td>(n= same 9 as at 15 yrs)</td>
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</tr>
</tbody>
</table>

(RANGE)
- (10) (47-100)
- (10) (64-100)
- (10) (17-98)
- (10) (21-100)
- (9) (81-100)
- (9) (56-100)
- (9) (81-100)
- (9) (46-100)
- (9) (40-100)
- (9) (29-100)
- (9) (14-100)
- (9) (38-100)
Representative of Colonies Grown from Defrosted 21 to 23.5 year Cryopreserved Cord Blood Cells (cells stimulated with Epo, SCF, IL-3, GM-CSF)
Replating Capacity of Multipotential (CFU-GEMM) and Granulocyte-Macrophage/Macrophage (CFU-GM/M) Progenitor Cell Colonies Isolated from Defrosts of 21 Year Old Cryopreserved Cord Blood \((n=2-3 \text{ exp})\)

<table>
<thead>
<tr>
<th>% Replates with at least one colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1(^0) Colony</strong></td>
</tr>
<tr>
<td>CFU-GEMM (Epo, SCF, IL-3, GM-CSF)</td>
</tr>
<tr>
<td>CFU-GM/M (Epo, SCF, IL-3, GM-CSF)</td>
</tr>
<tr>
<td>CFU-GM/M (GM-CSF, SCF, FL)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th># 2(^0) Colonies/ 1(^0) Colony Replated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-GEMM (Epo, SCF, IL-3, GM-CSF)</td>
</tr>
<tr>
<td>CFU-GM/M (Epo, SCF, IL-3, GM-CSF)</td>
</tr>
<tr>
<td>CFU-GM/M (GM-CSF, SCF, FL)</td>
</tr>
</tbody>
</table>

Primary colonies grew in presence of Epo, SCF, IL-3, GM-CSF or GM-CSF, SCF, FL. Single colonies replated in presence of Epo, SCF, IL-3, GM-CSF.
Percent Engraftment of Primary and Secondary NOD/SCID IL-2Rγnull (NS2) Mice with CD34+ Cells Purified from Human Cord Blood Stored Frozen for 18.5 to 22 Years

Exp #1
2.0x10^4 CD34+ cells/ 10 NS2 mouse

Exp #2
2.5x10^4 CD34+ cells/ 10 NS2 mouse

Exp #3
10x10^4 CD34+ cells/ 10 NS2 mouse
### CD4 T cells

#### Day 1

- **Count:** 10
- **Isotype control:** 9.5%
- **CD25 staining:** 38%

#### Day 5

- **Count:** 10
- **Isotype control:** 47.7%
- **CD25 staining:** 60%

### CD8 T cells

#### Day 1

- **Count:** 10
- **Isotype control:** 3.1%
- **CD25 staining:** 11%

#### Day 5

- **Count:** 10
- **Isotype control:** 44.0%
- **CD25 staining:** 44.0%

---

*Red line:* Isotype control  
*Black line:* CD25 staining
**Induced Pluripotent Stem Cells (iPS cells)**

Reviewed: Yamanaka, S. Cell 137:13-17, 2009

**iPS Cells Generated From Numerous Somatic Cell Types**


**iPS Cells Generated from Cord Blood (CB)**

[endothelial cells derived/generated from human CB]

[CD133+ CB; used CB frozen 5 years]

[CD34+ CB; used CB frozen up to 8 years]
iPS cell induction protocol for CD34+ cells isolated from CB cells stored frozen for 20+ years

Initial CD34 cell No.: 5x10^5 cells
iPS like colonies: 25

* OSMK = Oct 4, Sox2, cMYC, KLF4

Days:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27

2nd Lentiviral iPS infection OSMK*

1st Lentiviral iPS infection OSMK*

Initial colonies picked and transferred to murine embryonic fibroblasts (MEFs)

IMDM 10% FBS
TPO/hSCF/Flt3L

DMEM/F12-20% SR
Y- /VPA/SB/CF

Cord blood defrost: Scott Cooper
Isolation of CD34 fraction of cord blood cells: Dr. Young-June Kim
Viral infection: Dr. Nutan Prasain / Dr. Mervin Yoder
Cell culture: Dr. Man Ryul Lee
OCT4 promoters

Frozen CD – CD34+

FCB-iPS-1

FCB-iPS-2
Endoderm (Respiratory epithelium)

Mesoderm (Cartilage)

Ectoderm (Pigmented retinal epithelium)
Many unknowns remain regarding the realistic potential of iPS and other cell types for regenerative medicine.

The clinical potential and safety of these cells and their differentiated offspring have yet to be determined let alone whether CB may turn out to be a preferable source of starting material for iPS cell generation.

Generation of hematopoietic stem cells from iPS cell regardless of the starting cell population, may not be efficient enough to warrant their generation from iPS cells for clinical utility.

* It is possible that iPS cells may never be ready for “prime-time” clinical use.
Endothelial Progenitor Cells (EPCs)
(Collaboration with Dr. Mervin Yoder’s group)

Defrosts of 20+ year frozen unseparated cord blood cells

↓

EPC colonies formed from isolated CD34⁺ of defrosted cord blood

↓

EPC colonies formed, but the numbers were lower than expected [2-5/10⁷ mononuclear cells (= 1/5 - 1/10 numbers from fresh CB)]

↓

The size of colonies were smaller
Colonies derived from >20y cryopreserved cord blood emerge later and are smaller than fresh umbilical cord blood cells.
Conclusion:

Cryopreservation of cord blood EPCs may require modification of the freezing procedure.