Stem Cell Therapeutic Applications in Leukodystrophies

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Learning Objectives/Outline

• Define stem cells and different cell types
• Review mechanisms of action in cell-based therapy
• Review current clinical trials
• Review potential adverse events
Defining Stem Cells

• Stem cell: unspecialized cell that is capable of replicating itself but also differentiate into specialized cells.
Fig. 1 Various types of stem cells. Adapted from Mitalipov and Wolf [22]
Myeloid Derived Stem Cells

- **Hematopoietic stem cells** - CD34+ cells can give rise to all blood cell types, few controversial reports that under certain conditions in-vitro these cells can become neurons.

- **Mesenchymal stem cells** can be differentiated to a variety of different tissue cells in-vitro including neurons.

- 50,000 adult and pediatric patients/year worldwide have received bone marrow stem cells.
# Umbilical Cord Blood Derived Stem Cells

<table>
<thead>
<tr>
<th>Stem Cell Type</th>
<th>Definition</th>
<th>Putative mechanisms of action</th>
</tr>
</thead>
</table>
| Hematopoietic Stem Cells        | Multipotent cells that can give rise to all blood cell types including myeloid and lymphoid lineages and are CD45 positive | - immunomodulation  
- neurotrophic effect on endogenous cells  
- differentiation into microglia that may release defective enzymes (in metabolic diseases) |
| Mesenchymal Stem Cells          | Multipotent non-hematopoietic cells that differentiate into multiple mesenchymal lineages, and are CD34 negative and CD45 negative | - immunomodulation  
- neurotrophic effect of endogenous cells                                                    |
| Endothelial Progenitor Cells    | Cells that are able to form vessels when transplanted in immune deficient mice, express CD34 but do not express CD45 | - form new vessels in ischemic lesions                                                        |
| Aldehyde Dehydrogenase (ALDH) Positive Progenitor Cells | Cells enriched for both multipotent myeloid and endothelial colony-forming cells | - immunomodulation  
- release of growth factors  
- vessel formation                                                               |
| CD133+ Early Multipotent Stem Cells | Multipotent cells with ability to differentiate into various non-hematopoietic lineages (including neural and glial-like cells in vitro) | - promote axonal growth in  
- potential transdifferentiation into neural cells                                             |
Neural Stem Cells

- Neural stem cells can be derived from adult and fetal human cadavers.
- Mouse embryonic stem cells can be differentiated to neural stem cells in-vitro.
- Neural stem cells have the ability to become neurons, astrocytes, oligodendrocytes and endothelial cells.
Glial Precursor Cells

**Legend:**
- NG2−
- PDGFRα−
- OLIG2+/−
- SOX10−
- NG2− →+ PDGFRα+
  - O4−
  - OLIG2+
  - SOX10+
  - MBP−
- NG2+
  - PDGFRα+
  - Rat O4+/−
  - OLIG2−
  - SOX10−
  - Galactocerebroside−
  - MBP−
- NG2−
  - PDGFRα−
  - O4+
  - OLIG2+/−
  - SOX10+
  - Galactocerebroside+
  - MBP+
- Myelinating oligodendrocyte

**Diagram:**
- Neural stem cell → Polydendrocyte → Premyelinating oligodendrocyte → Myelinating oligodendrocyte
iPS Cells Make Viable Mice

Tetraploid Complementation Assay

Using iPSCs to Study Disease (example Rett syndrome)

Using iPSCs to Study Disease
(example Rett syndrome)
Issues with Reprogrammed Cells

Somatic cells
- De novo genetic variation
- Epigenetic variation
- Parental epigenetic memory
- X chromosome instability

iPSCs
- Clonal variation
- Optimal differentiation protocol

Differentiation
- Neuron
- Astrocytes
- Microglia

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How Exogenous Cells May Help

1. Integration into existing networks
2. Immunomodulation
3. Neuroprotection by trophic support
4. Cell-Based enzyme and gene delivery
Human Glial Precursors remyeliate the shiverer

Windrem MS et al. *Nat Med* 2004
Immunomodulatory Effects

Neural Spheres Migrate and Stimulate Endogenous Stem Cells

FIG. 2. In vivo fate of NDPs injected into ibotenate-lesioned brains. Injected NDPs migrate rapidly to the lesion site (A–D). One day after ibotenate administration and NDP infusion, Dii-positive cells (red) are mostly located in the injected ventricle, and individual cells can be detected in the cerebrospinal fluid. Some NDPs are juxtaposed to the ventricular wall, whereas others are already migrating away from the injected ventricle. On day 2, Dii-positive cells migrate toward the lesion (B, E). Migrating NDPs reach the corpus callosum on day 3 (C, F). On day 4, Dii-positive cells are visible at the level of the lesion (D, G). Blue staining, DAPI; red staining, Dii; V, ventricle; CC, corpus callosum. Color images available online at www.liebertonline.com/scd
Mesenchymal Stem Cell Injection Improves Outcome in Mice with Neonatal Hypoxia-Ischemia

Cells injected into the brain 3 days after HI

Stem Cell-Based Enzyme Delivery
Stem Cell-Based Gene Delivery
A tripotential glial precursor cell is present in the developing spinal cord

(stem cells/neuroepithelium/differentiation/development)

Mahendra S. Rao*, Mark Noble†, and Margot Mayer-Pröschel†‡

†Huntsman Cancer Institute, Biopolymers Building 570, Room 410, University of Utah, Salt Lake City, UT 84112; and *Department of Neurobiology and Anatomy, University of Utah Medical School, 50 North Medical Drive, Salt Lake City, UT 84132

Communicated by Raymond L. White, University of Utah, Salt Lake City, UT; December 5, 1997 (received for review September 4, 1997)
Glial Precursors Migrate Along the White Matter
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Bone Marrow Stem Cell Transplantation

• Has improved outcome in some neurometabolic disease.

• High risk of mortality (5-30%) pending on patient’s age, baseline disease and other factors.

• High morbidity due to chemotherapy and immunosuppressive treatment leading to infections and organ damage.
1. Immunosuppress recipient to prevent graft rejection
2. Reduce number of tumour cells
3. Reduce number of recipient haematopoietic cells
<table>
<thead>
<tr>
<th>Category</th>
<th>Diagnosis</th>
<th>HSCT reported</th>
<th>Current status of HSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucopolysaccharidosis</td>
<td>MPS I, severe phenotype</td>
<td>BMT(^1), UCBT(^2)</td>
<td>Standard of care</td>
</tr>
<tr>
<td></td>
<td>MPS II with CNS disease</td>
<td>BMT(^3), UCBT(^4)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>MPS III A-D</td>
<td>BMT(^5), UCBT(^6)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>MPS IV A-B</td>
<td>BMT(^7)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>MPS VI</td>
<td>BMT(^8), UCBT(^9)</td>
<td>If failed ERT</td>
</tr>
<tr>
<td></td>
<td>MPS VII</td>
<td></td>
<td>Investigational</td>
</tr>
<tr>
<td>Glycoproteinosis</td>
<td>Aspartylglucosaminuria</td>
<td>BMT(^10)</td>
<td>Standard of care</td>
</tr>
<tr>
<td></td>
<td>Fucosidosis</td>
<td>BMT(^11)</td>
<td>Standard of care</td>
</tr>
<tr>
<td></td>
<td>Alpha-Mannosidosis</td>
<td>BMT(^12), UCBT(^13)</td>
<td>Standard of care</td>
</tr>
<tr>
<td></td>
<td>Mucolipidosis II or I-cell disease</td>
<td>BMT(^14), UCBT(^15)</td>
<td>Standard of care</td>
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<tr>
<td>Sphingolipidosis</td>
<td>Fabry</td>
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<td>Not indicated</td>
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<td></td>
<td>Farber</td>
<td>BMT(^16)</td>
<td>Investigational</td>
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<tr>
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<td>Gaucher</td>
<td>BMT(^17)</td>
<td>Investigational for CNS involvement</td>
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<td>GM1 gangliosidosis</td>
<td>BMT(^18), UCBT(^19)</td>
<td>Investigational</td>
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<td>Niemann-Pick disease A and B</td>
<td>BMT(^20), UCBT(^21)</td>
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<td>Tay-Sachs disease</td>
<td>BMT(^22), UCBT(^23)</td>
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<tr>
<td></td>
<td>Sandhoff sisease</td>
<td>UCBT(^24)</td>
<td>Investigational</td>
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<tr>
<td></td>
<td>Globoid leucodystrophy</td>
<td>BMT(^25), UCBT(^26)</td>
<td>Standard of care</td>
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<td>Metachromatic leucodystrophy</td>
<td>BMT(^27), UCBT(^28)</td>
<td>Standard of care</td>
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<tr>
<td>Other lipidoses</td>
<td>Niemann-Pick disease C</td>
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<td>Not Indicated</td>
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<td>Wolman disease</td>
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<td>Ceroid lipofuscinosis</td>
<td>BMT(^30)</td>
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<tr>
<td>Glycogen storage disorders</td>
<td>GSD type II, early infantile</td>
<td>BMT(^31)</td>
<td>Investigational</td>
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<tr>
<td>Peroxisomal storage disorders</td>
<td>Adrenoleucodystrophy</td>
<td>BMT(^32), UCBT(^33)</td>
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<td>Adrenomyeloneuropathy</td>
<td>BMT, UCBT(^34)</td>
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<tr>
<td>Other</td>
<td>Pelizaeus-Merzbacher disease</td>
<td>UBCT(^34)</td>
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<tr>
<td></td>
<td>Lesch-Nyhan</td>
<td>UCBT(^34)</td>
<td>Investigational</td>
</tr>
</tbody>
</table>

'Standard of care' – Significant single institution or registry based studies demonstrating efficacy of HSCT. Each case should be evaluated for risk and benefits based on many factors including status of disease, functional status, donor availability, quality of graft amongst others.
Cord Blood Nucleated Cell Transplantation in Neurologic Diseases

• Recently used in neurometabolic diseases

• Associated with lower morbidity and mortality, easier to find a matched donor

• Role of autologous transplantation
Autologous Cord Blood Transplantation in Cerebral Palsy

• More than 170 children with diagnosis of cerebral palsy have received autologous CB units as compassionate care at Duke University

• Ongoing trial in USA, NCT01072370, Inclusion Criteria:
  – 1-12 years of age, placebo-controlled, FU after one year
  – Clinical evidence of a non-progressive motor disability due to brain dysfunction. The subjects will not have the ability to sit independently by one year of age or the ability to walk by 18 months of age.

• 2 other studies: Korea (allogeneic + Epo) and Iran (CD133+ autolog)
Autologous Cord Blood Transplantation in HIE

• Two ongoing trials listed at clinicaltrials.gov:
  NCT00593242 (Duke Univ. USA)
  - Term infants with moderate to severe encephalopathy that missed cooling receive autologous cord blood within first 14 days
  - Compared to historical controls
  - Outcome will be assessed at 9-12 months

NCT01506258 (Mexico)
  - Term infants with HIE within the first 48h of life
  - Compared to controls who refuse therapy
  - Outcome at one year
Central nervous system stem cell transplantation for children with neuronal ceroid lipofuscinosis

Clinical article

Nathan R. Selden, M.D., Ph.D.,1,8 Amira Al-Uzri, M.D.,2,8 Stephen L. Huhn, M.D.,9
Thomas K. Koch, M.D., 2,3 Darryn M. Sikora, Ph.D.,2 Mina D. Nguyen-Drivers, Psy. D.,2
Daniel J. Guillaume, M.D., M.Sc.,1,8 Jeffrey L. Koh, M.D.,4 Sakir H. Gultekin, M.D.,5
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Yakov Jacobs, Ph.D.,9 and Robert D. Steiner, M.D.,2,7,8

Departments of 1Neurological Surgery, 2Pediatrics, 3Neurology, 4Anesthesiology and Peri-Operative Medicine, 5Pathology, 6Diagnostic Radiology, and 7Molecular and Medical Genetics, Oregon Health & Science University; 8Doernbecher Children’s Hospital, Portland, Oregon; 9StemCells, Inc., Newark; 10Department of Pathology, Stanford University Medical School, Stanford; and 11Department of Pediatrics Palo Alto Medical Foundation, Los Altos, California
HuNSC Tx in Batten Disease

• In six patients with advanced stages of infantile and late infantile Neuronal Ceroid Lipofuscinosis due to PPT1 deficiency.
• Cells directly transplanted into two different sites in each hemisphere via stereotaxic surgery, three patients got 500mil cells, three got 1bil cells
• Patients immunosuppressed for 12 months
• Monitored for four years, three patients all due to NCL disease progression
• Autopsy no evidence of malignancy
Evidence of Cell Migration in Autopsy Case
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Fetal Stem Cell Injections Create Brain Tumors in Israeli Boy

BY LIFESITENEWS.COM
Fri Feb 20, 2009 12:15 EST | Comments (0)
Complications of Hematopoietic Cell Transplantation

- Acute and chronic graft versus host disease
- Engraftment syndrome
- Veno-occlusive disease
- Idiopathic pulmonary syndrome
- Immunosuppression associated infection
- Side effect of chemotherapeutics (preparative regimen)
Lessons Learned from Krabbe Disease
the ‘big thing in 2005’

Transplantation of Umbilical-Cord Blood in Babies with Infantile Krabbe’s Disease

Maria L. Escolar, M.D., Michele D. Poe, Ph.D., James M. Provenzale, M.D., Karen C. Richards, M.D., June Allison, R.N., Susan Wood, P.N.P., David A. Wenger, Ph.D., Daniel Pietryga, M.D., Donna Wall, M.D., Martin Champagne, M.D., Richard Morse, M.D., William Krivit, M.D., Ph.D., and Joanne Kurtzberg, M.D.
Long Term Follow Up Study of Same Cohort Published in 2009

• 16 presymptomatic children transplanted at Duke and elsewhere for early infantile Krabbe disease
• Two died
• All others spastic – three mild
• Five required gastostomies
• All were below 3% with height and weight
• All have abnormal expressive language
• 50% walk with assistive devices
• 25% don’t walk

Summary

• Currently ongoing cell based therapy for a number of neurometabolic conditions.
• Advanced cell engineering methods open the door to new therapeutic approaches.
• Expect to have many more trials to come within the next 5-10 years.
• Not all patients will be ideal candidates.
• Stem cell therapy can be harmful, very dangerous complications including death.
• Need for careful investigations to determine who will benefit and in whom it may be harmful.
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