Stem Cell Therapy
Applications in pediatric neurology

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The Author has nothing to disclose in relevance to this lecture
HYPE OR HOPE??

FORGET BLOOD-GET INTO STEM CELLS.
LEARNING OBJECTIVES/OUTLINE

• THE TARGET DISEASE
• DEFINE STEM CELLS AND DIFFERENT CELL TYPES
• REVIEW MECHANISMS OF ACTION IN CELL-BASED THERAPY
• REVIEW CURRENT CLINICAL TRIALS
• REVIEW POTENTIAL ADVERSE EVENTS
Rule of thumb for therapies

• Each condition will require targeted treatment. Stem cells will not cure all diseases.

• If a disease is static, regenerative therapy is much more likely to be effective than if a disease is progressive.

• Need for surrogate markers and proper controls to evaluate therapies.

• DO NO HARM! Need for careful monitoring for adverse events which may outweigh benefits.
TIMING AND MODE OF INTERVENTION IS DISEASE SPECIFIC

- Age of patient (changes in immune response)
- Time of disease onset (more likely to be effective if treatment is early to prevent destruction)
- In rodent studies for different disease models different time points have been determined, but extrapolation to humans very difficult
LEARNING OBJECTIVES/OUTLINE

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• DEFINE STEM CELLS AND DIFFERENT CELL TYPES
• REVIEW MECHANISMS OF ACTION IN CELL-BASED THERAPY
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• REVIEW POTENTIAL ADVERSE EVENTS
Defining stem cells

- Stem cell: unspecialized cell that is capable of replicating itself but also differentiate into specialized cells.
Different cell types

• Human body after birth has > 200 cell types
• All cells derived from embryonic stem cells
• Organs also contain stem cells in niches referred to as ‘somatic stem cells’.
Embryonic stem cell
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 world-wide 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

Neural stem cell → Polydendrocyte → Premyelinating oligodendrocyte → Myelinating oligodendrocyte

- NG2– PDGFRα– OLIG2+/− SOX10–
- NG2– →+ PDGFRα+ O4– OLIG2+ SOX10+ MBP–
- NG2+ PDGFRα+ Rat O4+/− OLIG2+ SOX10+ MBP–
- NG2– PDGFRα– O4+ OLIG2+ SOX10+ Galactocerebroside+ MBP–
- NG2– PDGFRα– O4+ OLIG2+/− SOX10+ Galactocerebroside+ MBP+
“Traditional” Sources of Stem Cells

- Embryos created by research
- Supernumerary embryos from in vitro fertilisation
- Inner Cell Mass
- Aborted foetuses
- Foetal tissues
- Umbilical cord
- Cord blood
- Cell removal


- Embryonic SCs
- Foetal SCs
- Adult SCs

Muscle cells, Liver cells, Blood cells, Neural cells, Skin cells, Endothelial cells
Induced Pluripotent Stem Cells (iPSC)

Shinya Yamanaka

Nobel Prize 2012 in Physiology or Medicine
Induced Pluripotent Stem Cells (iPSC)

- cells that have been genetically reprogrammed to an embryonic stem cell–like state
- initial method based on using 4 different lentiviral vectors containing OCT4, Sox2, Klf4, c-Myc.
- Mouse iPSCs were first reported in 2006, and human in late 2007.
- iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors.
iPS cells make viable mice

Tetraploid Complementation Assay

OUTLINE

• THE TARGET DISEASE
• DEFINING STEM CELLS AND DIFFERENT CELL TYPES
• MECHANISMS OF CELL-BASED THERAPY
  • Clinical trials
  • Potential Adverse Events
How exogenous cells may help

1. Restore tissue by becoming neurons or glial cells and integrate into the neuronal network
2. Restore tissue by promoting activation of endogenous stem cells
3. Alter inflammatory response

Unlikely to have an effect in brain malformations (schizencephaly, lissencephaly, Holoprosencephaly etc.)
Restoration by Differentiation

- Embryonic, neural and CD133+ stem cells are all thought to be able to differentiate into neurons when transplanted directly into the CNS in rodents
Injury

Cell death; disruption of the blood–brain barrier and inflammation

Neurogenic signals \(\to\) Gliogenic signals

Transplantation of multipotent cells

Differentiation

- Partially injured regions: neurons
- Neuron-depleted regions: glia
- Partially injured regions: neurons
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, Dil-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, Dil-positive cells migrate toward the lesion (B, E). Migrating NDPs reach the corpus callosum on day 3 (C, F). On day 4, Dil-positive cells are visible at the level of the lesion (D, G). Blue staining, DAPI; red staining, Dil; V, ventricle; CC, corpus callosum. Color images available online at www.liebertonline.com/scd
INTEGRATION EXAMPLE
Human Glial Precursors remyeliante the shiverer

Windrem MS et al. *Nat Med* 2004
Glial precursors migrate and differentiate along the white matter.

<table>
<thead>
<tr>
<th>Patterns Observed</th>
<th>Animals with OPC transplant assessed at 2 months post-transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Animals with GFP+ cells</td>
<td>13/16</td>
</tr>
<tr>
<td>Animals with GFP+ cells Migrating along corpus callosum</td>
<td>11/16</td>
</tr>
<tr>
<td>Animals with cells migrate laterally</td>
<td>6/16</td>
</tr>
<tr>
<td>Animals with cells migrate medially</td>
<td>8/16</td>
</tr>
<tr>
<td>Animals with cells crossing midline to contralateral hemisphere</td>
<td>6/16</td>
</tr>
</tbody>
</table>
Limited co-localization of GRPs with astrocytes at 1 month

Fatemi A. et al. 2012
Glial precursors migrate and differentiate along the white matter
GRPs differentiate into mature Oligodendrocytes at 2 months

Fatemi A. et al. 2012
How exogenous cells may help

1. Restore tissue by becoming neurons or glial cells and integrate into the neuronal network.
2. Restore tissue by promoting activation of endogenous stem cells.
3. Alter inflammatory response.

Unlikely to have an effect on defects of brain formation or neuronal migration.
Trophic effects

• Many studies show that transplanted animals often have better outcome even if no transplanted cells are found in the brain.
• Many stem cells
  – release growth factors
  – have immuno-modulatory effects.
Mesenchymal stem cell injection improves outcome in mice with neonatal hypoxia-ischemia

Cells injected into the brain 3 days after HI

Mesenchymal stem cell injection increases endogenous stem cell proliferation after neonatal hypoxia-ischemia

Cells injected into the brain 3 days after HI

How exogenous cells may help

1. Restore tissue by becoming neurons or glial cells and integrate into the neuronal network
2. Restore tissue by promoting activation of endogenous stem cells
3. Alter inflammatory response

Unlikely to have an effect on defects of brain formation or neuronal migration
Immunomodulatory effects

OUTLINE

• THE TARGET DISEASE
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• MECHANISMS OF CELL-BASED THERAPY

• CLINICAL TRIALS
  • Potential Adverse Events
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 I. HSCT for various IMD: Reports from literature and our recommendations.

<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>
</tr>
<tr>
<td>Sphingolipidosis</td>
<td>Fabry</td>
<td></td>
<td>Not indicated</td>
</tr>
<tr>
<td></td>
<td>Farber</td>
<td>BMT(^16)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>Gaucher</td>
<td>BMT(^17)</td>
<td>Investigational for CNS involvement</td>
</tr>
<tr>
<td></td>
<td>GM1 gangliosidosis</td>
<td>BMT(^18), UCBT(^19)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>Niemann-Pick disease A and B</td>
<td>BMT(^20), UCBT(^21)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>Tay-Sachs disease</td>
<td>BMT(^22), UCBT(^23)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>Sandhoff sisease</td>
<td>UCBT(^24)</td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>Globoid leucodystrophy</td>
<td>BMT(^25), UCBT(^26)</td>
<td>Standard of care</td>
</tr>
<tr>
<td></td>
<td>Metachromatic leucodystrophy</td>
<td>BMT(^27), UCBT(^28)</td>
<td>Standard of care</td>
</tr>
<tr>
<td>Other lipidosis</td>
<td>Niemann-Pick disease C</td>
<td></td>
<td>Not indicated</td>
</tr>
<tr>
<td></td>
<td>Wolman disease</td>
<td>BMT(^29)</td>
<td>Standard of care</td>
</tr>
<tr>
<td></td>
<td>Ceroid lipofuscinosis</td>
<td>BMT(^30)</td>
<td>Investigational</td>
</tr>
<tr>
<td>Glycogen storage disorders</td>
<td>GSD type II, early infantile</td>
<td>BMT(^31)</td>
<td>Investigational</td>
</tr>
<tr>
<td>Peroxisomal storage disorders (PSD)</td>
<td>Adrenoleukodystrophy</td>
<td>BMT(^32), UCBT(^33)</td>
<td>Standard of care</td>
</tr>
<tr>
<td></td>
<td>Adrenomyeloneuropathy</td>
<td>BMT, UCBT(^34)</td>
<td>Investigational</td>
</tr>
<tr>
<td>Other</td>
<td>Pelizaeus-Merzbacher disease</td>
<td>UBCT(^34)</td>
<td>Investigational</td>
</tr>
<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 disease.
• 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 year of age, placebo-controlled, FU after 1 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

• 2 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 1 year
Phase I (safety) trial of Neural Stem Cells in USA

- First FDA approved study of human fetal tissue derived neural stem cells, was conducted by a biotech company.
- In 6 patients with advanced stages of infantile and late infantile Neuronal Ceroid Lipofuscinosis due to PPT1 deficiency.
- Study completed in 2008, data presented as poster no peer reviewed publication yet.
- Cells directly transplanted into different sites into the brain via stereotaxic surgery, 3 patients got 500mil cells, 3 got 1bil cells.
- Patients immunosuppressed for 12 months.
- Monitored for 4 years, 2 patients died presumably due to disease progression (autopsy: no sign of toxicity), others tolerated treatment well but all progressed.
- Autopsy showed “transplanted cells”, no details published.
Phase I Trial of Neural Stem Cell in a Leukodystrophy in USA

- conducted at a major academic institution in US in collaboration with a Biotech company
- enrolled 4 patients with genetically confirmed Pelizaeus-Merzbacher Disease
- immunosuppression for 9 months
- Cells again directly transplanted into different sites into the brain via stereotaxic surgery
- To be monitored first for 12 months intensely and then for 4 years.
- All patients have been enrolled, report of myelin signal in MRI around the injection site
OUTLINE

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• DEFINING STEM CELLS AND DIFFERENT CELL TYPES
• MECHANISMS OF CELL-BASED THERAPY
• Clinical trials

• POTENTIAL ADVERSE EVENTS
Considerations for Cell Based Therapy

• Stem Cells capable of forming tumors!
• Higher likelyhood with more immature cells like embryonic stem cells and neural stem cells
• Between 5-30% incidence in animals

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)
Graft versus Host disease

Graft cells attack body, very common in bone marrow transplant

• Acute:
  – Within 3 months
  – Diarrhea
  – Liver disease
  – Rash
  – Fever
  – in 19-66% of matched recipients

• Chronic:
  – After 3 months
  – Hairloss
  – Rash
  – Lung and digestive disease
  – Liver disease
  – Approximately one third of patients with matched recipient
Cell Based Therapy for Parkinson Disease

LESSONS LEARNED

- In 1987, a series of small open-label trials, in which only carefully selected patients were included, was initiated.
- Human fetal dopaminergic neurons survived and function for more than 10 years in the striatum of patients with PD, with benefits in some patients.
- 2 double-blind sham-surgery controlled trials were launched in the early 1990s turned out to be more disappointing than expected:
  - showing only modest efficacy was observed in the transplantation groups,
  - a significant number of patients developed worsening dyskinesias after transplantation.
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.
Longterm Follow Up study of same cohort published in 2009

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

Non-academic institutions worldwide currently offering ‘stem cell therapy’

- Often many different diseases are grouped into terms such as ‘cerebral palsy’
- No immunosuppressive therapy is discussed
- Almost all claim that there are no side effects
- Cell source and quality not clear
- No peer-reviewed publications of their claims
- High costs for patients
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 with Cerebral Palsy 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.