Motor Neurone Disease: *Is there a role for Stem Cell Therapies?*

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Introduction

- Motor Neurone Disease
- Exploitable human stem cell populations
- Current evidence from animal models
- Current clinical trials
Motor Neurone Disease (MND)

- Amyotrophic Lateral Sclerosis / Lou Gehrig’s disease
- Adult onset neurodegenerative disease – affecting people 50-70 years of age
- Prevalence = 5000 people in UK (1 in 400 recorded deaths)
- ‘Selective’ degeneration of motor neurones in cortex, brainstem & spinal cord
- Progressive muscle weakness, wasting, paralysis
- Death within 2-5 years of onset
Motor Neurone Disease

- Weakness
- Muscle wasting
- Problems with speech & swallowing
- Problems with breathing
- Sensation intact
- Mild cognitive impairment
Genetics of MND

- 5-10% of cases familial

<table>
<thead>
<tr>
<th>Gene</th>
<th>Familial Cases</th>
<th>Sporadic Cases</th>
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</thead>
<tbody>
<tr>
<td>C9orf72</td>
<td>40%</td>
<td>10%</td>
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<tr>
<td>SOD1</td>
<td>20%</td>
<td>2%</td>
</tr>
<tr>
<td>TARDBP</td>
<td>5%</td>
<td>&lt;1%</td>
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</table>

- Significant clinical & biological heterogeneity
- Why therapies do not translate well into wider disease?

Robberecht, 2013 Nature Rev Neurol. doi:10.1038/nrn3430
Complex pathophysiology of MND

- Toxic Glia
- Protein Aggregation
- Axonal Transport Defects
- Oxidative Stress
- Mitochondrial Dysfunction
- Excitotoxicity

Current therapies for MND

- Extend survival by only 3-7 months
Rationale for stem cell therapies in MND

- Complex pathophysiology – no single ‘drug-able’ pathway
- Loss of a specific cell population – open to replacement
- Role for support cell toxicity / loss of trophic signals – chimerisation of support cell population
Exploitable Human ‘Stem Cell’ Populations

1. Adult Central Nervous system
2. Embryonic / Fetal stem cells
3. Adult Mesenchymal tissues
4. iPS cells
   - Self-renewing ✓
   - Any lineage vs lineage restricted ✓ / ?
   - Teratoma formation in-vivo X
Adult Neurogenesis
Adult Neurogenesis

- Subventricular Zone, Subgranular Layer Hippocampus & within cortex (NG2 cells)
- Astrocytic in origin
- Generate neurones, astrocytes, oligodendrocytes \textit{in-vitro}
- Generate Hippocampal excitatory neurones & Olfactory Bulb interneurones \textit{in-vivo}
- Migratory
- Altered in disease states – Alzheimers, MND, Ageing
- Could be harnessed, mobilized & targeted \textit{in-vivo}?

Morrens, 2012, \textit{Glia} 60:159-174
Transplanting Adult Neural Progenitors

- Neural progenitors from Adult GFP-Mouse
- Injected into spinal cord of SOD-1\textsuperscript{G93A} mouse
- Survive, differentiate & protect endogenous motor neurones
- Supportive niche is important
- Expansion to therapeutically useful numbers may be a problem

Embryonic / Fetal ‘Neural Progenitor’ Cells
Human Embryonic / Fetal NPCs

- Commonest therapeutic model studied *in-vivo (>20 studies)*
- Derived from Blastocyst or 8-12 week old fetus cortex/spinal cord
- Expanded *in-vitro* an conditioned towards neural fate using bFGF, Sonic Hedgehog, Retinoic Acid
- Can form mature phenotypes *in-vitro* (e.g. CHAT+ motor neurones)

Human NPC transplants

- Cervical & Lumbar injections into late pre-symptomatic SOD-1 rats
- Extended survival by 17 days (compare Riluzole = 14 days)
- Delayed decline in motor performance
- Formed functional synaptic connections *in-vivo*

Xu, 2009, *J. Comparative Neurology* 514:297–309
Rat lumbar cord at end-stage (immunosuppression with tacrolimus)

Graft cells co-labelling with neuronal marker *in-vivo*

Rat motor neurone decorated with human synaptophysin positive processes

EM of graft synapse formation with host axon

Clinical trials with Human NPCs

**Neural Stem** (Glass, 2012, *Stem Cells*, 30:1144–1151)

- Mixed population of neural & glial progenitors derived from **spinal cord** of 8-week old human fetus (NSI-566RSC)

- **Phase 1** – recruited 18 patients, range of disease severity, received 5 unilateral or 5 bilateral injections of 100,000 cells into cervical or lumbar cord

- Safe – main side-effects related to immunosuppressive regime

**hNSCALS**

- Italian trial with very little preclinical data to support it

- Cells from 10.5 week old human **fetal cortex**

- 18 patients, range of severity, intra-spinal transplant, no information on cell numbers or immunosuppressive regime
Considerations with Embryonic/Fetal Cells

- Ethical constraints
- Expansion to therapeutically useful numbers may be a problem
- Tetatoma formation *in-vivo*
- Immunosuppression (graft cell survival inconsistent in literature)
- Niche important for survival & differentiation – injured spinal cord is innately gliogenic
- Priming – *in-vivo* cells tend to form inter-neurones
- Slow maturation time vs Rapid disease progression
- Intra-parenchymal injection – multi-injection protocols feasible?
Mesenchymal ‘Stem Cells’
Mesenchymal Stem Cells

- Derived from bone marrow, adipose tissue, umbilical cord

- Express ‘characteristic’ markers: CD105+, CD90+, CD73+, CD45-

- In-vitro can ‘trans-differentiate’ into adipocytes, osteocytes, chondrocytes & neurones – relatively rapid

- Delivered intraparenchymally, intrathecally, intravenously or via whole bone marrow transplant

- Effective at symptomatic stages

Human MSC transplant

- Intrathecal delivery of Human BMD-MSCs into presymptomatic mice
- Increased survival, preservation of motor neurones, reduced microglial inflammatory reaction
- Released trophic factors - VEGF
- Graft cells survive & may differentiate into glial phenotypes (controversial)

Clinical trials utilizing MSCs in ALS

<table>
<thead>
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<th>Clinic Trial Number</th>
<th>Cell type</th>
<th>Intervention</th>
<th>Phase</th>
<th>Sponsor</th>
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<tbody>
<tr>
<td>NCT01051882</td>
<td>Autologous bone marrow-derived MSCs</td>
<td>Intramuscular or intrathecal injection</td>
<td>1/2</td>
<td>Hadassah Medical Organization</td>
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<tr>
<td>NCT00855400</td>
<td>Ficoll-separated mononuclear cells derived from autologous bone marrow</td>
<td>Intraspinal injection</td>
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<td>Fundacion para la Formacion e Investigacion Sanitarias de la Region de Murcia</td>
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<tr>
<td>NCT01759797</td>
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- 11 patients, 12 months follow-up, pathology showing preservation of motor neurones at injection sites

Considerations with MSCs

- Potential methodological flaw in animal models utilizing whole bone marrow transplant
- Autologous cell transplants may not alter disease course
- Therapy limited to ‘bystander effect’ – trophic factors not cell replacement (conflicting reports)
- Cells easily revert back to MSC state *in-vitro*
- Attractive because of ‘ease’ of use
- Immunomodulatory function – allografts possible without immunosuppression?
**Induced ‘Neural’ Cells (iPS cells)**

- Utilize somatic patient cells e.g. fibroblasts
- Various routes of derivation – Yamanka, direct-lineage, small molecule-based
- Widely used to study disease pathophysiology
- Attractive for treatment because:
  - Autologous transplants
  - Don’t have ethical limitations of ESCs
  - Can be genetically corrected *in-vitro*

- Inefficient generation, epigenetic memory, chromosomal abnormalities & teratoma formation *in-vivo* are *darker sides*

- Parkinson’s disease leading the way – only one therapeutic study in MND rats (Popescu, 2013, *Stem Cells Translational Medicine*, 2:167-174)
- Rat iPS dopaminergic neurones mimic primary neurones biochemically & electrophysiologically, engraft into the striatum of lesioned rats & restore rotational defects (Kim, 2011, Cell Stem Cell, 9:413-419)
Summary

- Therapeutic neural stem cells are available from a variety of sources
- Effectively isolated & expanded *in-vitro* & differentiated into functionally mature phenotypes
- Transplanted *in-vivo*, survive & differentiate
- Alter disease progression in well characterized models of familial MND
- On-going clinical trials in humans
Challenges ahead

- Understanding how cell transplants work – trophic factors vs cell replacement
- Ethics & Good Manufacturing Practice
- Delivery
- Safety & Tumourgenicity
- Initiating therapy at symptomatic stage
- How much is a MSC or ESC like a mature neurone?
- Can we form functional connections to NMJ

SITraN Stem Cell & Regenerative Medicine Group
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