To Oz...and back?  
Perspectives on stem cell therapies for ALS  

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Objectives

• Review the basics of stem cell biology
• Introduce common stem cell translational strategies
• Discuss past, present, and anticipated ALS stem cell trials
Stem Cell Basics

What is a stem cell?

A single cell that can

replicate itself, or...

differentiate into many cell types.

Stem Cell Basics

http://juanv.files.wordpress.com/2008/01/stemcells2.gif
Molecular cell biology 101: Making the vector
Molecular cell biology 101: Transformation
Molecular cell biology 101: Transfection and Transduction

HEK 293 cells
Three Common Translational Stem Cell Strategies

- *Ex vivo, in vitro* disease models
- Cell replacement
- Biopharmacologic agents
  - Intrinsic cell characteristics
  - Genetically altered
Cellular reprogramming: Yamanaka factors

Driving to pluripotency: Viral transfection of cells with 4 Genes: Oct4, Sox2, Myc, Klf4

Possible disadvantages:
• Reactivation of viral transgenes (tumorigenesis)
• Random integration could disrupt regulatory regions
Neurodifferentiation

EB suspension
EB plating
Rosette plating
Dissociate into NPCs

SSEA4
Tra-1-60
SOX2
OCT4
Nanog

D11
D5
D12
D15
Co-culture

Human Astrocyte (HA)
Derived neurons with HA

Unpublished data: Jeanie Liu PhD

4 weeks
Safer iPSC Technology
virus free – integration free

Marchetto et al, 2009 PLoSOne
**Translation Milestones**

**Year 1**

CIRM Grants: Translational Award 3 years → Disease Team Award 4 years

- **Phase 1:** Identify development candidate, *in vitro* testing

**Year 7**

- **Phase 2:** Pre-clinical efficacy testing in a small animal model

- **Phase 3:** Develop GMP quality master cell bank

- **Phase 4:** GLP Pre-clinical efficacy/safety in a small and large animal model

IND application
?Stem Cell Therapy?

• Which stem cell population do we use?
  – ESC vs. iPSC vs. adult stem cell (NSC vs. MSC vs. terminally differentiated cells)
• How do we deliver the cells?
  – Intravascular vs. intramuscular vs. surgical implantation
• How many cells do we deliver?
• How often will treatment need to be given?
• Etc.………..
Mesenchymal stem cells
First ALS Stem Cell Trial

7 spinal onset ALS patients treated (4 women and 3 men)
Autologous MSC re-suspended in 2ml of CSF
Thoracic laminectomy with T6-T9 parenchymal injection
No serious adverse events reported
Response


ORIGINAL ARTICLE

Is it too soon for Mesenchymal Stem Cell trials in people with ALS?

IRINA BADAYAN & MERIT E. CUDKOWICZ

• Insufficient pre-clinical evidence to support a clinical trial
• Insufficient pre-clinical evidence to determine an appropriate dose
• Heterogenous study population without controls
• Non-validated outcome measures
Current: MSC trials in the US

Safety/Efficacy Study for the Treatment of Amyotrophic Lateral Sclerosis (ALS)

Status: Ongoing; not recruiting participants
Sponsor: TCA Cellular Therapy
ClinicalTrials.gov Identifier: NCT01082653
Information First received: March 1, 2010

Design: Phase I, single center, prospective, non-randomized, open label, safety/efficacy study of the infusion of autologous bone marrow-derived stem cells, in 6 patients with Amyotrophic Lateral Sclerosis according to established criteria (1), (2) with a moderate to severe diagnosis of ALS according to the World Federation of Neurology El Escorial criteria.
The primary purpose of this study is to evaluate safety of the infusion procedure, as assessed by absence of complications at the site of infusion or the appearance of new neurologic deficit not attributed to the natural progression of the disease.
Objective: Determine the safety of intrathecal delivery of autologous mesenchymal stem cells (MSCs) to the cerebrospinal fluid (CSF) of patients with ALS using a dose-escalation study.
Recruitment goal: 25 adult, non-ventilator-dependent patients with clinically definite amyotrophic lateral sclerosis (ALS).
Treatment: Adipose derived MSC expanded ex vivo for ~8 weeks, intrathecal (IT) autologous delivery of MSC.
5 treatment groups of 5 patients each with escalating dose and repeat dosing.
First ALS stem cell trial in the US

Lumbar Intraspinal Injection of Neural Stem Cells in Patients with Amyotrophic Lateral Sclerosis: Results of a Phase I Trial in 12 Patients

Jonathan D. Glass, a Nicholas M. Boulis, b Karl Johe, c Seward B. Rutkove, d Thais Federici, b Meraida Polak, a Crystal Kelly, a Eva L. Feldman e

Table 2. Patient groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient numbers</th>
<th>Characteristics</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>A1</td>
<td>1-3</td>
<td>Nonambulatory, FVC &gt;60% or trach/vent</td>
<td>5 injections, unilateral</td>
</tr>
<tr>
<td>A2</td>
<td>4-6</td>
<td>Nonambulatory, FVC &gt;60% or trach/vent</td>
<td>10 injections, bilateral</td>
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<tr>
<td>B</td>
<td>7-9</td>
<td>Ambulatory, FVC &gt;60%</td>
<td>5 injections, unilateral</td>
</tr>
<tr>
<td>C</td>
<td>10-12</td>
<td>Ambulatory, FVC &gt;60%</td>
<td>10 injections, bilateral</td>
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Abbreviation: FVC, forced vital capacity.
First ALS stem cell trial in the US

Microinjection platform developed by Nicholas Boulis, M.D.  
Department of Neurosurgery, Emory University, Atlanta, Georgia

Riley et al., 2012 Neurosurgery
First ALS stem cell trial in the US

<table>
<thead>
<tr>
<th>Table 3. Patient demographics</th>
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<tr>
<td>Patient</td>
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<tr>
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<tr>
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<th>Table 4. Serious adverse event</th>
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<tr>
<td>SAE name (related to study)</td>
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<tr>
<td>Transient encephalopathy</td>
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<td>Pulmonary emboli</td>
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<tr>
<td>CSF leak</td>
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<tr>
<td>Wound dehiscence</td>
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<td>Bronchitis/pneumonia</td>
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<tr>
<td>Dyspnea</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
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<tr>
<td>Vomiting</td>
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<tr>
<td>Basal cell carcinoma</td>
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</table>

Abbreviations: CSF, cerebrospinal fluid; SAE, serious adverse events.
First ALS stem cell trial in the US

Glass et al., 2012 Stem Cell
Evidence of Long-Term Cell Survival
NIH Funding for Phase II

Neuralstem Investigator Presents New ALS NSI-566 Data at International Symposium on ALS/MND

December 10, 2012 9:16 AM ET

ROCKVILLE, Md., Dec. 10, 2012 /PRNewswire/ -- Neuralstem, Inc. (NYSE MKT: CUR) announced that Jonathon Glass, MD, Director of the Emory ALS Center, presented new data from the Phase I trial of Neuralstem's human spinal cord stem cells, NSI-566, in amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) at the International Symposium on ALS/MND in Chicago, sponsored by the Motor Neurone Disease Association. In a Thursday presentation, "RESULTS OF PHASE 1 TRIAL OF SPINAL CORD TRANSPLANTATION OF NEURAL PROGENITOR CELLS IN ALS (THE NEURALSTEM, INC. TRIAL)." Dr. Glass revealed that researchers were able to establish the long-term survival of Neuralstem's transplanted spinal cord stem cells in autopsied patients, through a technology called DNA fingerprinting. Dr. Glass, who is the principal site investigator of the trial at Emory, also announced that the study team has received a grant from the National Institutes of Health (NIH) to cover a majority of the cost of an upcoming Phase II trial.
On the horizon

- PI: Don Cleveland, PhD
- June 2010
- Requested funds: $10,857,762
- ESC derived human astrocyte precursor cells for CNS transplantation
- Plan where to have IND for a phase I trial by 2014

- PI: Clive Svendsen, PhD
- February 2013
- Requested funds: $17,842,617
- Allogeneic Neural progenitor cells secreting glial derived neurotrophic factor into the lumbar spine
- Complete pre-clinical and phase I trial in 4 years
All Stem Cell Trials Listed on http://clinicaltrials.gov
Synapse directed treatment

Kaspar B et al. Science 2003
Fischer L et al. Exp Neurol 2004
Suzuki M et al. Mol Ther 2008
Develop MSC–IGF cellular product and determine the \textit{in vitro} characteristics
Vector induction is visible by 18 hours after in vitro exposure to doxycycline

11.5 MSC-IGF-eGFP vector induction over 72 hours
In vivo confirmation of IGF-1 vector induction
Thank you