Cervical Targeted Intraspinal Microinjection
Preclinical Morbidity Threshold Assessment

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Emory University Department of Neurological Surgery

Disclosures and Conflicts

- Nicholas Boulis, MD
  - Medtronic, Ceregene, Genzyme, Neuralstem, ACDF
- Eva Feldman, MD PhD
  - Neuralstem
- Jonathan Glass, MD
  - Neuralstem
- Jonathan Riley, MD
  - No disclosures

Introduction

I – Overview of Intraspinal Microinjection
II – Phase I Trial Design and Outcomes
III – Defining Toxicity, The Next Preclinical Effort
IV – The Next Horizon, A Phase IIIB Trial

I – Overview of Intraspinal Microinjection
Preclinical Safety Data and Device Development

II – Phase I Trial Design and Outcomes
A Risk Escalation Approach

Patient Groups for Stem cell ALS trial

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Unilateral (n=3) and bilateral (n=3) stem cell injections in the lumbar enlargement of non-ambulatory patients</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>Unilateral stem cell injections in the lumbar enlargement of ambulatory patients (n=6)</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>Bilateral stem cell injections in the lumbar enlargement of ambulatory patients (n=3)</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>Unilateral stem cell injections in the cervical enlargement of fully ambulatory patients (n=3)</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>Bilateral stem cell injections in the cervical enlargement and unilateral stem cell injections in the cervical enlargement of fully ambulatory patients (n=3)</td>
<td>3</td>
</tr>
</tbody>
</table>

Appendix - Trial Design Details
II – Phase I Trial Design and Outcomes

Surgical Approach

Intra-Operative Findings

Group

Ambulation Status

Pt #

Unilateral vs Bilateral Injection Side

Operative Time (min)

Injection Tracts Per Patient

Intra-Operative Cord Hemorrhage

Other

A

Non-Ambulatory

1

2

3

Unilateral (n=3)

R

R

R

1)

311

2)

247

3)

199

B

Ambulatory

7

8

9

Unilateral (n=3)

R

L

L

7)

205

8)

172

9)

146

C

Ambulatory

10

11

12

Bilateral (n=3)

10

11

12

10)

274

11)

281

12)

273

Pt

4, 2 tract hemorrhages

Pt

5, 3 tract hemorrhages

Pt

4, 20 min 50% SSEP reduction

Pt

5, Sclerotic pia

Pt

9, 1 tract hemorrhage

Pt

11, 1 tract hemorrhage


II – Phase I Trial Design and Outcomes

Post-Operative Neurologic Outcomes

• All non-ventilated patients (n=9) were successfully extubated in OR
• (n=5) patients with lower extremity sensory changes
  – Resolved in 4/5 by time of discharge
  – Radicular in one patient
  – Resolved in 5/5 by time of two week 2nd appt
• All patients (n=12) at motor baseline by time of discharge (t=4-5d)
• Post-operative ileus resolved by discharge in (n=3) patients
• One patient required foley replacement, weaned by time of D/C

II – Phase I Trial Design and Outcomes

Serious Adverse Events

Surgical

• CSF leak (n=1)
  – Failed LD conservative therapy
  – Washout, reclusion

• Suprafascial wound dehiscence (n=1)
  – Failed iodineform packing and Wound Vac
  – Prominent Spinous process rongeured, reclosed

Non-Surgical

Two Deaths

• Ph 5 (13mo post-op) → respiratory failure 2/1 disease progression
• Ph 6 (8 mo post-op) → arrhythmia, ventricular hypertrophy, bicuspid aortic valve

III – Defining Toxicity, The Next Preclinical Effort

• A Segue into Phase II Efficacy Studies
  • Risk Escalation vs Dose Escalation
  • Defining Maximum Tolerated Dose (MTD)
• A GLP-level Preclinical Dose Escalation Series
**III - Experimental Design**

<table>
<thead>
<tr>
<th>A (n=6)</th>
<th>B (n=6)</th>
<th>C (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td></td>
<td>C3</td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td>C4</td>
</tr>
<tr>
<td>C5</td>
<td></td>
<td>C5</td>
</tr>
<tr>
<td>Conc</td>
<td>Vol/ing</td>
<td>Inj #</td>
</tr>
<tr>
<td>(2x10^6 cells/ul)</td>
<td>10ul/inj</td>
<td>10 unil. inj</td>
</tr>
<tr>
<td>(2x10^5 cells/ul)</td>
<td>10ul/inj</td>
<td>20 bilateral inj</td>
</tr>
<tr>
<td>(3x10^5 cells/ul)</td>
<td>20ul/inj</td>
<td>6x10^5 cells</td>
</tr>
</tbody>
</table>

**III – Defining Toxicity, The Next Preclinical Effort**

No lasting neurologic sequelae were observed with either:

- Increasing number of microinjections (Group B vs A)
- Bilateral vs unilateral microinjections (Group B vs A)
- With increasing payload concentration (Group C vs Group B)

All animals returned to neurologic baseline by POD 1.

**IV - The Next Horizon, A Phase IB Trial**

**Appendix 1**

**Patient Demographics**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gender</th>
<th>Age (yrs)</th>
<th>BMI (kg/m²)</th>
<th>Diagnosis</th>
<th>Target stem cell count</th>
<th>Date of Enrollment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>57.7</td>
<td>28.5</td>
<td>N/A</td>
<td>N/A</td>
<td>January 2012</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>58.1</td>
<td>29.2</td>
<td>N/A</td>
<td>N/A</td>
<td>May 2013</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>57.5</td>
<td>28.5</td>
<td>N/A</td>
<td>N/A</td>
<td>April 2010</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>65.2</td>
<td>27.7</td>
<td>N/A</td>
<td>N/A</td>
<td>June 2010</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>66.1</td>
<td>28.5</td>
<td>N/A</td>
<td>N/A</td>
<td>September 2013</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>59.0</td>
<td>28.5</td>
<td>N/A</td>
<td>N/A</td>
<td>August 2015</td>
</tr>
</tbody>
</table>

**Appendix 2**

**Injection Parameters**

- **Vol (10µl/injection)**
- **Conc [1x10^5 cells/ul]**
- **Rate 5µl/min**

**Immunosuppression Regimen**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate (Solumedrol®)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Tacrolimus (Prograf®)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mycophenolate mofetil (Cellcept®)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Exclusion Criteria**

- General exclusion criteria
- Group-specific exclusion criteria

**Acknowledgements**

**Trial Investigators**
- Jonathan Glass, MD
- Eva Feldman, MD PhD
- Nicholas Boulis, MD

**Trial Coordinator**
- Miranda Polack

**Trial Industry Sponsor (Neuralstem, Inc.)**
- Karl Jaffe, PhD

**Boulis Laboratory**
- Thais Federici, PhD

**NSgy Residents**
- Jason Taek, MD
- Bethwel Raore, MD

**Appendix – Trial Design (cont)**

**A Risk Escalation Approach**

- **Trial Design**
  - 5 patient groups (n=3/group) except group A (n=6) (next slide)
  - Unilateral lumbar (non-ambulatory) + bilateral lumbar microinjection (ambulatory)
  - Demographics (see Appendix 1)

- **Injection Parameters**
  - Vol (10µl/injection)
  - Conc [1x10^5 cells/ul]
  - Rate 5µl/min

- **Immunosuppression Regimen**
  - Methotrexate (Solumedrol®)
  - Tacrolimus (Prograf®)
  - Mycophenolate mofetil (Cellcept®)

- **Exclusion Criteria**
  - General exclusion criteria
  - Group-specific exclusion criteria
Appendix 2a
General Inclusion Criteria

- Have the ability to understand the requirements of the study, provide written informed consent, understand and provide written authorization for the use and disclosure of Protected Health Information (PHI) (Personal Health Information) and comply with the study procedures.
- Male and female at least 18 years old.
- Capable of providing informed consent and complying with study procedures.
- Women must have a negative serum pregnancy test and practice an acceptable method of contraception for at least 2 years or be in a long-term, permanent monogamous relationship.
- Self-assessment of height and weight and the ability to travel to the site for study visits.
- Presence of a walking aid or similar.
- Diagnosis of ALS based on examination by the site PI, meeting E. F. Nord’s criteria for laboratory supported probable, probable or definite ALS (Appendix A).
- Vital capacity of at least 50% of predicted for age, height and gender measured in the seated position at the time of screening and >60% of predicted for age, height and gender measured prior to the 7-day period in surgery (for Group B only), static forces >3 lbs/ml, antispasticity and inspiratory exclusion.
- Inability to ambulate (<60% by nomogram measured at the time of screening and VC < 120 mL) for secondary to extremity weakness due to ALS.
- Failure of antispasticity medications is defined as clinical inability to walk independently after maximum PT and OT intervention.
- Vital capacity >60% of predicted normal for age, height and gender measured in the seated position at the time of screening and >90% of predicted normal for age, height and gender measured prior to the 7 days prior to surgery (B unable for at least 6 months with tracheostomy and invasive ventilation).
- For Group B and C, arthrodensis or arthrodesis were performed on the same day (Group C only), static forces >3 lbs/ml, antispasticity and inspiratory exclusion.
- For Group A and B, the site should confirm the following conditions: a) Amputations or amputation of long bones with injured soft tissue; b) Extensive soft tissue trauma; c) Extensive skin breakdown; d) Skin infections; e) Protruding unhealed osteomyelitis; f) Presence of areas with the following conditions: i) Cardiac disease or stroke; ii) Uncontrolled systemic or infection; iii) Uncontrolled diabetes; iv) Uncontrolled hypertension; v) Uncontrolled hyperlipidemia; vi)IDADE (Not allowed for at least 6 months with tracheostomy and invasive ventilation).
- Failure to provide informed consent as determined by the PI.
- Inpatient status for surgery at the University of Alabama at Birmingham – see B above.

Appendix 2b
Group-Specific Inclusion Criteria

For Group B:
- Failure in walking evaluated for ≥2 weeks secondary to lower extremity weakness and/or spasticity due to ALS. The ALS Functional Rating Scale—Revised (ALSFRS-R) lower extremity subscore must be ≤12.
- Fitted to one or more portable devices of lower extremity, such as a stander (Triforma, Rifton, etc.) with at least 7 feet of travel and having a sit-to-stand function.
- Failure in walking by nomogram measured at the time of screening and VC < 120 mL.
- Failure of antispasticity medications is defined as clinical inability to walk independently after maximum PT and OT intervention.
- Vital capacity >60% of predicted normal for age, height and gender measured in the seated position at the time of screening and >90% of predicted normal for age, height and gender measured prior to the 7 days prior to surgery (B unable for at least 6 months with tracheostomy and invasive ventilation).
- For Group B and C, arthrodensis or arthrodesis were performed on the same day (Group C only), static forces >3 lbs/ml, antispasticity and inspiratory exclusion.
- For Group B and C, the site should confirm the following conditions: a) Amputations or amputation of long bones with injured soft tissue; b) Extensive soft tissue trauma; c) Extensive skin breakdown; d) Skin infections; e) Protruding unhealed osteomyelitis; f) Presence of areas with the following conditions: i) Cardiac disease or stroke; ii) Uncontrolled systemic or infection; iii) Uncontrolled diabetes; iv) Uncontrolled hypertension; v) Uncontrolled hyperlipidemia; vi)IDADE (Not allowed for at least 6 months with tracheostomy and invasive ventilation).
- Failure to provide informed consent as determined by the PI.
- Inpatient status for surgery at the University of Alabama at Birmingham – see B above.

Appendix 2c
Exclusion Criteria

- Failure of antispasticity or weakness due to ALS.
- VC > 120 mL protected normal for age during the 7 days prior to surgery.
- Current or actual use of Riluzole (Rilutek®) due to uncontrolled ALS.
- Previous use of antispasticity medications for >90 days (any indication for surgery).
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- Failure to provide informed consent as determined by the PI.
- Inpatient status for surgery at the University of Alabama at Birmingham – see B above.