Cervical Targeted Intraspinal Microinjection

Preclinical Morbidity Threshold Assessment

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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

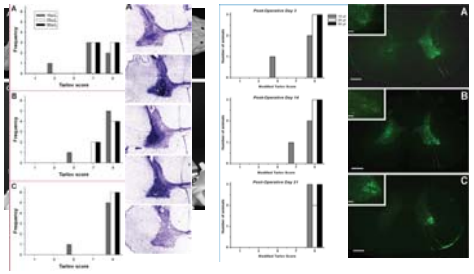
II – Phase I Trial Design and Outcomes

III – Defining Toxicity, The Next Preclinical Effort

IV – The Next Horizon, A Phase IB Trial

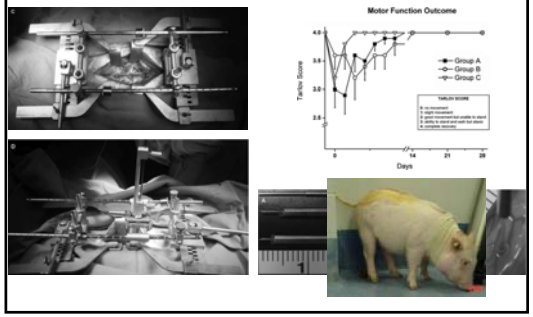
I – Overview of Intraspinal Microinjection

Preclinical Safety Data and Device Development



I – Overview of Intraspinal Microinjection

Preclinical Safety Data and Device Development



II – Phase I Trial Design and Outcomes

A Risk Escalation Approach

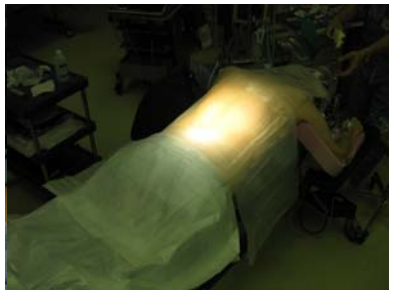
Patient Groups for Stem cell ALS trial

Group A :	Unilateral (n=3) and bilateral (n=3) stem cell injections in the lumbar enlargement of non-ambulatory patients
Group B:	Unilateral stem cell injections in the lumbar enlargement of ambulatory patients (n=3)
Group C:	Bilateral stem cell injections in the lumbar enlargement of ambulatory patients (n=3)
Group D:	Unilateral stem cell injections in the cervical enlargement of fully ambulatory patients (n=3)
Group E:	Bilateral stem cell injections in the lumbar enlargement and unilateral stem cell injections in the cervical enlargement of fully ambulatory patients (n=3)

Appendix - Trial Design Detail

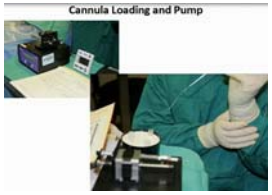
II – Phase I Trial Design and Outcomes

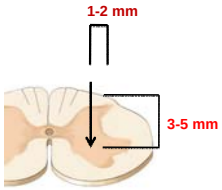
Surgical Approach

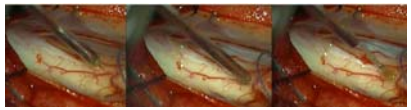


II – Phase I Trial Design and Outcomes

Surgical Approach







II – Phase I Trial Design and Outcomes

Intra-Operative Findings

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	Unilateral	R	1) 311	5	None Observed	
		2	(n=3)	R	2) 247			
		3		R	3) 199			
		4	Bilateral		4) 288	10	Pt 4, 2 tract hemorrhages	Pt 4, 20 min 50% SSEP reduction
		5	(n=3)		5) 213		Pt 5, 3 tract hemorrhages	Pt 5, Sclerotic pia
		6			6) 226			
B	Ambulatory							
		7	Unilateral	R	7) 205	5		
		8	(n=3)	L	8) 172			
		9		L	9) 146		Pt 9, 1 tract hemorrhage	
C	Ambulatory							
		10	Bilateral		10) 274	10	Pt 10, 3 tract hemorrhages	
		11	(n=3)		11) 281		Pt 11, 1 tract hemorrhage	
		12			12) 273			

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 f/u 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, reclosure
- Suprafascial wound dehiscence (n=1)
  - Failed iodoform packing and Wound Vac
  - Prominent Spinous process rongeured, reclosed

Non-Surgical

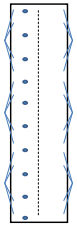
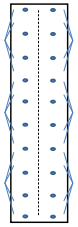
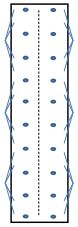
Two Deaths

- Pt 3 (13mo post-op) → respiratory failure 2/2 disease progression
- Pt 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

	A (n=6)	B (n=6)	C (n=6)
			
Conc	[2x10^4 cells/ul]	[2x10^4 cells/ul]	[3x10^4 cells/ul]
Vol/inj	10ul/inj	10ul/inj	10ul/inj
[Total]/inj	[2x10^{5}cells/inj]	[2x10^{5}cells/inj]	[3x10^{5}cells/inj]
Inj #	10 unilateral inj	20 bilateral inj	20 bilateral inj
Total #	**2x10^{6}cells**	**4x10^{6}cells**	**6x10^{6}cells**

C3 C4 C5 (left) C3 C4 C5 (right)

III – Defining Toxicity, The Next Preclinical Effort

No lasting neurologic sequelae were observed with either :

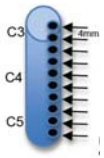
- Increasing number of microinjections (Group B vs A)
- Bilateral vs unilateral microinjections (Group B vs A
- With increasing payload concentration (Group C vs Group B)

All animals returned to neurologic baseline by POD 1.

IV - The Next Horizon, A Phase IB Trial

Table 2: Dose escalation description

Dose #	# cells/ inj	uni (1) or bilateral (2)	# inj	Total # cells inj
1	1x10^5	1	5	0.5x10^6
2	1x10^5	1	10	1x10^6
3	2x10^5	1	5	1x10^6
4	2x10^5	1	10	2x10^6
5	1x10^5	2	15	1.5x10^6
6	1x10^5	2	20	2x10^6
7	2x10^5	2	15	3x10^6
8	2x10^5	2	20	4x10^6



Modifiable Parameters

1) number of penetrations (injections)
2) number of cells/injection,
3) unilateral vs. bilateral injections

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Eva Feldman, MD PhD
Nicholas Boulis, MD



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Meraida Polack

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Thais Federici, PhD

NSgy Residents
Jason Taub, 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) through…
- •bilateral lumbar microinjection + unilateral cervical microinjection (ambulatory)
- •Demographics (see Appendix 1)

Injection Parameters

- •NSI-566RSC (Neuralstem, Inc)
- •Vol (10µl/injection) Conc [1x10^4 cells/µl] Rate 5µl/min
- •5 inj per side

Immunosuppression Regimen	Dose		
Methyprednisolone (Solumedrol®)	125 mg IV	(pre-op)	60, 40, 20, 10 (mg) 28d taper
Basiliximab (Simulect®, East Hanover, NJ)	20mg IV	intra-op	20mgIV POD4
Tacrolimus (Prograf®)	0.1mg/kg/day	(BID, POD1)	troughs followed
Mycophenolate mofetil (Cellcept®)	500mg	(BID, POD1)	up to 1gm BID over 2wks

Inclusion/Exclusion Characteristics

General Inclusion Criteria (see Appendix 2a)
Group-Specific Inclusion Criteria (see Appendix 2b)
Exclusion Criteria (see Appendix 2c)

Appendix 1

Patient Demographics

PATIENT	GROUP	GENDER	AGE AT SURGERY	Dz DUR at SGY (Yrs)	IMPLANT DATE
1	A1	Male	61.7	5.2	January/2010
2	A1	Male	43.4	12.7	March/2010
3	A1	Male	51.1	2.1	April/2010
4	A2	Male	37.5	2.0	May/2010
5	A2	Male	66.2	2.2	June/2010
6	A2	Male	55.0	2.2	August/2010
7	B	Male	59.0	1.6	October/2010
8	B	Male	41.1	5.5	November/2010
9	B	Male	54.5	3.5	December/2010
10	C	Male	48.9	11.7	January/2011
11	C	Male	39.3	1.6	March/2011
12	C	Male	65.0	3.0	April/2011

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) [per Health Insurance Portability and Accountability Act (HIPAA) Privacy Ruling] and comply with the study procedures.
- Men and women 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 or be of non-childbearing potential (post-menopausal for at least 2 years or who have undergone hysterectomy or oophorectomy or surgical sterilization).
- Geographic accessibility to the study center and the ability to travel to the clinic for study visits.
- Presence of a willing and able caregiver.
- Diagnosis of ALS based on examination by the site PI, meeting El Escorial criteria for laboratory-supported probable, probable or definite ALS (Appendix A).
- Vital capacity ≥ 60% of predicted for age, height and gender measured in the seated position at the time of screening and ≥ 50% of predicted for age, height and gender measured supine during the 7 days prior to surgery (OR for Group A only: stable for at least 3 months with tracheostomy and invasive ventilation).
- Not taking riluzole (Rilutek®) or on a stable dose for ≥30 days.
- All required vaccinations current: tetanus/diptheria (TDAP), herpes zoster/shingles (Zoatavax®: within last 10 years and must be prior to surgery), pneumonia (Pneumovax®), seasonal/H1N1 flu vaccines (as appropriate for season) for Groups B-E.

Appendix 2b

Group-Specific Inclusion Criteria

- For Group A:
  - Inability to walk unassisted 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 1 or less. Patients who are non-ambulatory because of severe spasticity must have failed standard antispasticity treatement with baclofen (up to 120 mg/day) and/or tizanadine (up to 12 mg/day), physical therapy (PT), and occupational therapy (OT). Failure of antispasticity medications is defined as continued inability to walk unassisted even at the highest tolerated doses of these medications. Failure of PT and OT means continued 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 ≥ 50% of predicted normal for age, height and gender measured supine during the 7 days prior to surgery OR stable for at least 3 months with tracheostomy and invasive ventilation.
- For Groups B and C:
  - Ambulatory subjects with impaired gait and approximately symmetrical lower extremity weakness and/or spasticity due to ALS and an ALSFRS-R gate subscore ≥ 2.
  - Vital capacity ≥ 60% of predicted normal for age, height and gender measured in the seated position at the time of screening and ≥ 50% of predicted normal for age, height and gender measured supine during the 7 days prior to surgery.
- For Groups D and E, the same requirements as Group B with the addition of demonstrable arm weakness with an ALSFRS-R arm subscale between 1 and 3.
- Able to undergo lumbar or cervical laminectomy operation as determined by the site PI, neurosurgeon and anesthesiologist.
- Able to tolerate, as determined by the site PI, an immunosuppression regimen consisting of basiliximab, tacrolimus, mycophenolate mofetil, and methyprednisolone.
- Agrees to the visit schedule as outlined in the informed consent.

Appendix 2c

Exclusion Criteria

- Etiology of paraplegia or weakness is due to causes other than ALS.
- VC < 60% predicted normal by standard nomogram measured seated at the time of screening and VC < 50% predicted normal for age during the 7 days prior to surgery measured in the supine position.
- Current or peak Panel Reactive Antibody (PRA) due to alloantibodies > 20% receiving their first allograft.
- Any known immunodeficiency syndrome.
- Receipt of any investigational drug, device or biologic within 30 days of surgery.
- Any concomitant medical disease or condition limiting the safety to participate:
  - Coagulopathy
  - Active uncontrolled infection
  - Hypotension requiring vasopressor therapy
  - Previous spinal surgery at the site of planned transplantation except for anterior cervical dissection fusion (ACDF).
  - Skin breakdown over the site of surgery
  - Malignancy (except for non-melanoma skin cancer)
  - Spinal stenosis.
- Creatinine >1.5, liver function tests (SGOT/SGPT, Bilirubin, Alk Phos) > 2x upper limit of normal, hematocrit/hemoglobin < 30/10, total WBC < 4000, uncontrolled hypertension (systolic > 180 or diastolic > 100) or uncontrolled diabetes (defined as hemoglobin A1C >8), evidence of GI bleeding by hemoccult test, tuberculosis (TB test: PPD/Mantoux), serologic evidence of current infection with a hepatitis virus or human immunodeficiency virus.
- Presence of any of the following conditions:
  - Current drug abuse or alcoholism
  - Unstable medical conditions
  - Unstable psychiatric illness including psychosis and untreated major depression within 90 days of screening
- Any condition that the site PI feels may interfere with participation in the study.
- Any condition that the surgeon feels may pose complications for the surgery.
- Known hypersensitivity to basiliximab, tacrolimus, mycophenolate mofetil, or methyprednisolone/prednisone.
- Inability to provide informed consent as determined by site PI.
- **Inadequate family or caregiver support as determined by the site PI.** Redundant – see #4 above.