Regenerative Therapies for Spinal Cord Injury: Rationale, Evolution and Current Status

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Director Spinal Cord Injury Center

Medical College of Wisconsin

Acknowledgements

- Departmental Staff (Fellows, Residents, Clinical Nurses)
- Operating Room
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- Department of Neurosurgery and PMR Faculty
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- Neuroscience Research Center
- Center for Imaging Research
- **Peers and Students**
- Froedtert/ MCW Leadership

Acknowledgements

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  - 1R43NS079001-01
  - 1RO1NS085405 (Budde)

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International Colleagues for Guidelines Development to Manage Spinal Cord Injury

Fehlings MG (U Toronto); Harrop J (Jefferson, Delaware Valley); Aarabhi B (U Maryland, Shock Trauma); Kurpad SN (MCW, CNTR); Kwon B (ICORD, UBC, Vancouver); Kotter M (U Cambridge)

Neurological Surgery

The Art and Science of “Restoring Humanity to Human Beings”

Sir Henry Marsh

“Do No Harm: Stories of Life, Death and Brain Surgery” Orion Books 2014

The Status Quo Sucks

George Carlin

Academic Medicine is an opportunity for a doctor to leave the field better that (s)he found it.

knowledge changing life

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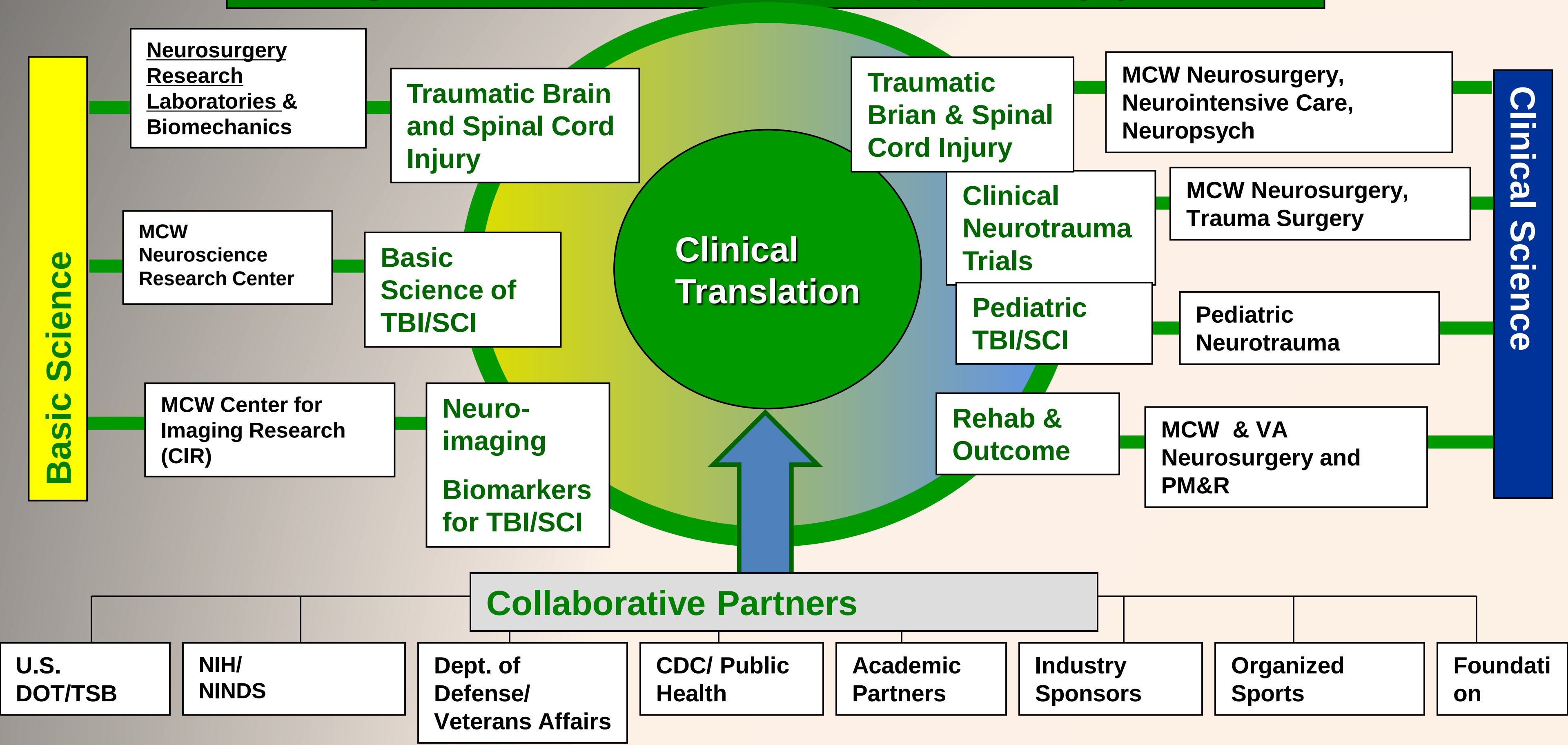
CENTER OF NEUROTRAUMA RESEARCH

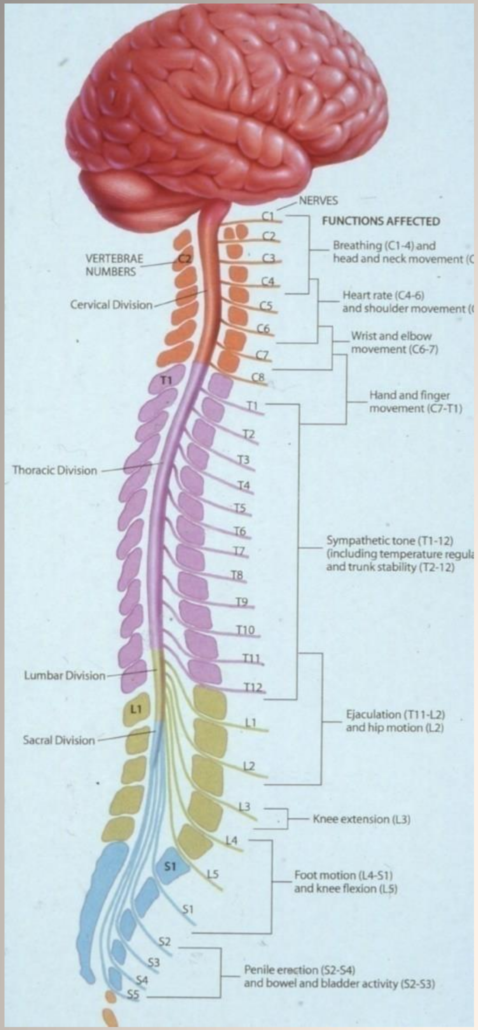
TRANSLATIONAL NEUROTRAUMA RESEARCH

Advancing the Science of Neurotrauma: Brain and Spinal Cord Injury



Spinal Cord Injury
Facts and Figures

If you drive a car…Or ride a motorcycle



- ***Incidence***
- **3-5/100.000 in the US**

- ***New cases***
- **12.000/year in the US, 250,000 total**
- **with deficits**

- ***Survival***
- **90%, near-normal life span**

- ***Costs***
- **$ 6 billion/year in the US**

- ***Age***
- **Average: 33.4 years**
- **most common age: 19 years**

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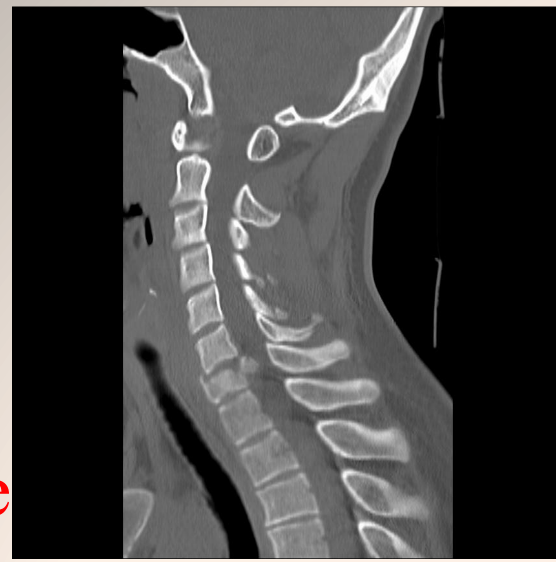
The Dark Side of Summer

24 Year Old Male
Medical Student

Diving Accident

Quadriplegic at Scene
and in ER



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Topics

- History
- Developing the “Drug”: 2001-2015 and ??
- Developing the “Map”: 2006-Present
- Delivering the “Drug”: Surgical Considerations
- Funding!

Edwin Smith Papyrus (2500-1700 BC)

- Five Cases reported
- Crushed vertebra- “He is unconscious of neck and arms, speechless and urine dribbles”..**An ailment not to be treated**
- Sprained vertebra- Treat with application of fresh meat and honey

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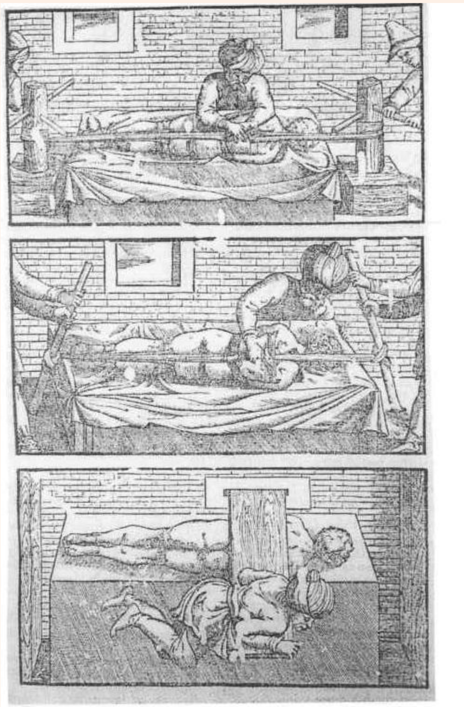
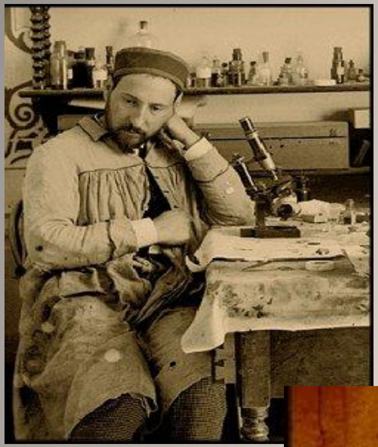
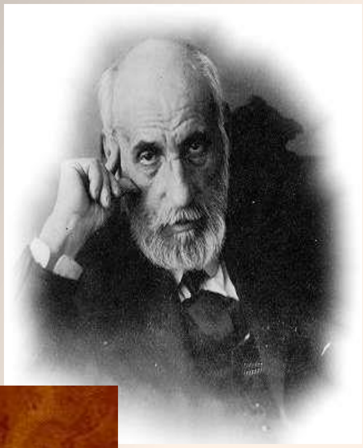
Galen of Pergamon (AD 129-210)

- Lived under Emperors Antonius Pius and Marcus Aurelius
- Studied Gladiators in the Coliseum
- Transection of spinal cord results in paralysis
- Injury to one half of spinal cord results in paralysis on same side of the body (earliest concept of localization)
- **First to Treat Spinal Cord Injury (Traction)**



Neuron Doctrine and Concept of Localization (1850-1910)
Santiago Ramon Y Cajal and Charles Eduoard Brown Sequard

- Spinal Cord composed of Predictable and Definable Nerve Cell Connections
- Different Parts of the Spinal Cord Serve Different Functions
  - Front or Ventral – Motor
  - Back or Dorsal - Sensory

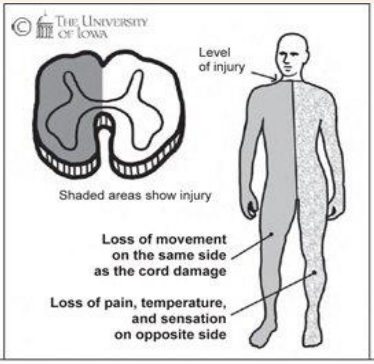



Figure 12. Brown-Séquard Syndrome

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What Has Been Available?

- Early Diagnosis
- Intravenous Medication (Methylprednisolone)
- Surgical Reconstruction of the Spine (if indicated)
- Long Term Rehabilitation

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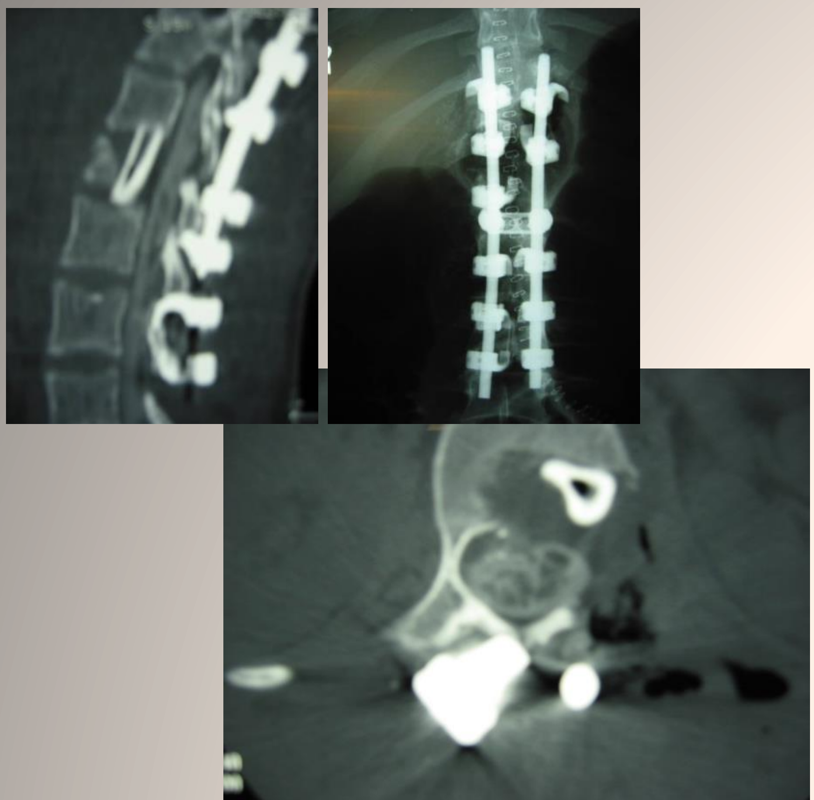
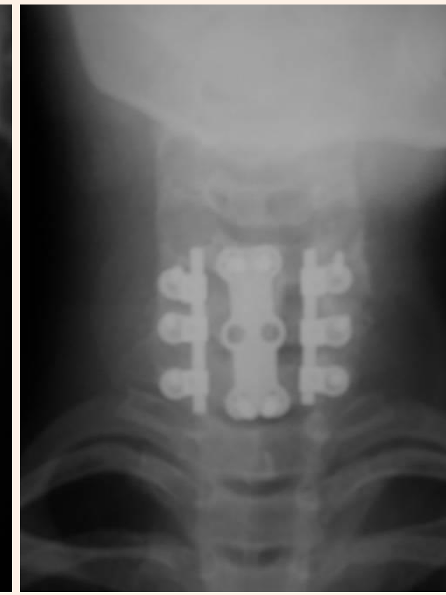
What Do We Do?





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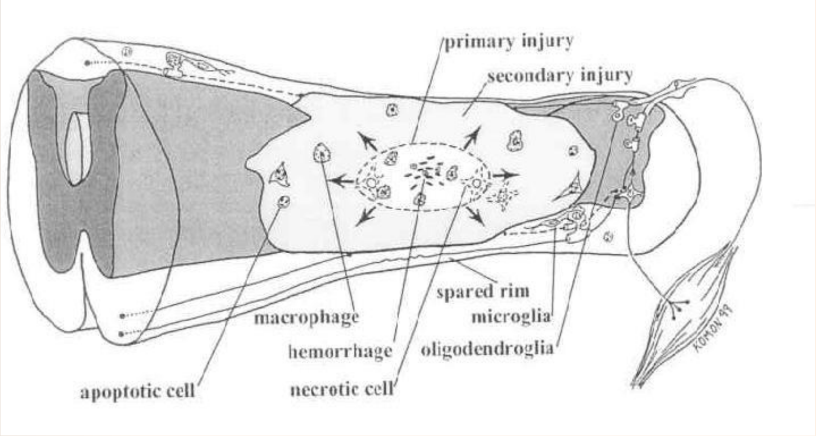
- **Plus Long Term Rehabilitation and Re-integration**

What Happens after Spinal Cord Injury?

- Tissue Swelling from Inflammation
- Release of toxic substances into the zone of injury
- Loss of normal tissue
- Disruption of normal nerve connections
- Scar Formation
- Creation of environment hostile to regrowth of nerves



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1. Developing the “Drug”


17 December 1999
Science
Vol. 286 No. 5448
Pages 2221–2416 $8
Breakthrough
of the Year
Stem Cells
Show Their
Potential

Timeline (Stem Cell Strategies)

- 2005-2008: Application of concept to Human Stem Cells to generate Transplantable Myelin Making Cells
- 2009: GERON Stem Cell Study Starts. Terminated in 2011 (FUNDING Shortages!)
- 2015: Asterias Stem Cell Study Starts
  - Human Embryonic Stem Cells
  - Genetically Engineered to form Oligodendrocytes

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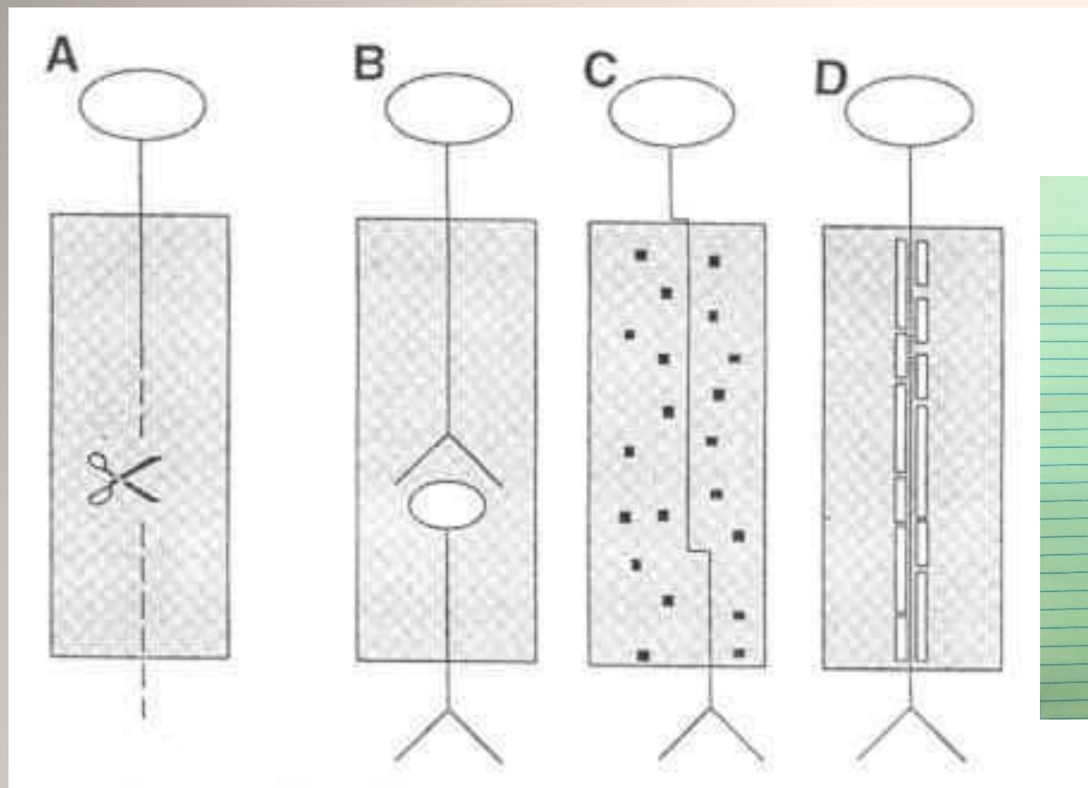
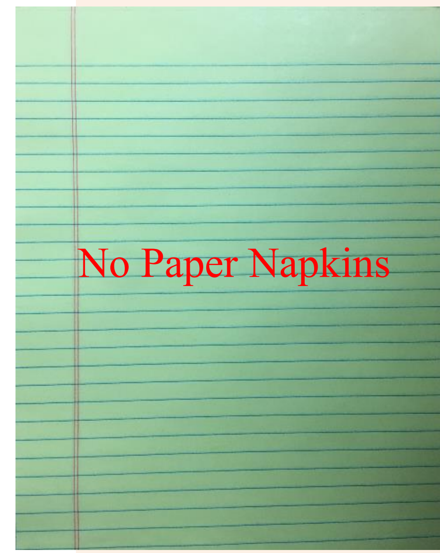
Strategies for Spinal Cord Repair

Karolinska Institutet 2001





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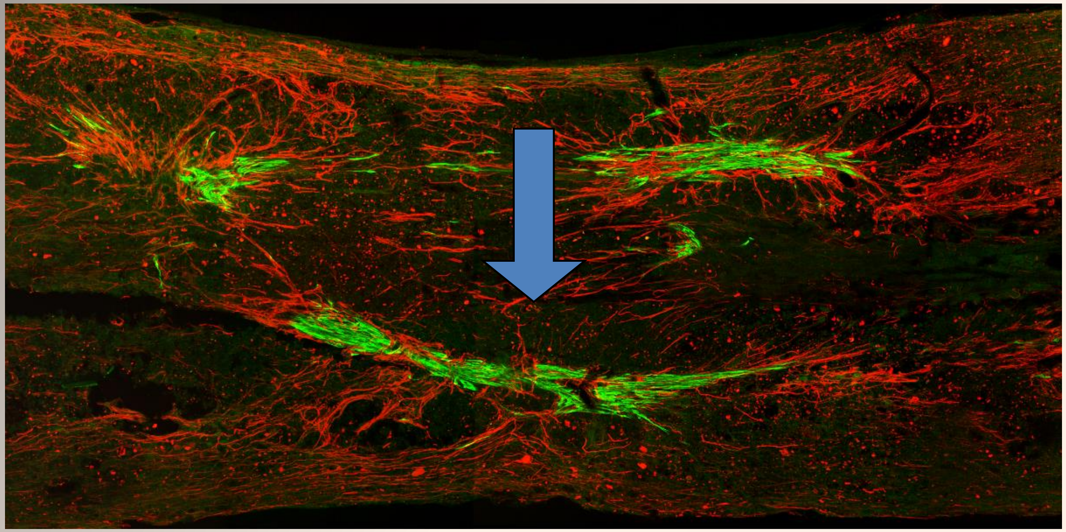
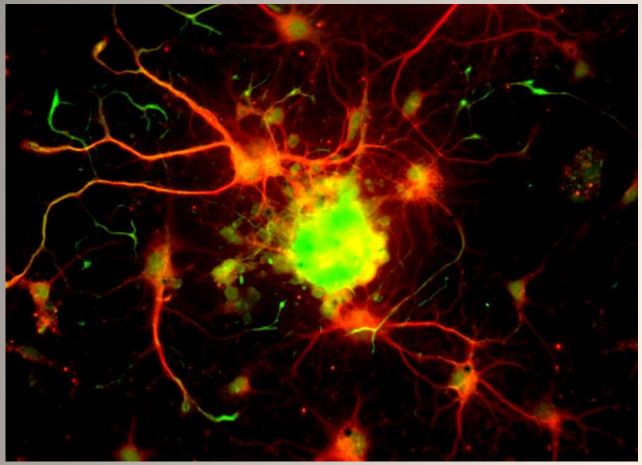
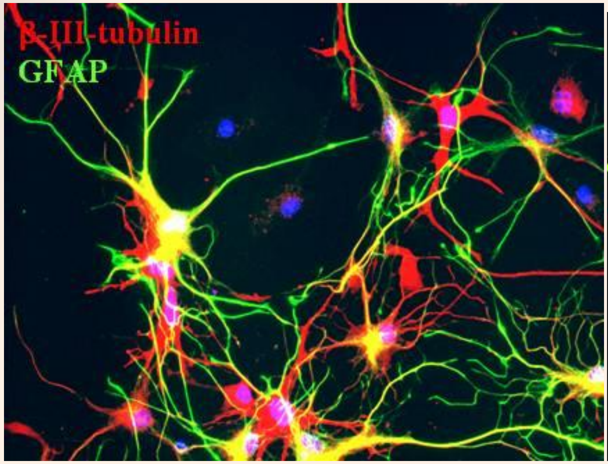
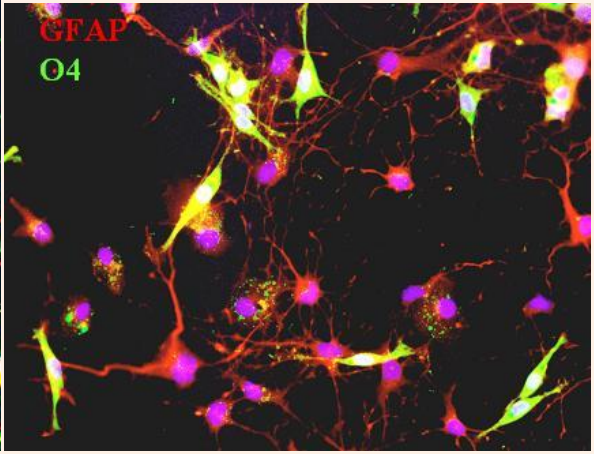
Neural Stem Cells integrate into the spinal cord

Green: GFP in stromal cells; Red: Neurofilament-IR









Experimental Neurology

Available online at www.sciencedirect.com ScienceDirect

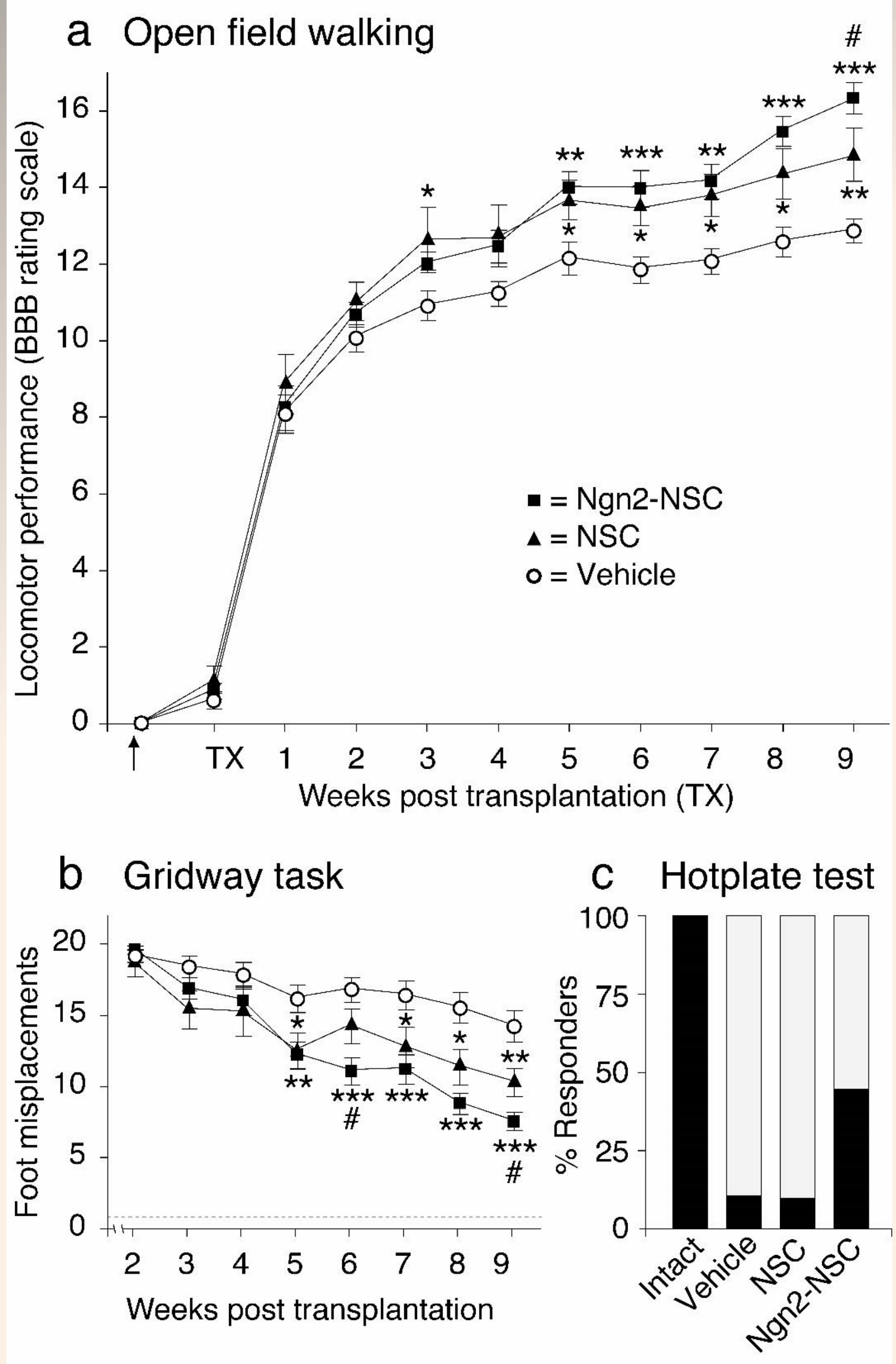
ELSEVIER

Experimental Neurology 201 (2006) 335–348

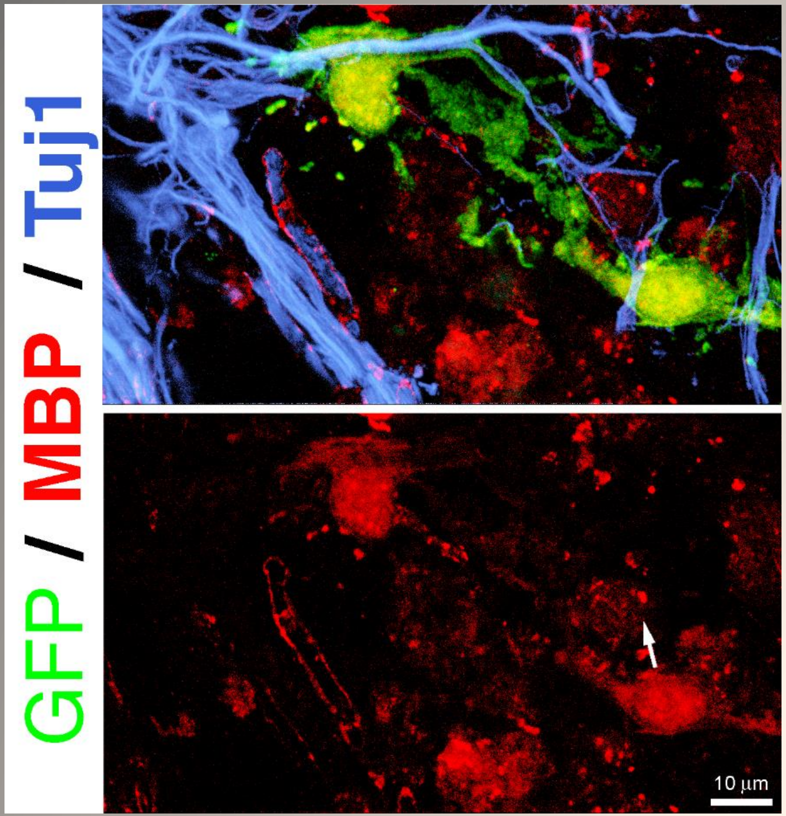
Pain with no gain: Allodynia following neural stem cell transplantation in spinal cord injury

Melissa Y. Macias, Mara B. Syring, Michael A. Pizzi, Maria J. Crowe, Arshak R. Alexanian, Shekar N. Kurpad ∗

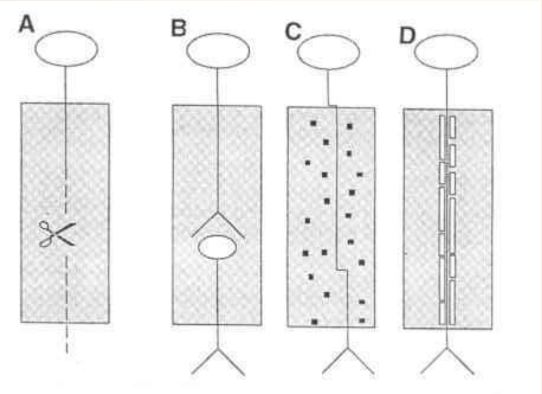
Grafting of neural stem cells genetically engineered to express Neurogenin2 allows for recovery of hindlimb sensory function



Grafting Ngn-2 transduced neuronal stem cells into the injured spinal cord



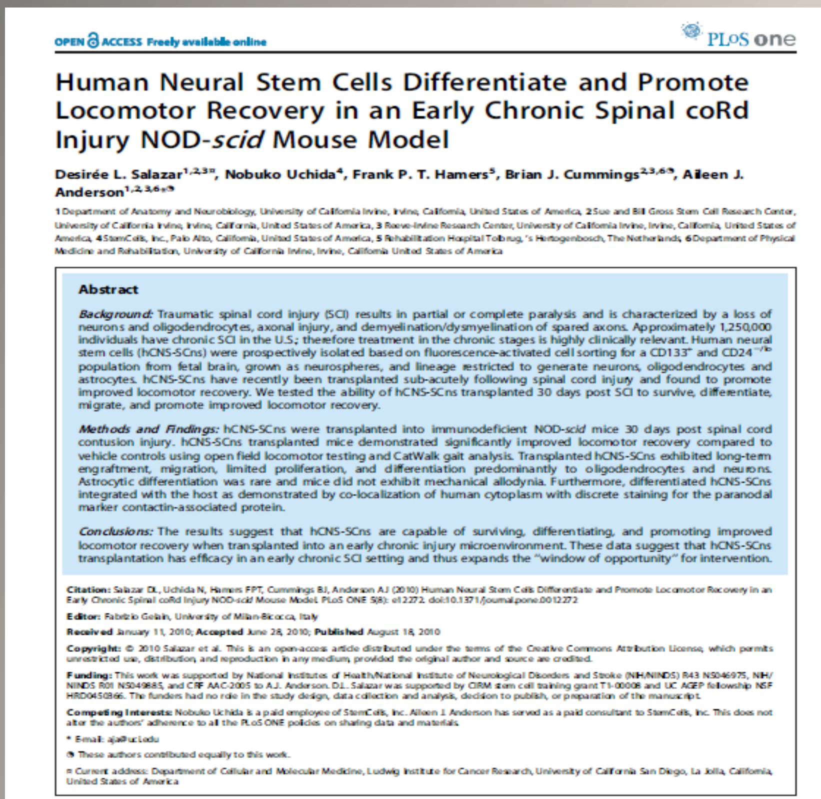
Ng-2 stem cells (GFP) have become oligos (MBP) ensheathing host nerve fiber (Tuj1)



Nature Neuroscience 2005 Mar;8(3):346-53. **Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome.**
Hofstetter CP, Holmstrom NA, Lilja JA, Schweinhardt P, Hao J, Spenger C, Wiesenfeld Hallin Z, Frisen J, Olson L, Kurpad SN

What about Chronic Spinal Cord Injury?

STEMCELLS



OPEN ACCESS Freely available online PLoS one

Human Neural Stem Cells Differentiate and Promote Locomotor Recovery in an Early Chronic Spinal coRd Injury NOD-*scid* Mouse Model

Desirée L. Salazar[1,2,3¤], Nobuko Uchida[4], Frank P. T. Hamers[5], Brian J. Cummings[2,3,6,9], Aileen J. Anderson[1,2,3,6*,9]

1 Department of Anatomy and Neurobiology, University of California Irvine, Irvine, California, United States of America, 2 Sue and Bill Gross Stem Cell Research Center, University of California Irvine, Irvine, California, United States of America, 3 Reeve-Irvine Research Center, University of California Irvine, Irvine, California, United States of America, 4 StemCells, Inc., Palo Alto, California, United States of America, 5 Rehabilitation Hospital Tolbrug, 's Hertogenbosch, The Netherlands, 6 Department of Physical Medicine and Rehabilitation, University of California Irvine, Irvine, California United States of America

Abstract

Background: Traumatic spinal cord injury (SCI) results in partial or complete paralysis and is characterized by a loss of neurons and oligodendrocytes, axonal injury, and demyelination/dysmyelination of spared axons. Approximately 1,250,000 individuals have chronic SCI in the U.S.; therefore treatment in the chronic stages is highly clinically relevant. Human neural stem cells (hCNS-SCns) were prospectively isolated based on fluorescence-activated cell sorting for a $CD133^{+}$ and $CD24^{-/lo}$ population from fetal brain, grown as neurospheres, and lineage restricted to generate neurons, oligodendrocytes and astrocytes. hCNS-SCns have recently been transplanted sub-acutely following spinal cord injury and found to promote improved locomotor recovery. We tested the ability of hCNS-SCns transplanted 30 days post SCI to survive, differentiate, migrate, and promote improved locomotor recovery.

Methods and Findings: hCNS-SCns were transplanted into immunodeficient NOD-*scid* mice 30 days post spinal cord contusion injury. hCNS-SCns transplanted mice demonstrated significantly improved locomotor recovery compared to vehicle controls using open field locomotor testing and CatWalk gait analysis. Transplanted hCNS-SCns exhibited long-term engraftment, migration, limited proliferation, and differentiation predominantly to oligodendrocytes and neurons. Astrocytic differentiation was rare and mice did not exhibit mechanical allodynia. Furthermore, differentiated hCNS-SCns integrated with the host as demonstrated by co-localization of human cytoplasm with discrete staining for the paranodal marker contactin-associated protein.

Conclusions: The results suggest that hCNS-SCns are capable of surviving, differentiating, and promoting improved locomotor recovery when transplanted into an early chronic injury microenvironment. These data suggest that hCNS-SCns transplantation has efficacy in an early chronic SCI setting and thus expands the "window of opportunity" for intervention.

Citation: Salazar DL, Uchida N, Hamers FPT, Cummings BJ, Anderson AJ (2010) Human Neural Stem Cells Differentiate and Promote Locomotor Recovery in an Early Chronic Spinal coRd Injury NOD-*scid* Mouse Model. PLoS ONE 5(8): e12272. doi:10.1371/journal.pone.0012272

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Competing Interests: Nobuko Uchida is a paid employee of StemCells, Inc. Aileen J. Anderson has served as a paid consultant to StemCells, Inc. This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

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9 These authors contributed equally to this work.

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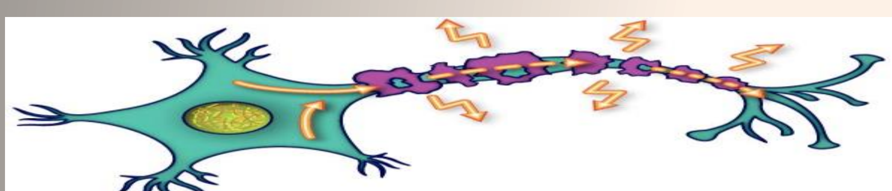
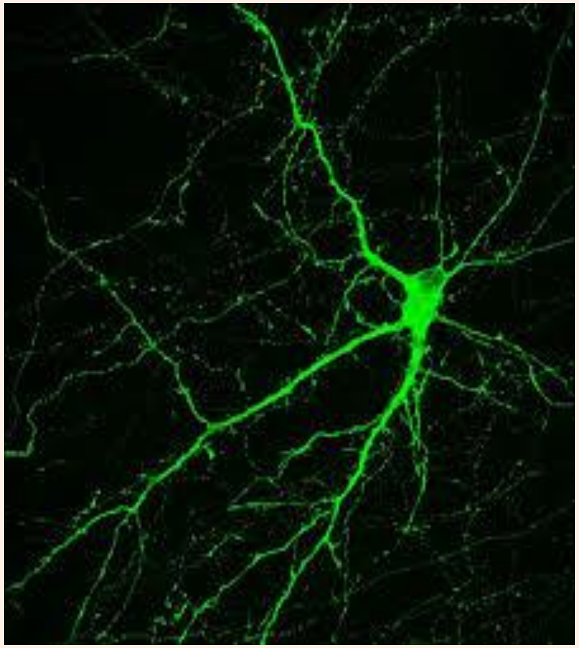


Study	N	Cell Dose	Location	Follow-up as of 7/2015
Lysosomal storage disorder Age range: 2 to 9 yrs	6/6	500 M to 1 B	BRAIN: Frontal lobe Parietal lobe Lateral ventricle	> 5 year (n=3)
Hypomyelination disorder Age range: 12 mo. to 5 yrs	4/4	300,000	BRAIN: Frontal lobe	> 4 years (n=4)
Thoracic SCI Age range: 19 to 53 yrs	12/12	20 M	SPINAL CORD: thoracic intramedullary	> 12 months (n=12)
Age-related macular degeneration Age range: 63 to 92 yrs	15/15	200,000 to 1 M	EYE: subretinal space	> 12 months (n=14)

Clinical Trial Structure

- Age 18 to 60
- C5 – C7
- **Chronic cervical** spinal cord injury A-C
- 4-24 months from injury
- Injection fetal derived HuCNS-SC®
- Peri-lesional injections
- Immunosuppression x 6 months



Summary

- Neural Progenitors can be harvested and propagated in culture
- Regardless of source, NPC can be partially differentiated *in vitro* into OPC and can induce remyelination in animal models of SCI

2. Developing the “Map”

Imaging Biomarker Development

A Reverse Translational Model to Guide Prognostication and Drug Delivery

Index Case I

29 Year Old Male in Rollover MCA

Quadriplegic at Scene and ER

Large Body Habitus (380 lb)
Emergent OR

ORIF, Posterior Approach

ASIA E at 6 month Follow Up

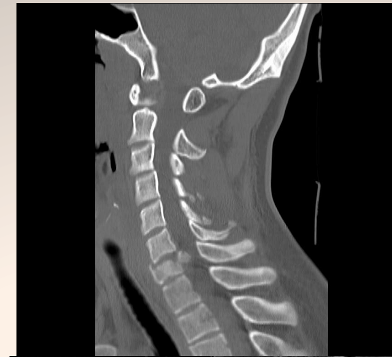
Index Case II

24 Year Old Male Medical Student

Diving Accident

Quadriplegic at Scene and in ER

ASIA A at 1 year



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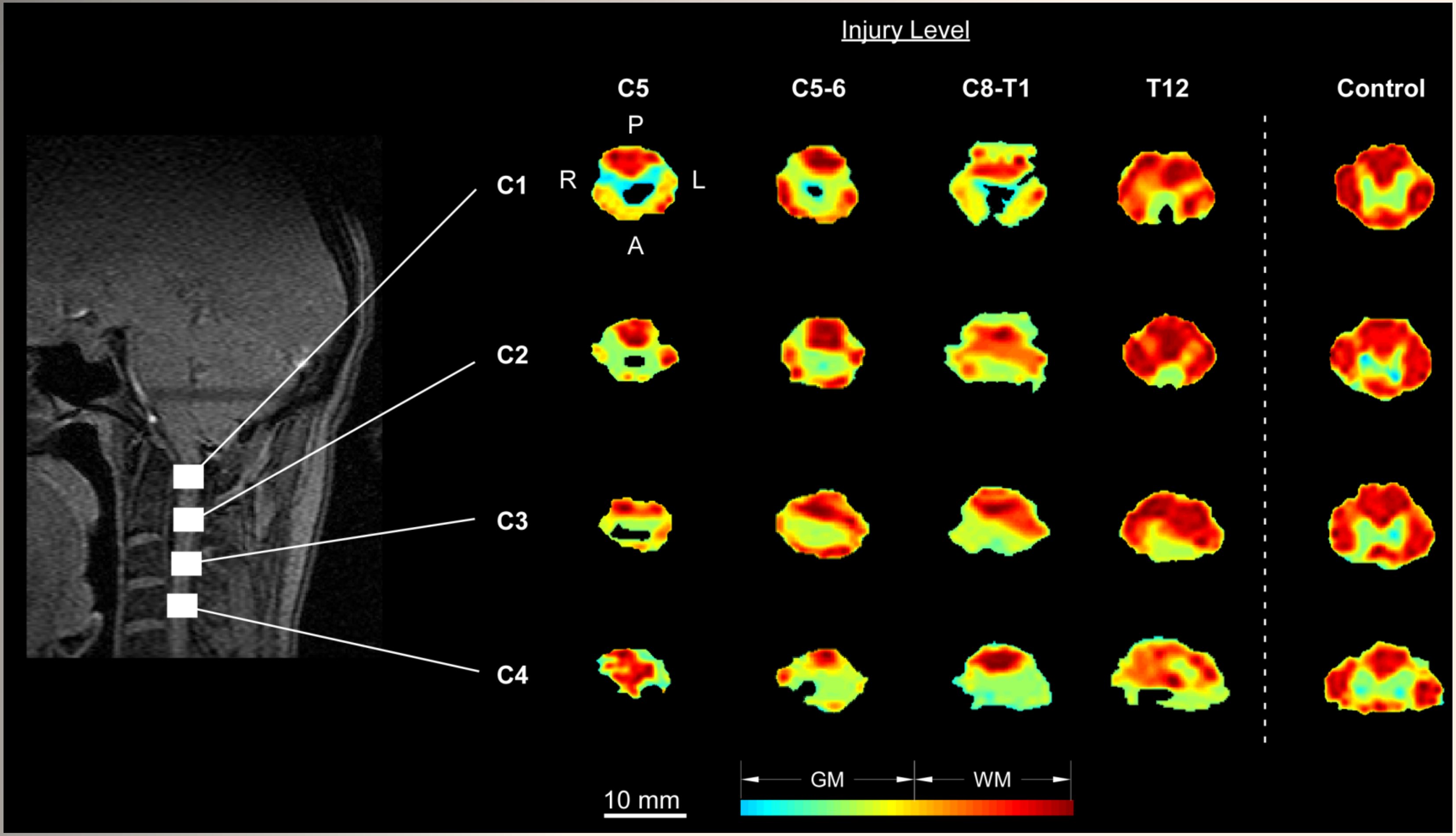
Human Spinal Cord DTI: C-Spine

(Ellingson et al AJNR 2008)

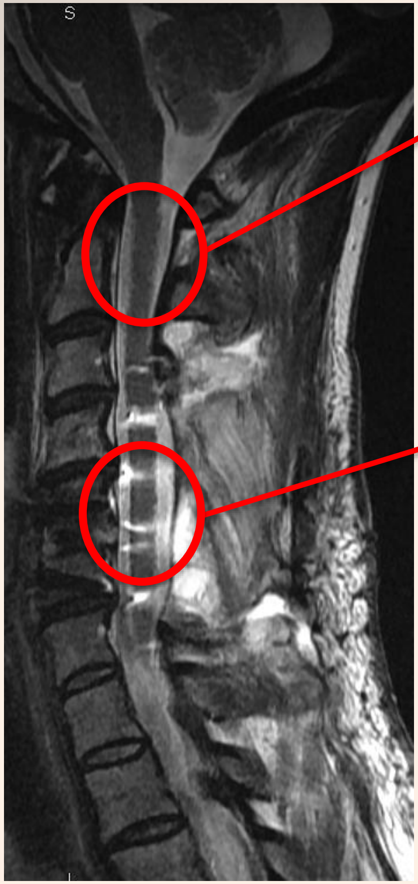


Translational Collaborative Efforts in Spinal Cord MRI (DTI)

CNTR/CIR

Acute Cord Injury Assessment
Matthew Budde, PhD

Disk Degeneration
Tugan Muftuler, PhD

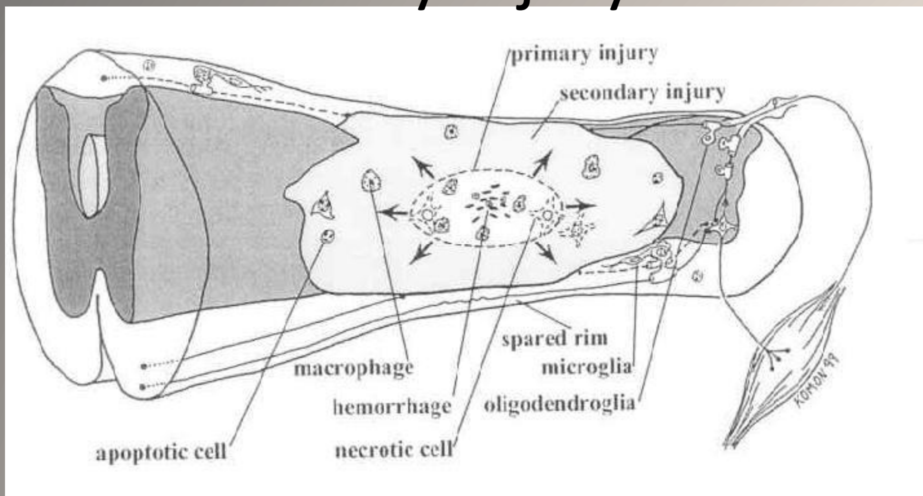


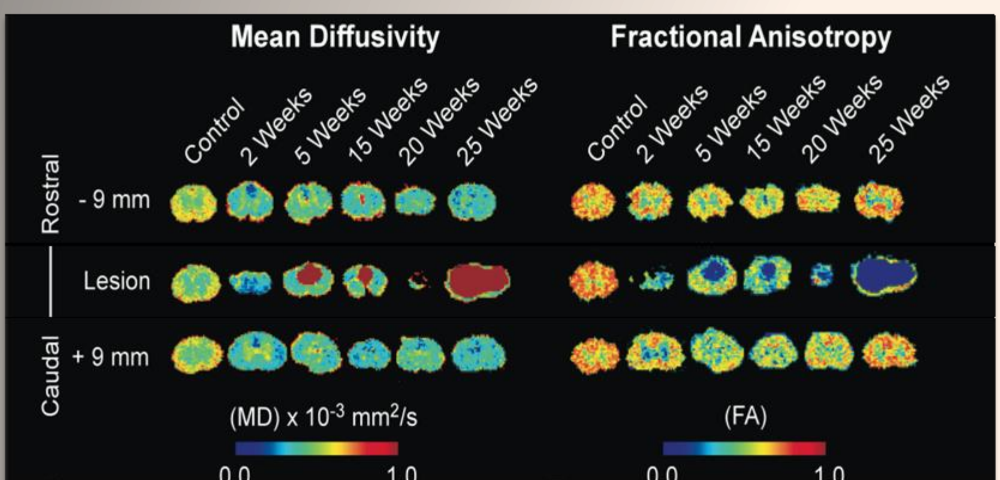
Remote Cord Imaging
Brian Schmit, PhD

Imaging Near Hardware
Kevin Koch, PhD

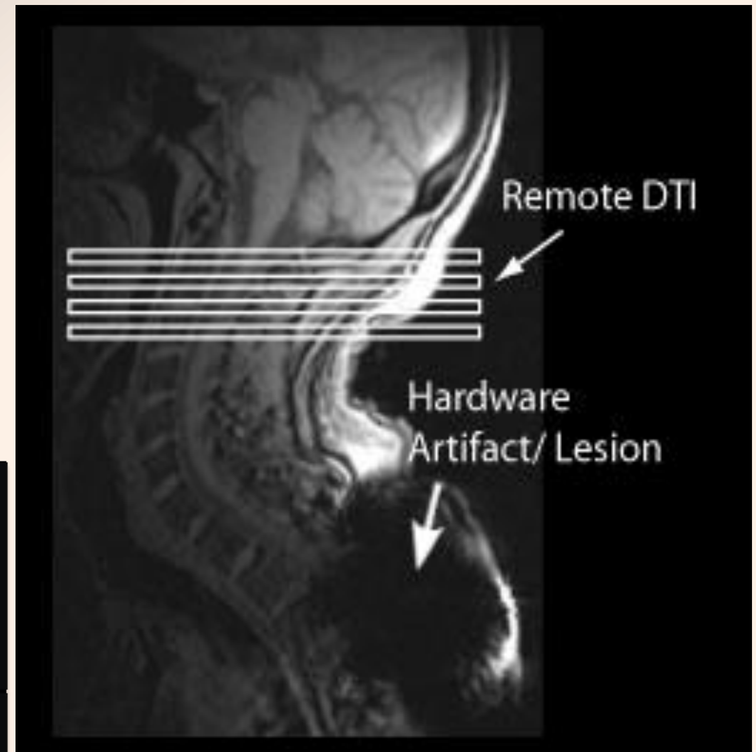
Lesion Site Imaging Limitation
Rationale for Remote Imaging

Secondary Injury in SCI





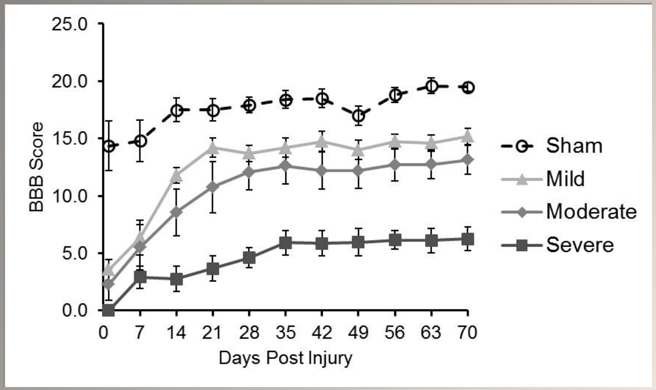
DTI Proximal to Injury Site



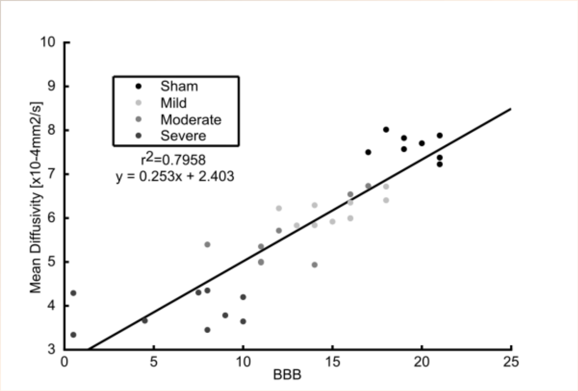
Cervical DTI Predicts Injury Severity in Thoracic SCI

Jirjis et al J Neurotrauma 2013

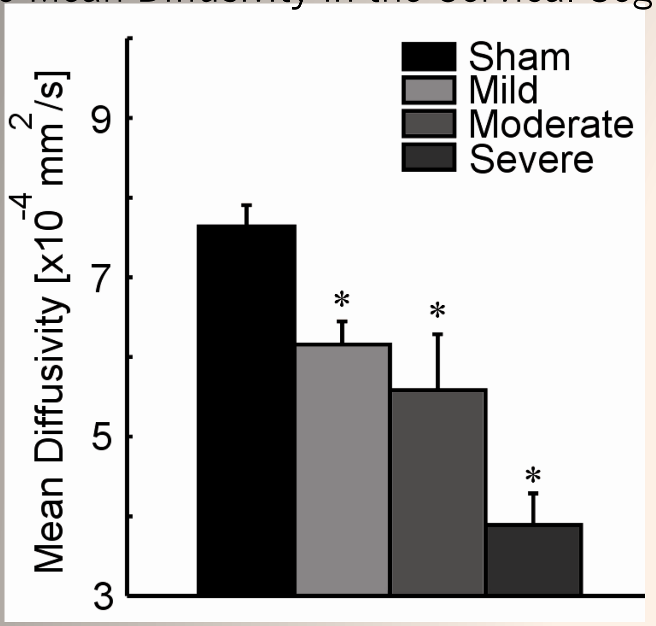
Hind Limb Motor Function



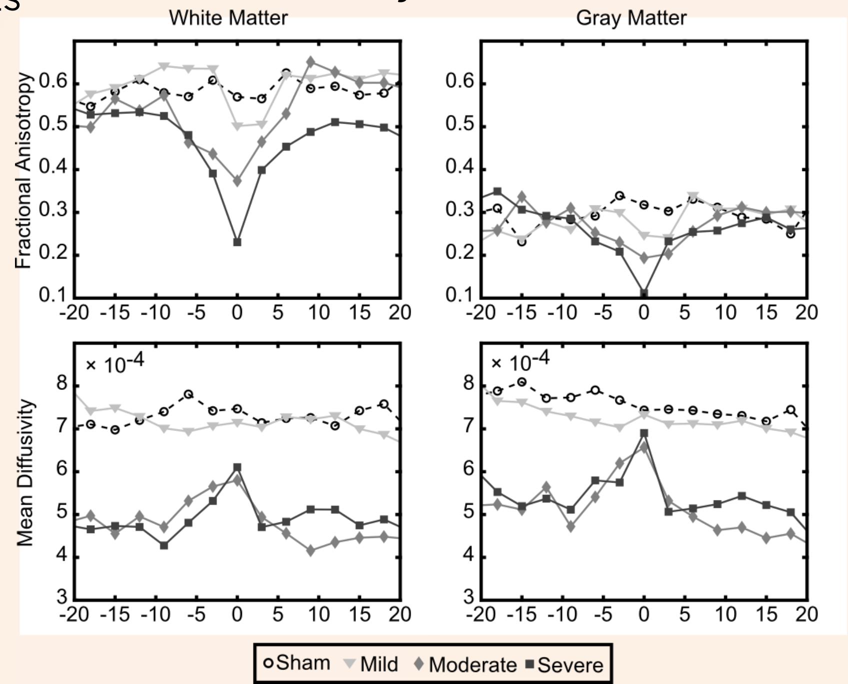
Correlation between MD and BBB



Average Mean Diffusivity in the Cervical Segments



White and Gray Matter Differences



Vedantam et al, World NS 2017

11 patients with cervical cord injury, 11 controls

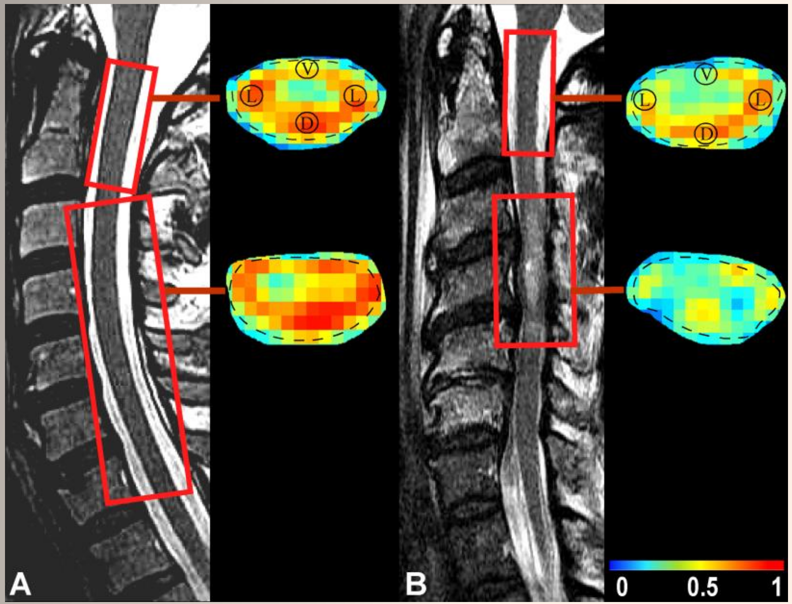
Underwent DTI of the cervical cord at a median duration of 3.5±0.9 days post- injury

DTI metrics measured at 2 levels:

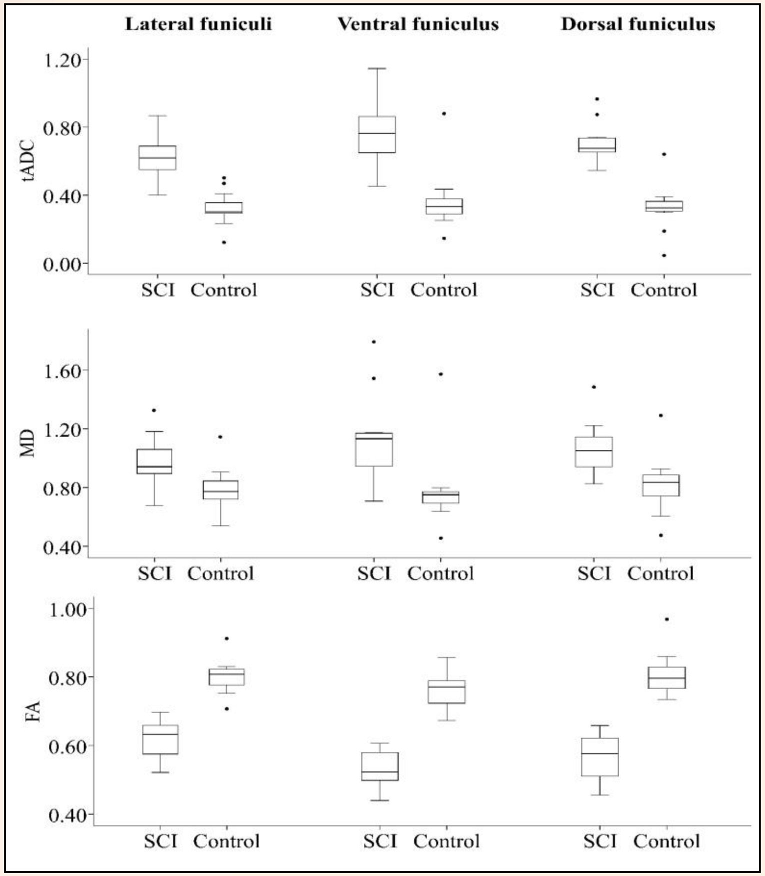
- High cervical cord (C1-C2 level)- hcDTI
- Injury zone- izDTI

Axial FA maps at the high cervical level and injury zone in a single acute SCI patient (right)

Corresponding FA maps in healthy control (left)



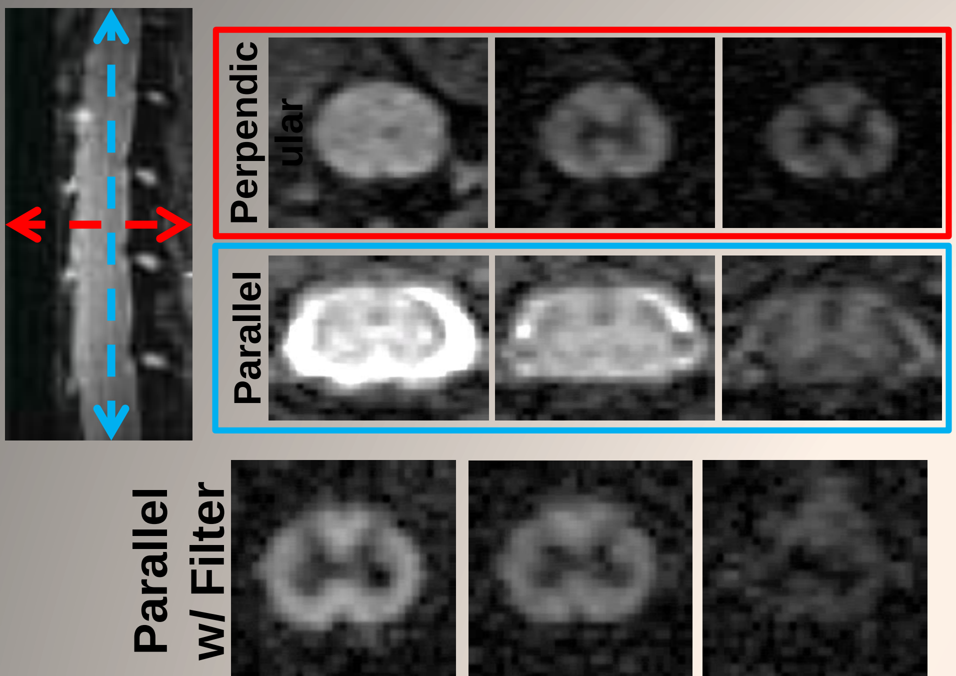
hcDTI metrics within white matter funiculi change predictably in SCI patients vs controls



Improving Specificity and Efficiency
Double Diffusion Encoding (DDE)

Isolated Evaluation of Spinal Cord WM

Budde MD et al



Perpendicular "Filter"
Suppress edema/CSF

Parallel "Detection"
Probes axonal injury

Combined
Measure axonal injury without contamination from edema/CSF

Main Confounding Signals:

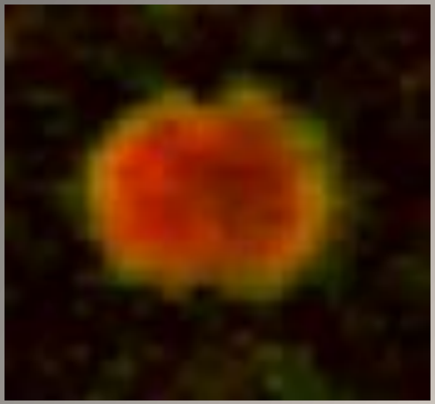
CSF
Edema
Other Inflammation

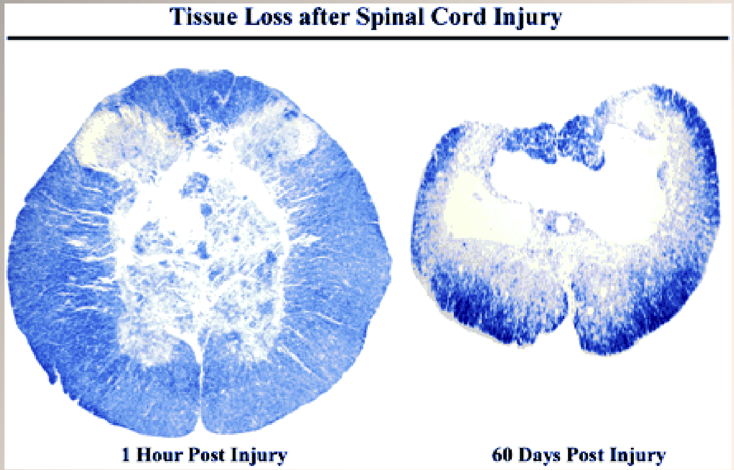
- **Reduced Acquisition time (< 3 mins)**
- **No regions of interest**
- **Immediate quantification**
- **No post-processing**
- **Eliminates variance between scanners**

Clinically Viable

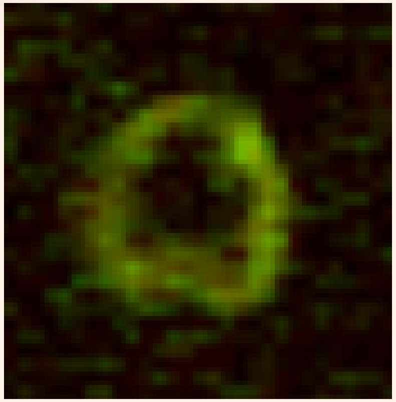
rFOV DDE

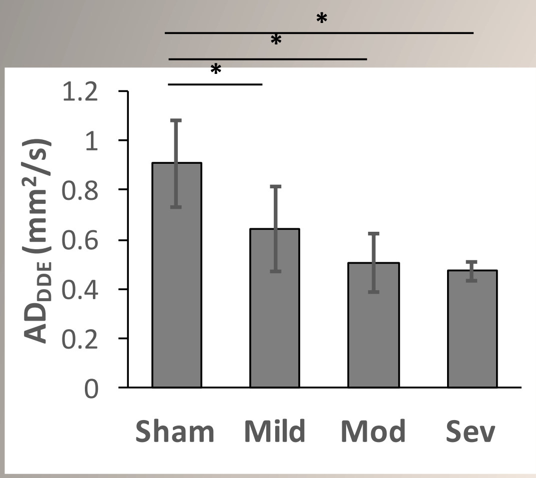
Acute (48 hrs)



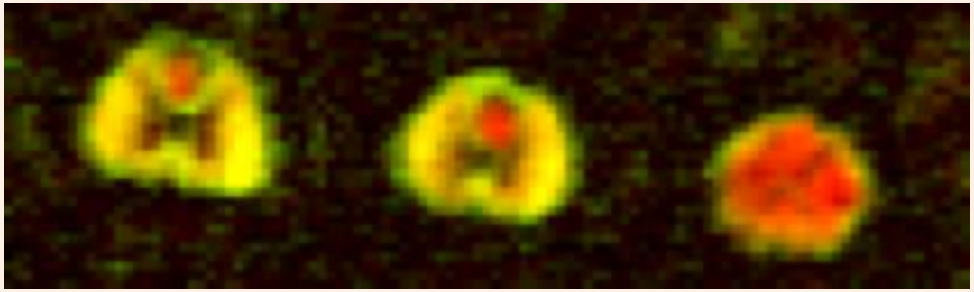


Chronic (30 days)





8 mm Rostral
4 mm Rostral
Lesion Site



Summary

- Current Gray Scale MRI Imaging is not useful as a planning or a prognostic tool
- Diffusion Imaging of the Spinal Cord shows promise in both and is already being harnessed in clinical trials as a secondary outcome measure

3. Delivering the "Drug": Surgical Considerations

Bringing it Together for use in Patients

Evaluation of AST-OPC1 in Subacute Cervical SCI

A Phase 1/2a Dose Escalation Study of AST-OPC1 in Subjects With Subacute Cervical Spinal Cord Injury

Six Sites Currently Enrolling

ClinicalTrials.gov: NCT02302157

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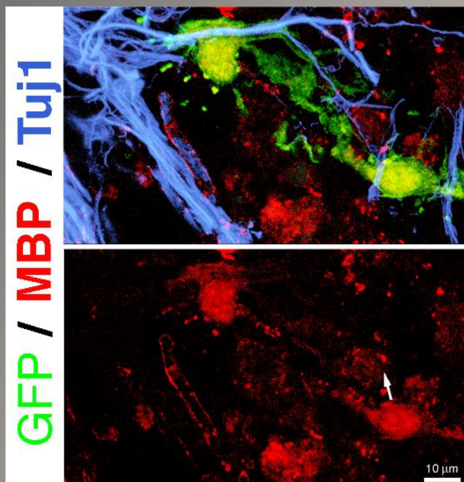
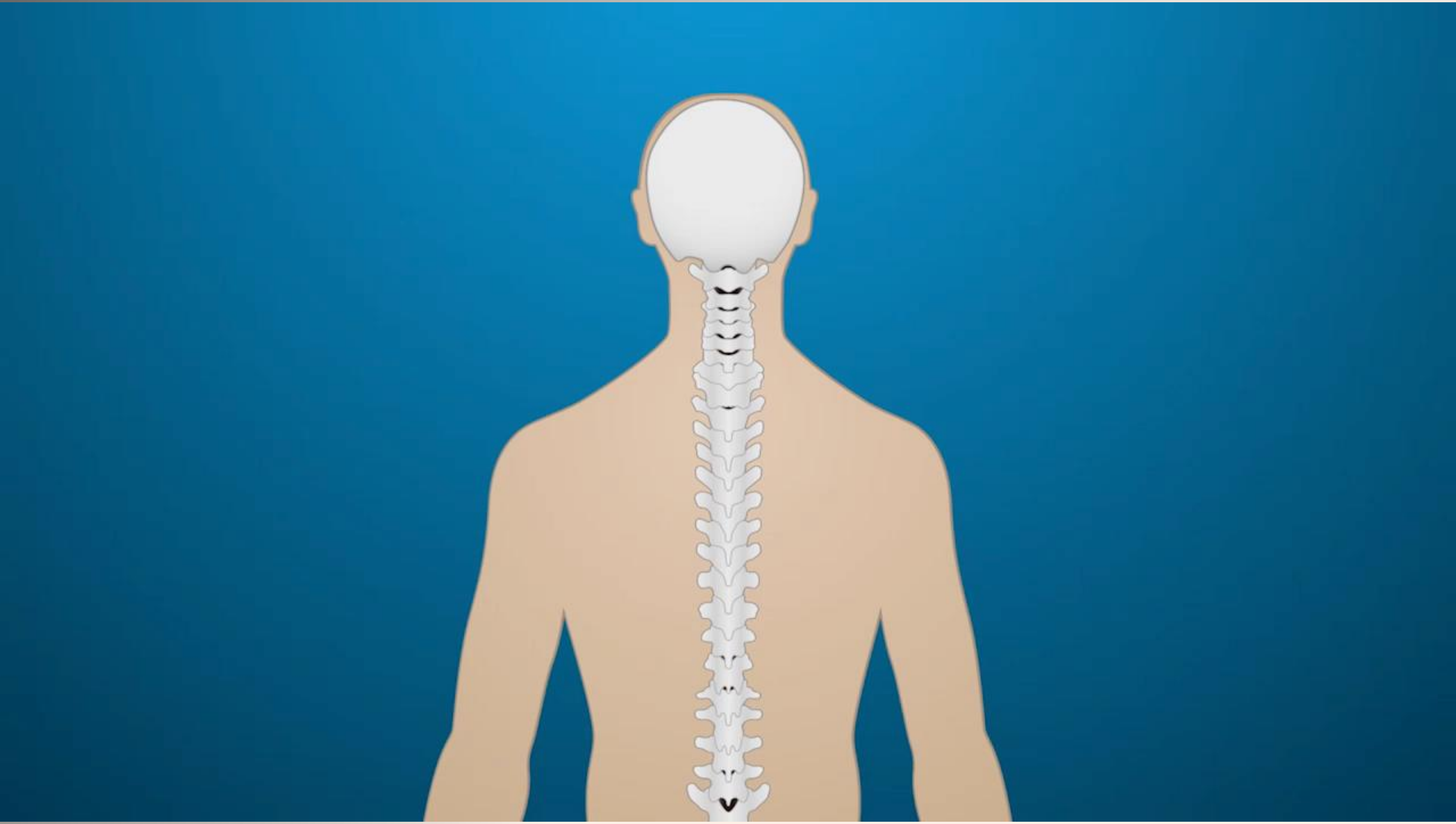
AST-OPC1: hESC-Derived Oligodendrocyte Progenitor Cells

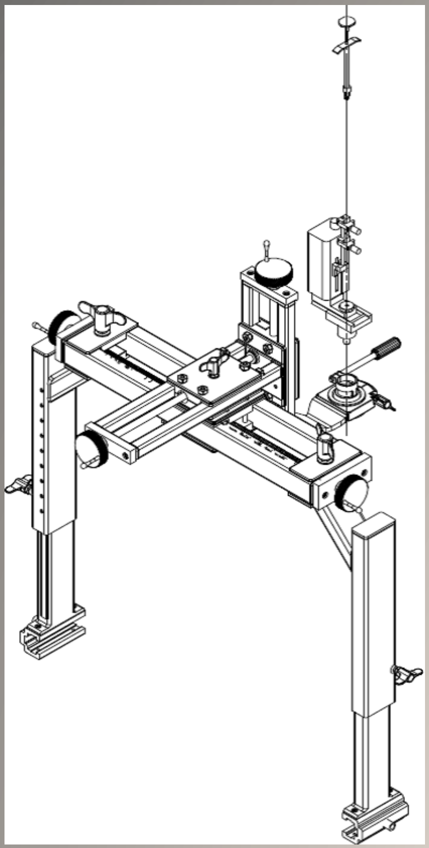


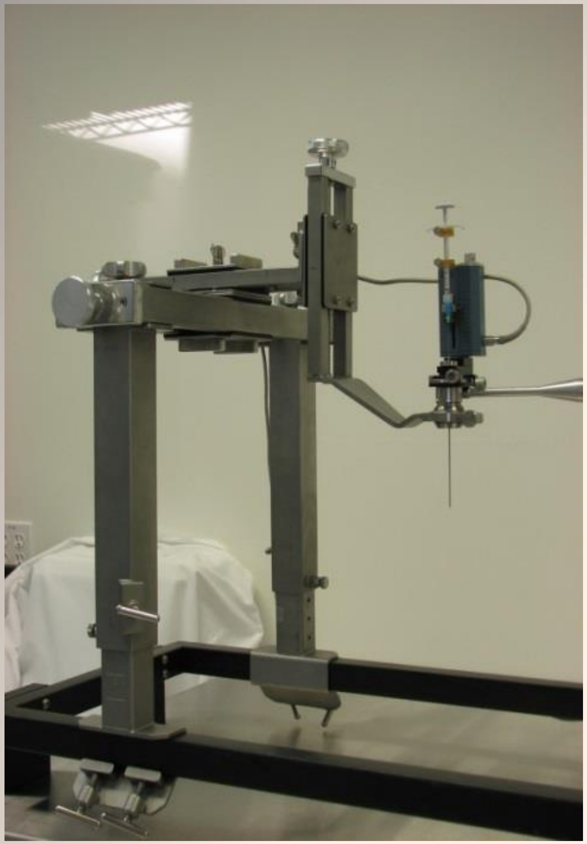
AST-OPC1

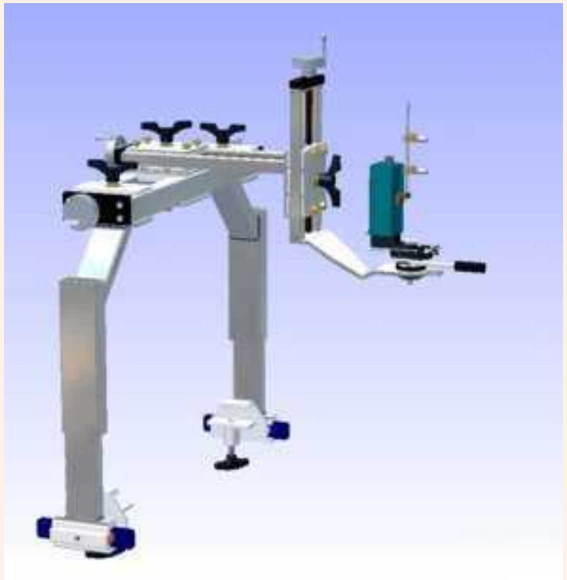
- Cryopreserved Allogeneic Cell Population
- Derived from Human Embryonic Stem Cells (hESCs)
- Characterized Composition of Cells:
  - Oligodendrocyte progenitors
  - Neural progenitors
  - Infrequent mature neural cells and
  - Rare other characterized cell types
- Three identified functions
  - Produces neurotrophic factors
  - Induces remyelination
  - Induces vascularization
- "Off the shelf" administration
- First indication: spinal cord injury
- Potential line extensions in other neurodegenerative diseases



Syringe Positioning Device

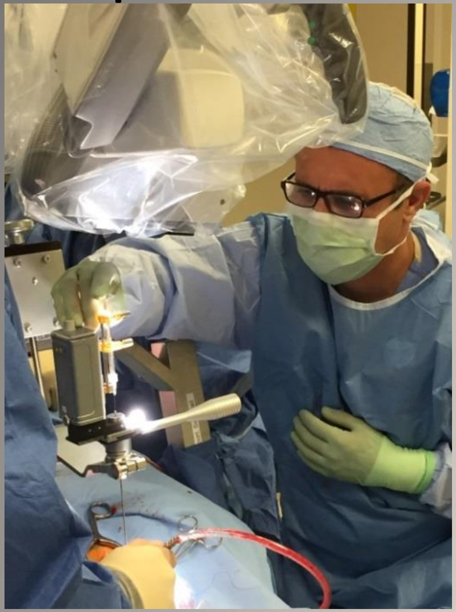




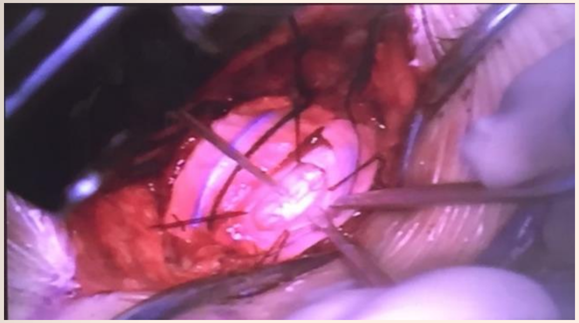


AST-OPC1 Injection Procedure

Shepherd Center



Rush University



- Injections performed using a table-mounted syringe positioning device (SPD)
- Direct intra-parenchymal injection into the spinal cord lesion
- Single 50µL injection for both the 2M & 10M doses; Two injections for the 20M dose
- No intraoperative complications to date

Summary of Findings from First in Human Study of AST-OPC1

All 5 Patients Now Followed for > 5 Years

Well Tolerated

- AST-OPC1 well tolerated, with no SAEs to date deemed related to the cells, delivery method, or immunosuppressive regimen

No Immune Responses

- No evidence of immune responses to AST-OPC1, even 10 months after removal of all immunosuppression
- Despite significant HLA mismatches between AST-OPC1 and subjects
- Suggests low dose, transient immunosuppressive regimen may be sufficient to enable long term engraftment of cells

Engraftment

- MRI results consistent with reduced cavity formation at injection site in 4 of 5 subjects

No Changes Neurological Function

- No evidence of significant changes in neurological function
- No evidence for ascending loss of function from cells or delivery
- Efficacy not anticipated in this study due to low dose (5-10x below predicted efficacious range) and suboptimal patient population (complete thoracic injuries)

AST-OPC1 Clinical Development Plan in Cervical SCI

Objectives of Trial

- Establish safety of AST-OPC1 in cervical sensorimotor complete SCI
- Assess effects on upper extremity motor function
- Investigate effects on additional measures of neurological function

Dosing complete

Currently enrolling

Future enrollment

AIS-A

Cohort 1

3 subjects with C5-C7 cervical SCI

- **Dose $2x10^6$ AST-OPC1**

Dose Escalation → Confirm Low Dose Safety Activity

Cohort 2

5 subjects with AIS-A C5-C7 cervical SCI

- **Dose $1x10^7$ AST-OPC1**

Dose Escalation → Confirm Mid Dose Safety Activity

Cohort 3

5 subjects with AIS-A C5-C7 cervical SCI

- **Dose $2x10^7$ AST-OPC1**

Confirm High Dose Safety Activity

AIS-B

Cohort 4

5 subjects with AIS-B C5-C7 cervical SCI

- **Dose $1x10^7$ AST-OPC1**

Dose Escalation

Cohort 5

5 subjects with AIS-B C5-C7 cervical SCI

- **Dose $2x10^7$ AST-OPC1**

ASTERIAS BIOTHERAPEUTICS

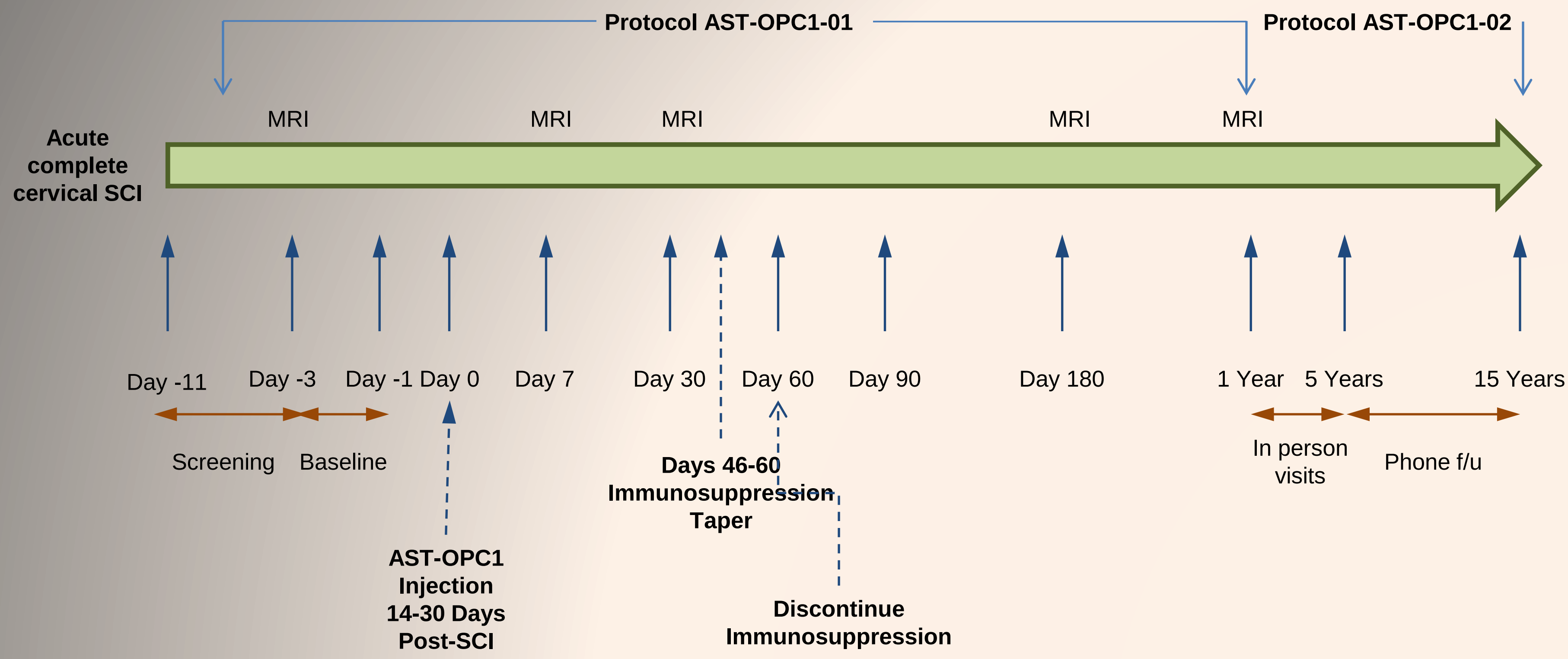
AST-OPC1 Cervical Phase 1/2a Study Schema

- Open Label Trial
- Multi-Center (8-12 sites)
- Complete cervical SCI (C5-C7)
- Temporary Immunosuppression

Primary Assessment: Safety

Secondary Assessment: ISNCSCI exams

Exploratory Assessments: SCIM, GRASSP



Cervical Phase 1/2a Clinical Trial: Enrolling Sites To Date

Enrolling Sites

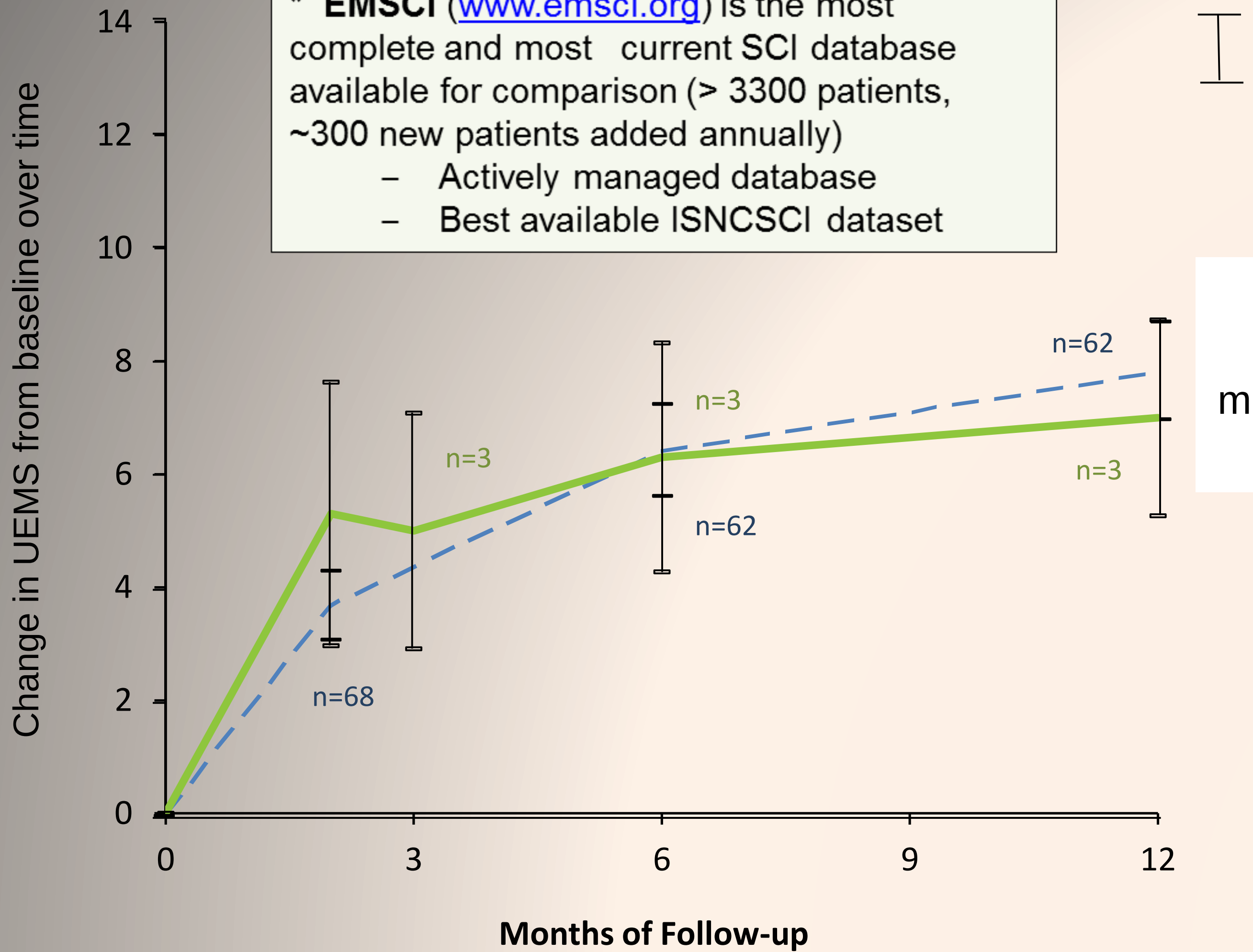
Shepherd Center	**Dr. Donald Leslie**
Rush University Medical Center	**Dr. Richard Fessler**
Stanford / Santa Clara Valley Medical Center	**Dr. Gary Steinberg** **Dr. Steve McKenna**
Rancho Los Amigos National Rehabilitation Center / USC University of Southern California	**Dr. Charles Liu**
Medical College of Wisconsin	**Dr. Shekar Kurpad**
Indiana University	**Dr. Eric Horn**

Low Dose 2 Million Cell Cohort Has Motor Recovery Similar to Matched Historical Controls

Cohort 1 data supports safety of AST-OPC1

* **EMSCI** (www.emsci.org) is the most complete and most current SCI database available for comparison (> 3300 patients, ~300 new patients added annually)

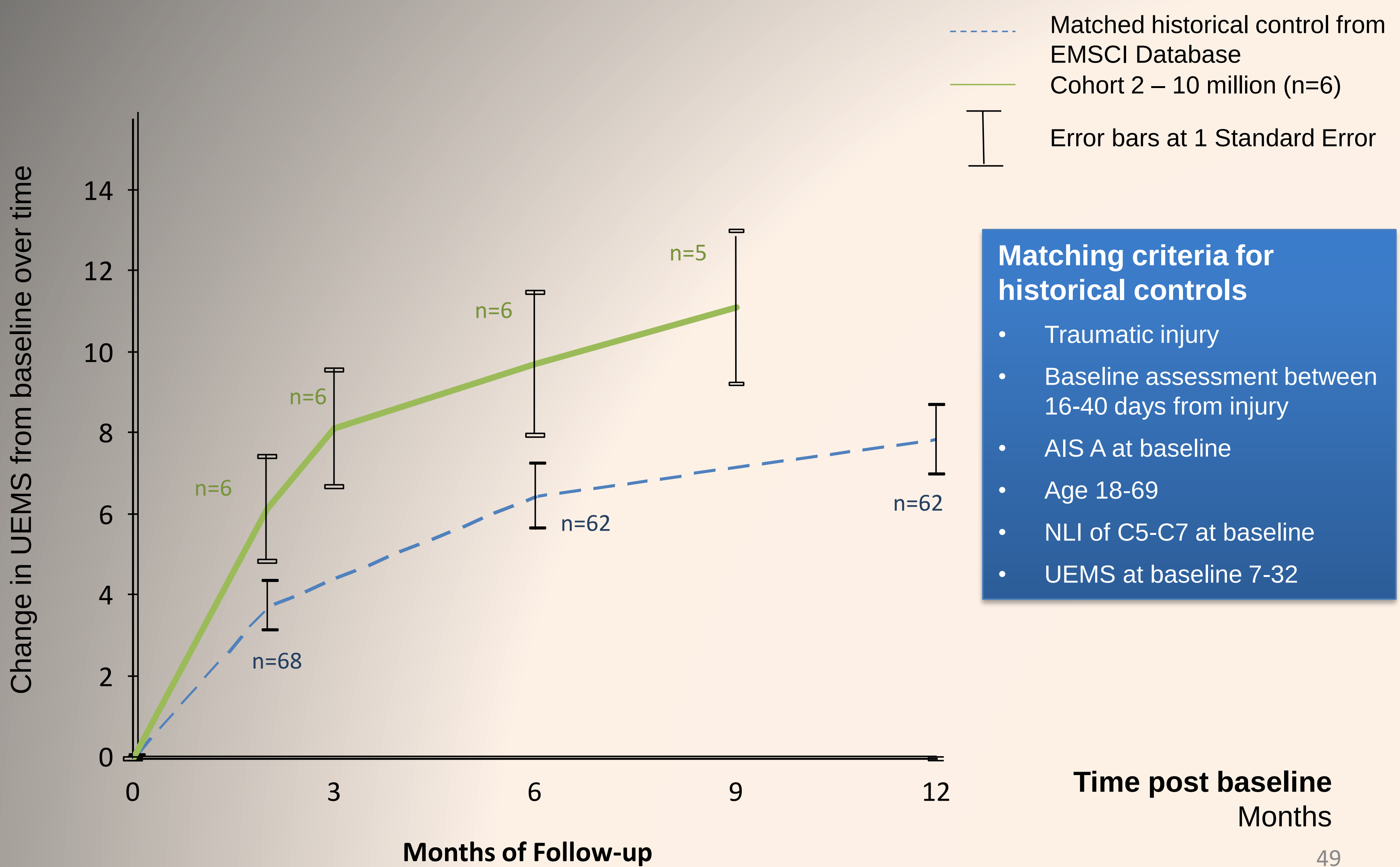
- Actively managed database
- Best available ISNCSCI dataset



As expected, UEMS recovery in low dose 2 million safety cohort tracks with historical controls

Time post baseline
Months

AIS-A 10 Million Cell Cohort Experienced Greater UEMS Recovery than Matched Historical Control Group

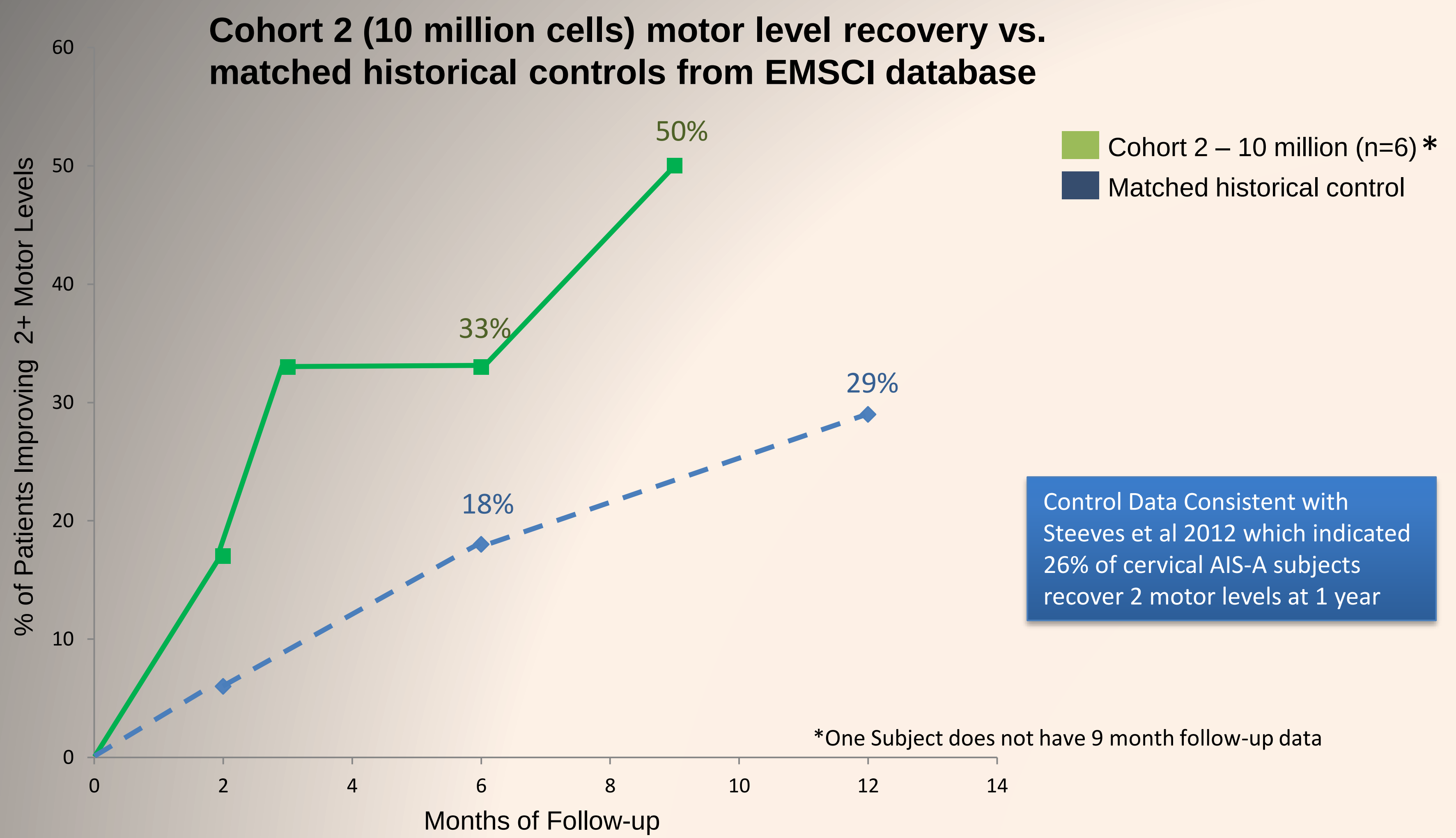


Matching criteria for historical controls

- Traumatic injury
- Baseline assessment between 16-40 days from injury
- AIS A at baseline
- Age 18-69
- NLI of C5-C7 at baseline
- UEMS at baseline 7-32

Time post baseline
Months

Cohort 2 Motor Level Recovery for 6 Subjects at Latest Follow-up Visit Through 9 Months



Motor level improvement vs. baseline measurement

Steeves et al., *Top Spinal Cord Inj Rehabil* 2012; 18(1): 1-14

HuCNSC: Stem Cells Inc Trial

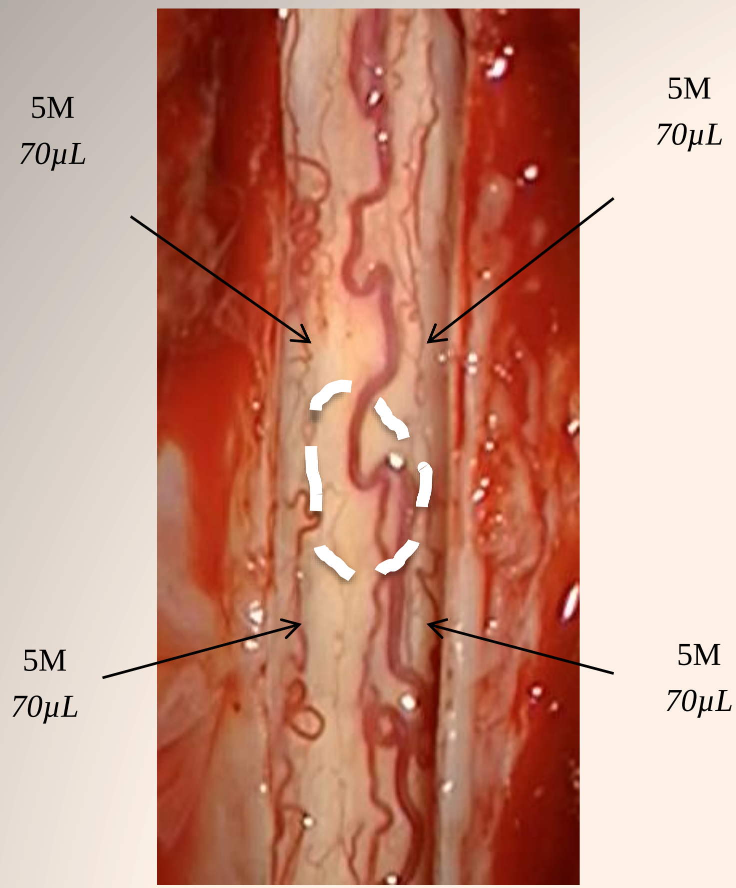
- Phase II Proof of Concept Trial in Cervical ASIA A patients
- Derived from Fetal Brain Tissue
- Cell Injection approx 14 weeks and up to 104 weeks Post SCI
- Objectives
  - Primary: Dose Escalation
  - Secondary: Imaging Biomarker Validation

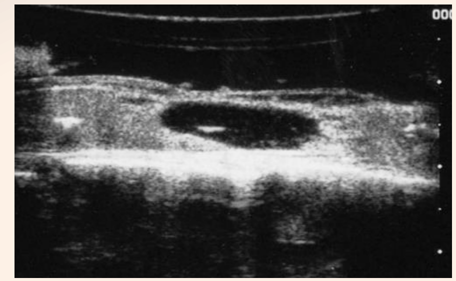
J Neurotrauma. 2018 Sep 5. doi: 10.1089/neu.2018.5843. [Epub ahead of print]
Clinical Outcomes from a Multi-Center Study of Human Neural Stem Cell Transplantation in Chronic Cervical Spinal Cord Injury.
Levi AD[1,2], Anderson KD[3], Okonkwo DO[4], Park P[5], Bryce T[6], Kurpad SN[7], Aarabi B[8,9], Hsieh J[10], Gant K[11].

Neurosurgery. 2018 Apr 1;82(4):562-575. doi: 10.1093/neuros/nyx250.
Emerging Safety of Intramedullary Transplantation of Human Neural Stem Cells in Chronic Cervical and Thoracic Spinal Cord Injury.
Levi AD[1], Okonkwo DO[2], Park P[3], Jenkins AL 3rd[4], Kurpad SN[5], Parr AM[6], Ganju A[7], Aarabi B[8], Kim D[9], Casha S[10], Fehlings MG[11], Harrop JS[12], Anderson KD[1], Gage A[13], Hsieh J[13], Huhn S[13], Curt A[14], Guzman R[15].

Phase I/II Thoracic SCI HuCNS-SC Transplantation

A phase 2 single-blind, randomized, parallel arm study of the safety and efficacy of HuCNS-SC transplantation in cervical spinal cord injury





Cell dose manually injected above and below injury based on MRI and ultrasound

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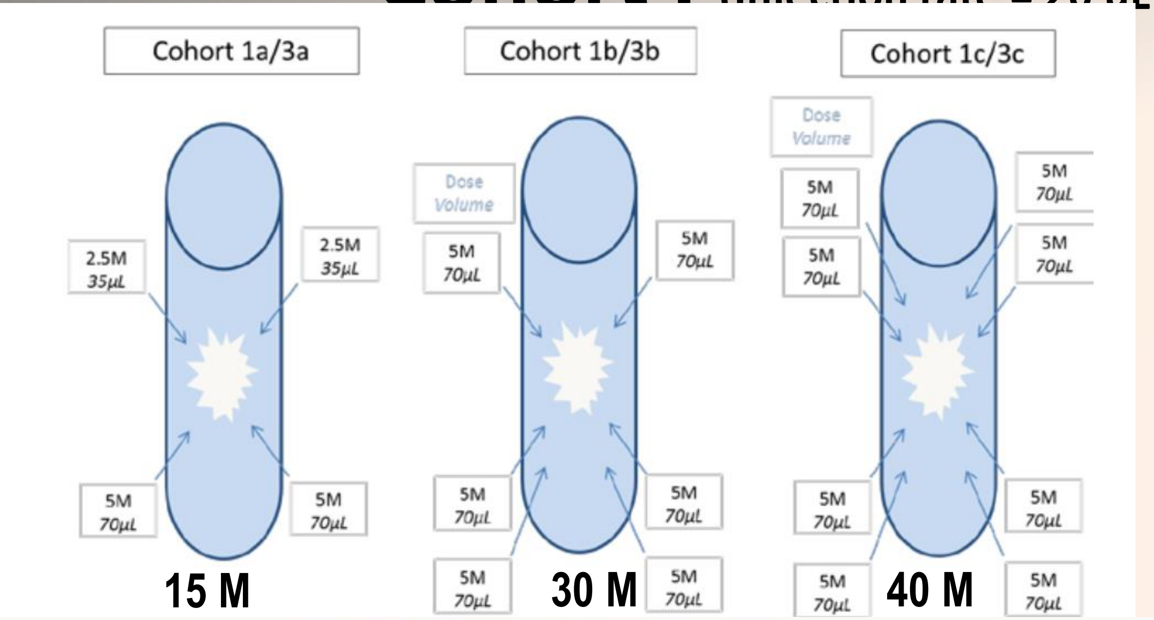
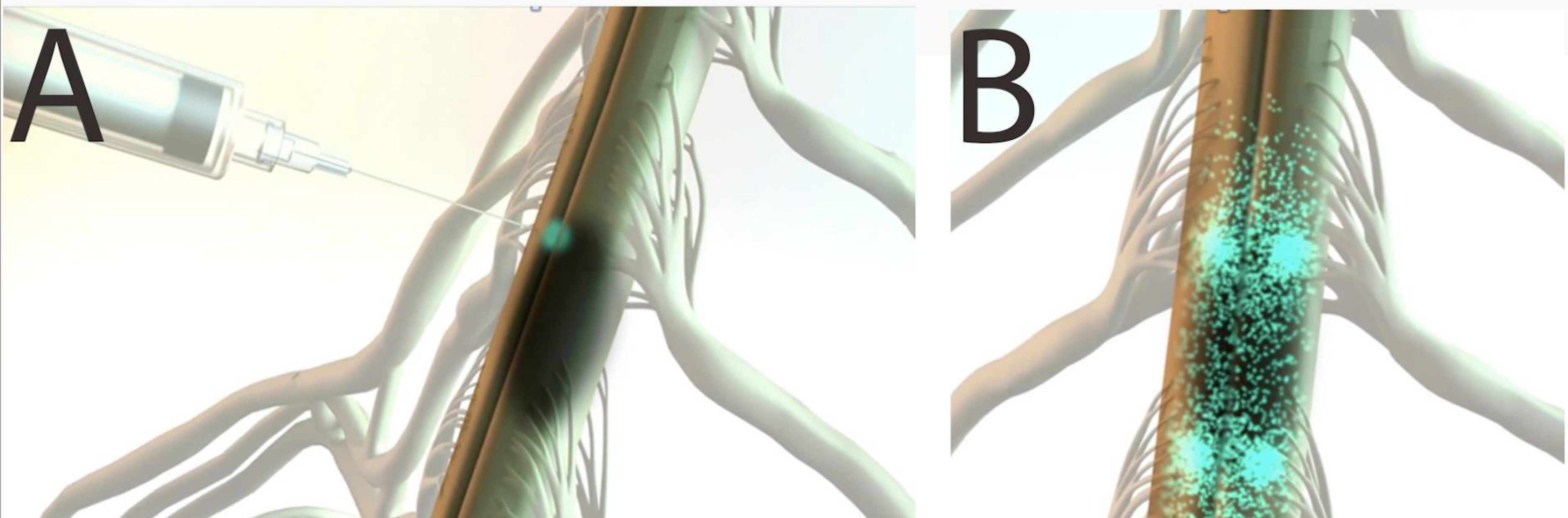
Cell Injection Strategy

Cohort I (Injection rate = 20 μL / minute)





Where are we NOW?

- AST-OPC1 cells are safe.
- No serious adverse effects so far
- Early recovery of **meaningful** function
  - Improved Arm and Hand Function
    Greater Independence in Self-care
  - Greater Independence in Transfers and Transport
  - Greater Independence in Activities of Daily Living

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Time and Resources for Outcome?

- Concept TO (2001) (Funding)
- Research TO (2001-10) (Funding)
- Clinical Trial TO (2010-Present) (Funding)
- Standard of Care

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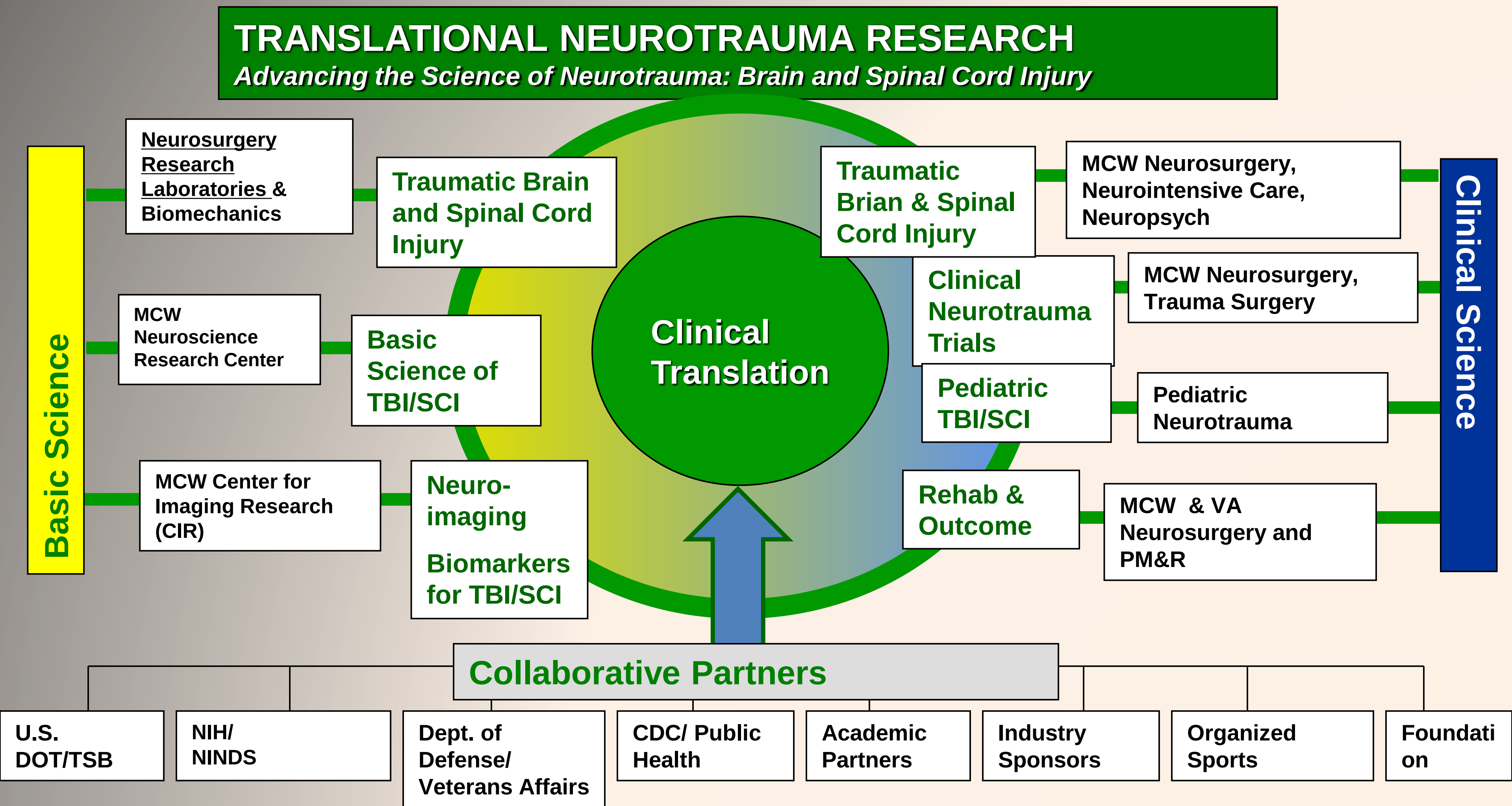
CENTER OF NEUROTRAUMA RESEARCH



Thank You