Stem cells for the treatment of neurodegenerative disease

Jan A. Nolta, Ph.D.

Professor, Director of the Stem Cell Program and Institute for Regenerative Cures
University of California, Davis

Neurons generated from human pluripotent stem cells in culture
Jeanie Liu, Nolta Lab
UC Davis Stem Cell Program Disease Teams-
Over 145 Basic, Translational, and Clinical Faculty Investigators working together

- Liver repair and regeneration, bioengineered livers
- Peripheral artery disease: revascularization to prevent amputation
- Eye degeneration/blindness
- Lung disease, lung repair and regeneration
- Skin: Non-healing ulcers, burn repair
- Bone repair, osteoporosis, cartilage regeneration
- Heart disease, infarction repair and stroke
- Neurodegenerative (Parkinson's, Huntingtons, Alzheimers, ALS)
- Neurodevelopmental disorders (autism spectrum, FXTAS, fragile x)
- Kidney repair and regeneration
- Bladder repair, bioengineered bladders
- Blood disorders, autoimmune disorders (Scleroderma, MS)
- HIV treatment using gene-modified stem cells
- Hearing, inner ear cilia repair
- Tumor stem Cells, Cell-based immunotherapy for Cancer
Neuroscience research at UC Davis: programs and centers devoted to advancing our understanding of the brain:

- The Center for Neuroscience
- The MIND Institute
- The Center for Mind and Brain
- The Alzheimer’s Disease Center
- Center for Visual Sciences
- The Imaging Research Center
- Adult stem cell therapies began in 1956
- Bone marrow transplantation
- Hundreds of thousands of lives have been saved through BMT
- Our team has processed cells for over 800 stem cell transplants and has performed 18 stem cell therapy clinical trials for tissue repair and treating genetic disease
Types of stem cells

<table>
<thead>
<tr>
<th>Adult Stem Cells</th>
<th>Pluripotent Cells</th>
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<tr>
<td>Blood forming (hematopoietic)</td>
<td>Embryonic</td>
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<tr>
<td>Mesenchymal (supporting cells)</td>
<td>Induced pluripotent stem cells</td>
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![Image showing adult stem cells and pluripotent cells](image-url)
Why use adult stem cells?

- Adult stem cells cannot create an entire tissue, as can embryonic cells.

- However, under the right conditions, adult stem cells can act as the “paramedics of the body”, rushing to areas of hypoxia and rapidly causing revascularization, early after tissue injury.
Mesenchymal Stem Cells (MSC): Reparative adult stem cells that can act as excellent “delivery vehicles” in the body.

Bone Marrow is harvested From a normal, healthy donor

Cells expanded in a clean room facility
Mesenchymal Stem Cells (MSC)

Expansion of MSC from bony spicules/trabeculae
Techniques detailed in:

Genetic Engineering of Mesenchymal Stem Cells. Kluwer
Nolta JA (Editor). Feb 2006
MSCs can be engineered to secrete copious amounts of protein factors for delivery to other cells and tissues in the body.
MSC delivering a protein product (red) throughout the tissue
(Meyerrose et al, Nolta lab, Published in Stem Cells 2008).

A – no MSC transplantation

B - MSC alone, making lower “innate” levels of the enzyme beta-glucuronidase

C – MSC engineered to deliver the protein. The entire tissue is corrected.
Nolta lab:
Papers since 1989 using Gene-modified Marrow Stromal Cells (MSC) to systemically produce cytokines, enzymes, and other factors in vivo
1987-2010 Nolta lab: Development of models to study biosafety and efficacy of engineered human Marrow Stromal Cells / Mesenchymal Stem Cells (MSC)

Decade-long biosafety studies: Bauer et al., Nolta lab 
*Mol Ther* 16, 1308 (2008)
Using Mesenchymal Stem Cells as “paramedics” engineered to treat Huntington’s disease
Clelland et al, 2008

Expanded CAG (exon 1 htt gene) → Mutant Huntingtin Protein → Transcriptional Dysregulation, Protein interactions & aggregation → Cellular Dysfunction → Autophagy, Apoptosis → Cell Death

Clinical Disease Phenotype
HD is not a “schema” like the prior slide. It is people, families, friends who suffer. Please visit sites like that from our friend Chris, below, to help understand it.

www.huntingtonsdance.org

Chris Furbee
• Currently, no preventative or curative treatments exist for HD.

• In addition to new drugs being developed to treat different aspects of the disease such as chorea, cellular therapy and gene therapy provide the best options for permanent cures.
In HD, a repeated sequence of DNA leads to a mutant RNA and then a toxic protein that leads to the death of neurons.

This disease is tough to treat because we need to restore the neurons but also to knock out the bad RNA and protein.

The field of “RNA interference” gives hope.
Studies Underway, Working Toward Cellular Therapy Trials for Huntington’s disease

1. MSC to promote neurorestoratorive effects: MSC and MSC/BDNF

2. MSC to continually secrete RNA interference molecules to reduce levels of the toxic huntingtin RNA and protein.
WHY plan to use Mesenchymal stem cells??

• Strong safety profile
• Neurorestorative effects
• Relative ease of isolation and expansion
Neurorestorative effects of MSC

- Reduce inflammation
- Increase vascularization
- Reduce death of damaged neurons
- Restore synaptic connections between damaged neurons

MSC can be safely delivered into the brain and spinal cord, in small and large animal models

• Following intravenous injection, only low levels of human MSC cross the blood brain barrier in chronic disease models (Meyerrose et al 2007).

• To effectively combat most neurodegenerative diseases we will need to deliver larger numbers of MSC directly into the brain tissue.

• We are validating the biosafety of catheter-based MSC delivery systems into the brains of rodents and non-human primates.
Brain: the final frontier

MSC use the external surface of the vasculature as “train tracks” to migrate throughout the brain tissue to deliver factors to damaged neurons.
FDA Gives TCA Cellular Therapy Green Light to Proceed with First ALS Adult Stem Cell Trial

TCA Cellular Therapy announced that the U.S. Food and Drug Administration (FDA) has approved its adult mesenchymal stem cell protocol to conduct Phase I clinical trials to treat Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig’s disease).

**Important consideration:**

**RISK**

**vs.**

**Potential BENEFIT**

**MSC will be injected into the spinal cords of patients.**

Dr. Miguell of TCA Cellular Therapy lifts the cover of the liquid nitrogen freezer where the adult stem cells are stored. (Staff Photos by Debbie Glover)
Medium Spiny Neuron

Damaged/Lost in HD- they control movement, cognition and emotion

Damaged neurons can “round up” and retract axons: this prevents effective signaling from cell to cell in the neural network.
Mesenchymal stem cells can restore synaptic connections between neurons by secreting factors (reviewed in Joyce et al, Nolta lab, 2010)
Adult Human Stem cells “home” to sites of tissue injury

Injjured leg

Labeled stem cells: 48 hours

healthy leg

Capoccia et al, Nolta lab 2009
MSC migrate to damaged striatum after implantation into the brain in an HD rat model

MSC (dark spots, white arrows) migrated from injection site at the red arrow to the area of striatal damage (white lesion)

Sadan et al, 2009
Human MSC (pink) in the brain of immune deficient mouse

Intracranial MSC implant
8 human cells per 30 murine in this field

Sham implant

Wirthlin et al, Nolta lab, MS in prep 2011
• Our ongoing studies show that human MSC, injected directly into the brains of immune deficient mice, survive for months and migrate readily throughout the neural tissue.

• MSC are still present in robust numbers and the brain tissue architecture is unaltered.
Intracranial injection of human mesenchymal stem cells into the most stringent biosafety model available:

1. After 5 months, human mesenchymal stem cells were still present in the brain tissue.

2. No tumors or other tissue abnormalities were detected.

SAFETY WAS DEMONSTRATED
Pre-IND paperwork was filed
written input was received from the FDA
Initial paperwork for the proposed HD clinical trial on its way to the FDA
Working toward clinical trial for HD following dialog with FDA, IND submission and approval

- MSC from healthy donors implanted near the affected portion of the brain in symptomatic HD patients.

- Evaluation of neuroprotective effects: slowing of disease progression as measured by Total Functional Capacity score and delay in volumetric MRI changes known to occur in HD. Clinical improvement in severity of movement disorders and cognitive impairment as measured by the Unified HD Rating Scale (UHDRS) and a battery of cognitive tests.

- Planned following the ongoing IND-enabling studies at UC Davis and conversations with the FDA/IND Application/ pending future FDA approval.

- HD Disease Team: UC Davis: Wheelock, Nolta, Tempkin, Shalaie, Olson, Annett, Bauer, Walker, other GMP and clinical staff, Jane Paulsen (MRI)

- Inclusion and exclusion criteria have been delineated

- Currently assembling advisory board and DSMB
MSC Therapy
For Neurodegeneration

Monitor patient for Potential improvement in UC Davis Movement Disorders Clinic

Cells produced under:
GOOD MANUFACTURING PRACTICE (GMP), with QUALITY CONTROL (QC) and QUALITY ASSURANCE (QA)
• MSC will exert neuroprotective/ neurorestorativa effects in the brain. We can engineer them to produce additional factors such as BDNF to further delay striatal neuron death in HD, and we are testing MSC biosafety in the brain.

• However, the toxic mutant htt protein will still be present in Huntington’s disease.

• To truly cure the disease the levels of this protein must be continually reduced.
Small interfering RNA (siRNA) molecules bind to the RNA messages transcribed from a specific gene and cause their destruction.

If the siRNA is effective, the protein levels will be greatly reduced from that targeted message.
Small interfering RNA (siRNA) strategies in mouse models of HD have been shown by ten different laboratories to specifically decrease mutant protein expression and aggregation, while sparing the normal protein and prolonging survival.

reviewed in:

How to deliver siRNA to the neurons of HD patients?

- Charged nucleic acids do not readily cross the blood–brain barrier.

- Our strategy uses human mesenchymal stem cells for sustained production and delivery of anti-mutant HTT siRNA (Olson, Wirthlin, Nolta, UC patent #2009-170, International patent filed 2009).

Funded by CIRM TR1-01257: (Nolta) translational grant
• Working with colleagues in the UC Davis Center for Biophotonics, we have shown that MSC infuse siRNA directly into damaged cells through tunneling nanotubules and exosomes.

• This technology could have far-reaching impact into any neurodegenerative disorder where a protein must be decreased in the brain.

Olson et al, Nolta lab 2010
Human MSC migrate rapidly and interact with many cells, infusing them with factors if needed. MSC Migration Video Courtesy of Scott Olson, Ph.D., Nolta lab
MSC to neuron transfer of alexa-dye labeled siRNA – standard microscopy (Pontow, Nolta lab 2010)
human MSC shown transferring labeled anti-huntingtin siRNA (red) into a target cell (green)

From Scott Olson, Nolta lab
Target cell (green) with anti-htt SIRNA (red) that had been infused into it by a neighboring MSC. Scott Olson 2010
High-resolution visualization of MSC-mediated siRNA transfer
Scott Olson, Nolta lab 2010
Center for Biophotonics, UCD
CIRM TR1-01257
Transfer of siRNA from MSC into neural progenitors can cause significant reduction in the levels of mutant htt protein in co-culture.

![Graph showing the reduction in levels of mutant htt protein](image-url)
Ongoing Testing:

Efficacy - HD mouse models (R6/2, NSG/HD)

Biosafety

The siRNA transfer project is funded by the California Institute for Regenerative Medicine CIRM TR1-01257
Studies Underway at the level of GLP, to Work Toward Cellular Therapy Trials for Huntington’s disease

1. MSC to test neurorestorative effects (+/- BDNF).

2. MSC to continually secrete siRNA to reduce levels of the toxic htt RNA and protein.
UC Davis Institute for Regenerative Cures

Opened March 2010

- 109,000 SF renovated building
- Former state fair building
- Over 200 scientists and MDs working together
Blue area=$20 million
Of the $60 mil total cost funded by CIRM
Good Manufacturing Practice ("clean room") Facility

- High flexibility, versatility
  - 6 manufacturing labs
  - 3 intermediate labs
- Nolta, Bauer, Annett: two decades of experience with stem cell gene therapy trials
  - 18 trials since 1994

Established contracts exist with Stanford, Industry, and other Institutions

MSC expansion is completed for phase 1 trials
iPSC expansion is underway (Stanford CIRM disease team grant for EB)
Stem Cell Clinical Trials Ongoing/ Pending at UC Davis

- Non-union bone fractures *Started in 2008*
- Cardiac infarction *Started Sept. 2009*
- Peripheral vascular disease *Started November 2010*
- Retinal Occlusion, causing blindness *pending, IND submitted*
- Gene-Modified MSC for Huntington’s Disease *pending, pre-IND submitted* and ALS *pending*
- Stem cell “Biological bandages” for non-healing ulcers *pending*
- Liver disease *pending*
- Cardiac patching with stem cell augmentation *pending*
UC Davis HD Team

- Vicki Wheelock
- Jan Nolta
- Terry Tempkin
- Geralyn Annett
- Scott Olson
- Louisa Wirthlin
- Gerhard Bauer
- Laurie Macintosh
- Jeannine McGee
- Karen Pepper
- Heather Stewart
- Alice Tarantal
- Amal Kambal
- Whitney Cary
- Jeanie Liu
- Gaela Mitchell
- Catherine Nacey
- Kari Pollock
- Suzanne Pontow
- Alice Tarantal
- Matt Lindsey
- Jeremy Tempkin

Advisors:
- Leslie Thompson, UCI
- Bob Deans, Athersys
- Willie Mays, Athersys

Barcelona Team:
- Jordi Munoz
- Jordi Alberch
- Josep Canals

Milan Team:
- Elena Cattaneo

Thank you HD patient advocates, patients and their families

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* * * *

* = Huntington’s disease team members
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Thanks!!
Jan.nolta@ucdmc.ucdavis.edu