Preclinical animal studies of gene-modified MSCs for the treatment of Huntington’s disease

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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 (Parkinsons, 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
• Huntington's disease (HD) is a fatal, dominant neurogenetic disorder resulting from a variable length polyglutamine (polyQ) repeat CAG expansion in exon 1 of the \textit{HD} gene.

• Repeats confer a toxic gain of function on the protein huntingtin (htt).
• 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.
Expanded CAG (exon 1 htt gene) → Mutant Huntingtin Protein → Cellular Dysfunction → Cell Death

- Transcriptional Dysregulation
- Protein interactions & aggregation

- Autophagy
- Apoptosis
- Ubiquitin Proteasome Pathway
- Mitochondrial Cell Death

Clelland et al, 2008
HD is not a “schema” like the prior slide. Please visit sites like this to help understand it.

www.huntingtonsdance.org

Chris Furbee
Last year we lost our precious friend, Emily, who had juvenile HD. Is not treating lethal diseases really “doing no harm”? For clinical trials we must always balance risk vs. benefit.
Cellular Therapy Studies Underway at the level of Good Laboratory Practice, to Work Toward Cellular Therapy Trials for HD

1. MSC to test neurorestorative effects.

2. MSC to secrete factors to better heal neurons (inducible BDNF).

3. MSC to produce siRNA to continually reduce the toxic htt protein.

Future:
- MSC plus medium spiny neuron progenitors (hESC-derived) to replace those that have already been lost.
- Once we believe that these approaches are safe, no teratoma – forming cells would be injected with the selected hESC derivative, and that the new neurons can effectively integrate.
WHY plan to use Mesenchymal stem cells??

• Strong safety profile
• Neurorestoratative effects
• Relative ease of isolation and expansion
Bone Marrow is harvested From a normal, healthy qualified donor

Cells can be readily expanded in a clean room facility
1987-2010 Nolta lab: Development of Immune deficient mouse models for tracking and biosafety of engineered human Marrow Stromal Cells / Mesenchymal Stem Cells (MSC)

Decade-long biosafety studies: Bauer et al., Nolta lab
Mol Ther 16, 1308 (2008)

Hofling et al, Blood 2003
Human IL-7 production by primary human stromal cells
Transplanted into immune deficient mice
Serum assessment by ELISA from 1-6 months

Long-Term Cytokine Production from Engineered Primary Human Stromal Cells
Influences Human Hematopoiesis in an In Vivo Xenograft Model
Mo A. Dao, Karen A. Pepper and Jan A. Nolta
Stem Cells 1997;15;443-454
Nolta lab:
Papers since 1989 using Gene-modified Marrow Stromal Cells (MSC) to systemically produce cytokines, enzymes, and other factors in vivo
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.
Adult MSC, injected into the bloodstream, lodge at low levels in multiple tissues in immune deficient mice, and survive long-term (Meyerrose et al, Stem Cells 2007).
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


FDA – approved clinical trial of MSC injection into the CNS for ALS – TCA therapeutics: safety to be tested in human trials
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).

MSC will be injected into the spinal cords of patients.

Important consideration:

RISK vs. BENEFIT

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)
Safety profile – MSC in cardiac clinical trials done in USA- preliminary results

Patients receiving MSC did better than those receiving only standard-of-care (Hare et al, 2009)

Fewer side effects and significant improvements in heart, lung and global function compared to the placebo group

Important step forward in the search for stem cell treatments for heart disease.

“With a strong safety profile that now includes more than 1,000 patients studied in clinical trials, MSC are in a position to make significant advances in the field of cardiology.”

Osiris Therapeutics trial NCT00114452
• 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.
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, *in Press* 2010).
Adult Human Stem cells “home” to sites of tissue injury

Capoccia et al, Nolta lab 2009
MSC migrate to striatum after IC implantation in an HD rat model of striatal neuron death.

Migration of Neurotrophic Factors-Secreting Mesenchymal Stem Cells Toward a Quinolinic Acid Lesion as Viewed by Magnetic Resonance Imaging

Ofer Sadan, Noam Shemesh, Ran Barzilay, Merav Bahat-Stromza, Eldad Melamed, Yoram Cohen, Daniel Offen

MSC injected into ipsilateral hemisphere (white circle), migrated to damage.
Microanatomical evidences for potential of mesenchymal stem cells in amelioration of striatal degeneration

Edalatmanesh Mohammad Amin*, Bahrami Ahmad Reza*, Behnam Rasuli Morteza†, Moghaddam Matin Maryam*, Moghimi Ali† and Neshati Zeinab*

Neurological Research 2008

Upon the stem cell implantation, the striatal atrophies were significantly reduced and consequently, the volume of the lateral ventricle almost returned to the normal condition
Genetically engineered mesenchymal stem cells reduce behavioral deficits in the YAC 128 mouse model of Huntington’s disease

Dey et al, Behavioural Brain Research 2010
• 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.
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 2010
Non-human primate biosafety testing of Intracranial MSC implantation was done.

MSC expanded under GLP conditions were transplanted into the brains of 3 fetal primates under ultrasound guidance on 07/08/09.

Brains and other organs were collected at term- 5 months later.
Intra-ventricular MSC transfer in fetal non-human primates. Sonogram in left panel shows route of transfer (arrow) through the coronal suture. MSC were injected at 70 days gestation; (early second trimester) during maximal neuronal proliferation and prior to development of the immune system. (Tarantal Lab, California National Primate Center, UC Davis)
Hu vs Mu centromeric DNA FISH probes – used to identify human stem cells transplanted into xenogeneic host (Zhou, Nolta, Hepatology 2009).

Ongoing in primate brain sections
Intracranial injection of human mesenchymal stem cells into fetal non-human primate brain— 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-pre-IND paperwork was filed
-written (non-binding) input was received from the FDA
Mesenchymal stem cells have been expanded in the UC Davis Good Manufacturing Practice Facility For our Good Laboratory Practice (GLP) – level preclinical studies
Specialized CNS-1 Catheters will deliver MSC through a tiny burrhole in the skull, under MRI guidance. The MSC should migrate to the damaged regions from the site of implantation. They naturally “home” to damaged areas and sick cells, as we and others have extensively published. Primate testing will be done initially.
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 non-human primate studies at UC Davis and conversations with the FDA/IND Application/pending future FDA approval.

- HD Disease Team: UC Davis: Wheelock, Nolta, Tempkin, Sigvardt, 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 will exert neuroprotective/ neurorestororative 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) 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, but can be directly injected into the brain, with cholesterol modification. This is a fleeting strategy, with low levels of uptake.

• We will use human mesenchymal stem cells, which integrate benignly into the brain tissue, and shelter themselves from the immune system, for sustained production and delivery of anti-mutant HTT siRNA (Olson, Wirthlin, Nolta, UC patent #2009-170, International patent filed 2009).

• CIRM TR1-01257: (Nolta) translational grant
MSC to neuron transfer of alexa-dye labeled siRNA – standard microscopy (Pontow, Nolta lab 2010)
• Working with colleagues in the UC Davis Center for Biophotonics, we have shown that MSC infuse siRNA directly into damaged cells through tunneling nanotubules.

• 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 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.
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
We are testing anti-mutant htt siRNA transfer efficiency from human MSC into damaged neurons, and efficacy in the brains of mice that overexpress the mutant human htt protein (immune deficient mhtt transgenics (NSG/HD) developed with the UC Davis Mouse Biology program).

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.

2. MSC to continually secrete siRNA to reduce levels of the toxic htt RNA and protein.
UC Davis Institute for Regenerative Cures at the UC Davis Medical Center in Sacramento
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
UC Davis/CIRM Institute for Regenerative Cures

Proposed Auditorium (naming opportunity exists!)

Shell space

Toxicology testing

GMP Facility

Translational Lab

Clinical Translational Science Center

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 and Bauer: 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 *Accruing in November 2010*
- Retinal Occlusion, causing blindness *pending, pre-IND submitted*
- Gene-Modified MSC for Huntington’s Disease *pending, pre-pre-IND submitted* and ALS *pending pre-IND*
- Stem cell “Biological bandages” for non-healing ulcers *pending pre-IND*
- Liver disease *pending pre-IND*
- iPSC expansion for Stanford EB CIRM disease team *pending pre-IND*
UC Davis HD Team

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- Jan Nolta
- Terry Tempkin
- Geralyn Annett
- Scott Olson
- Louisa Wirthlin
- Gerhard Bauer
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TR1-01257

Thank you HD patient advocates, patients and their families
Nolta lab tour guides at Institute for Regenerative Cures Grand Opening Event