Therapies using genetically modified human mesenchymal stem cells: from the bench to the bedside

Jan Nolta, Director
UC Davis Institute for Regenerative Cures

Using Mesenchymal Stem Cells to Deliver BDNF and RNA Interference Molecules for HD

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1987-2004: “bubble baby disease” trials

Baby 1st to Receive Gene Treatment:
Medicine: Childrens Hospital team uses the therapy on a newborn in historic surgery. Doctors hope to cure his rare immune disorder before disease sets in.

May 16, 1993 | SHERYL STOLBERG | TIMES STAFF WRITER

The historic experiment employed a novel technique: the use of blood from the infant’s own umbilical cord. The blood was drawn as soon as he was born. That night, laboratory technicians began the intricate process of altering the blood to include genes that would insert themselves into his stem cells, the parent cells of all other blood cells.

Gene therapy seems to have cured eight of 10 children who had potentially fatal “bubble boy disease,” according to a study that followed their progress after treatment. Times 2006
MSCs can be engineered to secrete copious amounts of factors for delivery to other cells and tissues in the body.

Nolta lab, 1987-present

Book published - 2006
Using MSCs as delivery factories for potential treatment of Huntington’s disease:
1. BDNF
2. RNA inhibition

Mesenchymal Stem Cells

- Adult stem cells that repair damaged tissues by responding to the scene of the injury and producing healing factors
- “Paramedics”
- Strong safety profile in clinical trials
Bone Marrow is harvested From a normal, healthy donor

Cells grown up to large numbers in a clean room facility, for transplantation to patients who need them

Mesenchymal Stem Cells (MSC):
Reparative adult stem cells that can act as excellent “delivery vehicles” in the body

Normal Healthy Donor MSCs are used in Therapies

Companies such as Osiris, Mesoblast, Aastrom and Athersys are in Phase I – III clinical trials using large lots of MSCs expanded from a single or several donors.

The cells are infused without tissue matching, due to their ability to shelter themselves from the immune system.

San-Bio is currently testing a gene-modified MSC therapeutic by delivery into the brain in Phase I clinical trials to treat stroke:

http://clinicaltrials.gov/ct2/show/NCT01287936?term=sb623&rank=1
• MSC can engraft stably in the brain and will exert neuroprotective/neurorestorative effects.
• However, the toxic mutant htt protein will still be present in Huntington’s disease.
• To truly cure the disease the levels of this protein should be continually reduced.

**Goal – eliminate mutant huntingtin protein**

Small interfering RNA (siRNA) molecules bind to the RNA messages made 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:

Regenerative Medicine 2010

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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 production and delivery of anti-mutant HTT siRNA (patent PCT/US2010/028712).

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.

MSC to neuron transfer of alexa-dye labeled siRNA – (Pontow, Nolta lab 2010)
High-resolution visualization of MSC-mediated siRNA transfer
Scott Olson, Nolta lab 2010
Center for Biophotonics, UCD
CIRM TR1-01257

Video - Human MSC migrate rapidly and interact with many cells
Advanced Drug Delivery Reviews 2010, Nolta lab
Video- transfer from MSC to neuron
Transfer of siRNA from MSC into neural progenitors can cause significant reduction in the levels of mutant htt protein in co-culture.
Examination of mesenchymal stem cell-mediated RNAi transfer to Huntington’s disease affected neuronal cells for reduction of huntingtin

Scott D. Olson, Arsal Kambal, Kari Pollock, Gaela-Marie Mitchell, Heather Stewart, Stefanos Kalomoiris, Whitney Cary, Catherine Nacey, Karen Pepper, Jan A. Nolta

Institute for Regenerative Cures, University of California Davis Health System, 2131 Stockton Blvd Room #200, Sacramento, CA 95817, USA
Brain-Derived Neurotrophic Factor (BDNF)

- Is a member of the nerve growth factor (NGF) family.
- Is essential for the survival of medium spiny neurons in the striatum.

Zuccato, Chiara and Elena Cattaneo. 2007. “Role of brain-derived neurotrophic factor in Huntington's disease.”

BDNF and HD

- Patients with HD have much lower levels of BDNF than usual.
- Mutant huntingtin protein blocks production of BDNF
- Low BDNF levels are a major contributing factor to the degeneration of affected brain structures.
- Evidence also links the lower BDNF levels to earlier onset and severity of the disease (as tested in mouse models of HD).

C. Zuccato, M. Valenza, E. Cattaneo, Physiol Rev 90, 905 (Jul, 2010).
Problems with BDNF delivery

• BDNF has a short half-life
• Does not cross blood-brain barrier well

• Our strategy, in collaboration with Gary Dunbar’s lab—produce BDNF from long-lived MSCs in the striatum

C. Zuccato, M. Valenza, E. Cattaneo, Physiol Rev 90, 905 (Jul, 2010)
N. D. Dey et al., Behavioural brain research 214, 193 (Dec 25, 2010)

Brain: the final frontier

MSCs use the external surface of the vasculature as “train tracks” to migrate throughout the brain tissue to deliver factors to damaged neurons
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 MSCs (green) making BDNF in mouse striatum

Kari Pollock, Nolta lab 2011
MSC engineered to secrete BDNF reduce behavioral deficits in the YAC 128 mouse model of HD

Dey et al, Behavioural Brain Research 2010

MSC engineered to secrete BDNF reduce behavioral deficits in the YAC 128 mouse model of HD

Dey et al, Behavioural Brain Research 2010
Genetically Engineered Mesenchymal Stem Cells as a Proposed Therapeutic for Huntington’s Disease

Scott D. Olson • Kari Pollo • Amal Kambal • Whitney Cary • Gaela-Marie Mitchell • Jeremy Tempkin • Heather Stewart • Jeannine McGee • Gerhard Bauer • Hyun Sook Kim • Teresa Tempkin • Vicki Wheelock • Geralyn Annett • Gary Dunbar • Jan A. Nolta

Table 3: GLP Biosafety studies of MSC/BDNF in murine models of HD (Nolta lab)

<table>
<thead>
<tr>
<th>Study #/Date</th>
<th>Experimental objective</th>
<th>Study duration</th>
<th>Route/ Dose</th>
<th>Human MSC</th>
<th>Mouse Strain</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>KP 12-27-10</td>
<td>MSC long-term 7 months</td>
<td>Intrastratal 250,000 cells/mouse in 5ul PBS</td>
<td>MSC = P3 human BM</td>
<td>NSG/NSG</td>
<td>NOG/SCID</td>
<td>Brain section slides were reviewed blindly by two licensed pathologists. Department of Comparative Pathology, UC Davis. No abnormalities were detected in brain tissue or other organs. No abnormal pathology was found on any slide.</td>
</tr>
<tr>
<td>CS 01-21-11</td>
<td>MSC long-term 4 months</td>
<td>Intrastratal 250,000 cells/mouse in 5ul PBS</td>
<td>MSC = P3 human BM</td>
<td>NSG/NSG</td>
<td>NOG/SCID</td>
<td>The NSG/HD strain has high levels of human hTR in brain tissue but a milder phenotype than R6/2, allowing longer-term biosafety studies. No abnormalities were detected. No abnormal pathology found on slides.</td>
</tr>
<tr>
<td>KP 02-10-11</td>
<td>MSC/BDNF Biosafety in rapidly progressing R6/2 HD model</td>
<td>Intrastratal 250,000 cells/mouse transduced human BM MSC P3 MOI 60</td>
<td>MSC = #2629 vector-transduced human BM MSC P3 MOI 60</td>
<td>R6/2 HD</td>
<td>Control age-matched littermates</td>
<td>This is the short-lived R6/2 strain, which limits the study duration. Biosafety was tested in the rapidly declining HD brain tissue, with 12 transplanted controls taken at the same time. No abnormalities were noted in brain tissue or other organs. No abnormal pathology on slides.</td>
</tr>
<tr>
<td>KP 05-12-11</td>
<td>MSC/BDNF Biosafety in rapidly progressing R6/2 HD model</td>
<td>Intrastratal 250,000 cells/mouse MOI 60</td>
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Conclusion: Biosafety has been demonstrated in the GLP studies of the MSC and MSC/BDNF product following intrastratia administration into rodent models of HD. No abnormalities in brain
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
-Initial paperwork was filed with the FDA

1. Three additional primates were transplanted using gene-modified cells, completed: safe- no abnormalities

   - funded by team KJ – THANK YOU

2. Additional primates will be infused in March

   - funded by NIH pilot grant through our CTSC

3. Then paperwork will go back to the FDA
Studies Underway to Work Toward Cellular Therapy Trials for Huntington’s disease

1. MSC and MSC/BDNF to test neurorestorative effects

2. In the Future, if in vivo efficacy can be demonstrated: MSC/RNAi

CIRM disease team planning grant received!

CIRM RFA 10-05: DISEASE TEAM THERAPY DEVELOPMENT – PART I PLANNING AWARDS

DR2-05415: MSC engineered to produce BDNF for the treatment of Huntington’s disease

Principal Investigator: Dr. Vicki Wheelock
Planning leader: Dr. Jan Nolta
Institution: University of California, Davis

CIRM disease team grant to potentially fund Phase 1 clinical trial Of MSC and MSC/BDNF infusion- Submitted January 25, 2012
Huntington’s Disease Team, University of California Davis
Grant Application DR2-05415

MSC engineered to produce BDNF for the treatment of Huntington's disease

Planned HD Clinical Trial: MSC/BDNF

Specialized Catheters will deliver MSC through a tiny burrhole in the skull, under MRI guidance. The burrhole will be sealed immediately after infusion.

The MSC will migrate to the damaged regions from the site of implantation.

They naturally “home” to damaged areas and sick cells to repair neurons.
Monitor patient for Potential improvement in UC Davis Movement Disorders Clinic

MSC Therapy For Neurodegeneration

Cells produced under: GOOD MANUFACTURING PRACTICE (GMP), with QUALITY CONTROL (QC) and QUALITY ASSURANCE (QA)

UC Davis Good Manufacturing Practice (GMP) Facility

Cellular product manufacturing
Six stem cell clinical trials are currently ongoing at UCD MSC and MSC/BDNF batches are banked
Current Focus: prepare and file an IND application to the FDA

UC Davis HD Team

- Vicki Wheelock
- Jan Nolta
- Terry Tempkin
- Geralyn Annett
- Kari Pollock
- Scott Olson
- Whitney Cary
- Heather Stewart
- Gaeta Mitchell
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- Bob Dean
- Willie Mays

UCSF:
- Phil Starr
- Dan Lim

Michigan:
- Gary Dunbar

Korea:
- Hyun Sook Kim

France:
- Anne Catherine Bachoud-Levi

Iowa:
- Jane Paulsen

Washington:
- Elizabeth Aylward

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Thank you HD patient advocates, patients and their families