Regenerative Medicine: Opening the Door to Innovations

As a leading research center, Mayo Clinic has embraced regenerative medicine and its potential to find innovative solutions for devastating neurodegenerative diseases. Research and clinical trials at all Mayo Clinic campuses are generating tools for the study of, and potential stem cell-based therapies for, diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) that currently lack treatments.

“Stem cells provide incredibly powerful ways to study disease, and the potential for trying therapy in patients is opening quickly,” says Anthony J. Windebank, M.D., a consultant in Neurology at Mayo Clinic in Rochester, Minnesota. “We’ve determined that we can put a person’s own cells into places where they don’t normally reside. The cells survive and don’t cause bad side effects.”

Among Mayo Clinic’s efforts in neuroregenerative medicine, recent developments include the discovery of a biomarker for the most common genetic risk factor for ALS and FTD, and clinical trials of stem cell therapy for ALS.

**Biomarker for ALS and FTD**

An innovative technique for generating induced pluripotent stem cells (iPSCs) from fibroblasts, developed at Mayo Clinic in Jacksonville, Florida, led to the discovery there of a biomarker — abnormal proteins resulting from a repeat expansion in a portion of the C9orf72 gene. The repeat expansion, which is the most common known genetic cause of ALS and FTD, causes RNA strands to misfold and form clumps, and produces an abnormal protein, called “c9RAN proteins” (Figure).

“The abnormal proteins only exist in patients carrying a C9orf72 repeat expansion, and they now represent the hallmark pathology of disorders collectively referred to as c9FTD/ALS,” says Leonard Petrucelli, Ph.D., chair of Neuroscience at Mayo Clinic’s campus in Florida.

Using iPSCs generated from people with the mutation, Mayo Clinic researchers developed in vitro models of C9orf72 pathology. In collaboration with The Scripps Research Institute in Jupiter, Florida, the researchers then designed bioactive molecules to target and bind to the aberrant RNA. In a study published in the Sept. 3, 2014, issue of *Neuron*, the researchers showed that the molecules significantly inhibited abnormal RNA translation and protein formation and that abnormal c9RAN proteins can be detected in the cerebrospinal fluid (CSF) of c9ALS patients.

“We’ve developed antibodies and assays that can detect the amount of this abnormal protein not only in the brain but also in CSF,” Dr. Petrucelli says. “In the future, a spinal tap could be used to measure protein levels in order to assess disease progression and to determine the efficacy of therapies designed to reverse pathological features associated with the mutation.”

*Figure.* The C9orf72 repeat expansion leads to the abnormal production of dipeptide repeat proteins that form inclusions inside cells of the brain. Shown here are inclusions formed of poly(GP) dipeptide proteins in neurons of the cerebellum.
Reprogramming the immune system

Stem cell transplants can induce sustained remission in patients with severe autoimmune diseases, such as systemic lupus erythematosus, that are refractory to standard therapies. Evidence suggests an autoimmune mechanism might be present in ALS. Researchers at Mayo Clinic in Phoenix/Scottsdale, Arizona, are developing a clinical trial using a similar strategy for ALS.

Although previous attempts to use stem cell transplantation for ALS failed to demonstrate improvement in motor function, “we believe the immune suppression in those early studies wasn’t strong enough to inhibit all of the disease process,” says Angel (A Arturo) A. Leis, M.D., a consultant in Neurology at Mayo Clinic’s campus in Arizona. “But now we have the capability to produce very intense immunosuppression, which we hope will abolish the immune mechanisms that may contribute to ALS progression.”

Immunosuppression is achieved through high doses of chemotherapy. Stem cells are removed from the patient’s bone marrow before chemotherapy starts and reinserted in the patient afterward. “The stem cells return to the bone marrow very rapidly and then start building up the patient’s blood and immune system again,” says Jose F. Leis, M.D., Ph.D., a consultant in Hematology/Oncology at Mayo Clinic’s campus in Arizona.

The procedure requires about three and a half weeks of hospitalization, from harvest of stem cells to regrowth of the immune system. “You have essentially erased that patient’s immune system, and then, with stem cells, developed a new immune system that is naive and self-tolerant,” says Dr. Angel Leis. “We are looking forward to this being a future mechanism that can halt the progression of ALS.”

Injecting stem cells into CSF

Through the Center for Regenerative Medicine at Mayo Clinic’s campus in Minnesota, Dr. Windebank is directing several studies involving injection of ALS patient stem cells into CSF in an effort to promote nerve regeneration. The work is co-directed by Nathan P. Staff, M.D., Ph.D., a consultant in Neurology at Mayo Clinic’s campus in Minnesota, and also involves Mayo Clinic’s Human Cellular Therapy Laboratory, which is conducting more stem cell trials than any other facility in the United States.

One of the Mayo Clinic studies, done in conjunction with Massachusetts General Hospital and the University of Massachusetts, uses stem cells that are harvested from an ALS patient’s bone marrow, treated in the laboratory for six weeks and then injected into the patient’s CSF. “The laboratory treatment makes the cells more neurotrophic,” Dr. Windebank says. Results from the phase IIA study are expected by the end of 2016.

Two other trials use adipose stem cells. The first — a dose-escalation safety trial — is nearly complete. “The safety profile is excellent,” Dr. Windebank says. “We’re up to the highest dose levels, and although we are noticing some side effects, they’re relatively minor and self-limited.” A phase IIA study involving all Mayo campuses is expected soon.

The second adipose trial is being developed in conjunction with the Karolinska Institutet in Stockholm; Paracelsus Medical University in Salzburg, Austria; Trinity College Dublin; and the National University of Ireland, Galway. “An international trial allows us to demonstrate that this kind of very novel cell therapy can be replicated in multiple centers,” Dr. Windebank says.

Regenerative medicine is conducive to finding treatment for ALS because the disease progresses quickly and affects a specific population of nerve cells in the brain and spinal cord. “In regenerative medicine you’re asking the body to heal itself,” Dr. Windebank says. “Of course the nervous system doesn’t regenerate quickly because it’s so complicated. Focusing on specific diseases such as ALS, in which a specific type of nerve cell is dying, simplifies the target.

“The next phase of our research is going to be even more exciting,” Dr. Windebank adds. “Our gene-editing technology — which allows us to genetically manipulate cells in very specific ways to make them do more of what we want them to do — is coming along very rapidly. In the future there might be a modification for ALS, for multiple system atrophy and maybe for Parkinson’s disease.”

For more information


Mayo Clinic. A dose-escalation safety trial for intrathecal autologous mesenchymal stem cell therapy in amyotrophic lateral sclerosis. ClinicalTrials.gov.
Seeking Strategies to Halt MS Progression

Although great progress has been made in recent decades in the treatment of relapsing-remitting multiple sclerosis (MS), progressive MS remains poorly understood. The natural history of MS (Figure 1) is notoriously variable; however, most patients eventually enter a progressive phase of the disease, resulting in ambulatory dysfunction that can be severe.

Researchers at Mayo Clinic are making significant advances in understanding the natural history and pathophysiology of MS, laying the groundwork for potential treatments. The goal is to slow or prevent the progressive phase of MS.

“The management of progressive MS remains frustrating, and progress is never fast enough for the people who are affected. But we are optimistic because developments are certainly moving in the right direction,” says Dean M. Wingerchuk, M.D., a consultant in Neurology at Mayo Clinic in Phoenix/Scottsdale, Arizona.

Specific efforts include studies of factors affecting disease progression among diverse patients, as well as strategies for preventing axonal damage and thus halting progression. “Relapses contribute only about 2 percent of MS-related severe disabilities,” notes Orhun H. Kantarci, M.D., a consultant in Neurology at Mayo Clinic in Rochester, Minnesota. “The remaining 98 percent happens with the progressive phase of the disease. None of the medications we have today impacts progression.”

Early start of pathology
Previous studies have suggested that primary-progressive MS portended a worse prognosis than relapsing-remitting MS or secondary-progressive MS. However, in a study published in the Jan. 6, 2015, issue of Neurology, researchers at Mayo Clinic’s campus in Minnesota showed that a clinical relapsing phase before the onset of progressive MS accelerates post-progression disability accumulation. Among patients studied, those with bout-onset progressive MS reached severe disability faster than did patients with primary-progressive MS.

“What happens after the first relapse, 15 or 20 years before progression starts, determines whether you’re going to have progressive MS,” Dr. Kantarci says. “If you don’t repair well early on, whatever we might do to prevent relapses is not going to affect that one lesion in a critical location that can induce progression. Pathology is not a process that starts at the time when progressive MS starts.”

In line with these findings, Mayo Clinic researchers are seeking greater understanding of radiologically isolated syndrome (RIS), in which intracranial abnormalities suggest MS in the absence of symptoms. Mayo Clinic is participating in an international study involving more than 500 patients in the U.S. and Europe, examining the feasibility of preventing the development of clinical MS in people with RIS. “We have to attack MS when it is crawling, not running,” Dr. Kantarci says.

Focus on axons
Much of MS research and drug development has focused on demyelination. Medications that prevent or slow relapses generally target acute demyelinating events, either by blocking immune system access to the central nervous system or by reducing inflammation in that event.

“Those drugs can suppress relapses, but they’ve never prevented progression because...”

Figure 1. MRI showing multiple sclerosis (MS) lesions in the brain.

Figure 2. Overview of Mayo Clinic’s axon protection project.
axons are still being injured. We focus instead on trying to understand the factors injuring the axon, particularly immune-mediated effectors,” says Charles L. Howe, Ph.D., director of the Translational Neuroimmunology Laboratory at Mayo Clinic’s campus in Minnesota.

In research published in the November 2013 issue of Neurobiology of Disease, Dr. Howe and colleagues presented a novel model of immune-mediated axon injury involving cytotoxic CD8+ T lymphocytes (Figure 2). The model suggests strategies for targeted, early therapeutic intervention aimed at protecting axons and preventing irreversible loss of neurological function.

“We’ve discovered that there is a granzyme B-dependent pathway that is involved in this damage,” Dr. Howe says. “Granzyme B is a protease that chews up very specific targets. It usually initiates an amplifying cascade of events that leads to cytotoxicity.”

Current efforts involve identifying specifically anti-axonal pathogenic CD8+ T cells as well as studying the mechanisms by which they injure axons. “Most of the field has studied that in the context of killing cells,” Dr. Howe says. “We’re studying these events in an axon, where the events involve altering the cytoskeleton and the mitochondrial function of the axon, leading either to a run-down of energy that causes the axon to stop functioning, or to degradation of the cytoskeleton — which means the axon loses its physical integrity and is no longer able to make a connection.”

**Growing human neurons**

In addition to animal models, the researchers have a chamber system (Figure 3) that allows them to facilitate myelination of axons in vitro. “We’ve succeeded in getting myelin to regrow on axons from mice in a dish,” Dr. Howe says. “Within two to three years, we will have human neurons growing in a dish with human oligodendrocytes added and putting myelin on axons.”

Ultimately, Dr. Howe hopes to set up a biobank with fibroblasts from patients with MS that can be used to grow the patients’ own neurons in the lab for individualized treatment. “We will then discover either that there is a common anti-axonal T cell that we can target globally across all MS patients or, perhaps more likely, we will find that each patient has his or her own unique anti-axonal T cell that we will have to design individual therapies to block,” Dr. Howe says.

This individualized medicine approach offers great potential benefit for patients newly diagnosed with MS. “At the moment we’re unable to predict with much confidence what will happen to any one individual,” Dr. Wingerchuk says. “In the early stages of the disease, a patient wants to know, ‘What’s going to happen to me?’ ”

**For more information**


Clinical indications for cEEG
In Mayo Clinic’s ICUs, cEEG involves the same noninvasive technique used in the inpatient epilepsy monitoring unit. Technicians monitor ICU patients on EEG around-the-clock. Common clinical indications for cEEG in Mayo Clinic’s ICUs include:

• Established seizures or status epilepticus, to guide titration of anti-epileptic drug therapy
• Screening for subclinical seizures among patients deemed to be at high risk because of suspected encephalitis, hypoxic-ischemic encephalopathy, traumatic brain injury or stroke
• Screening for seizures among patients who are paralyzed and deemed at risk of seizures
• Characterization of paroxysmal events suspected to represent electrographic seizures

Depending on the underlying causes of their disorders, patients might undergo cEEG for periods ranging from 12 to 24 hours to several weeks.

“Kids under 2 to 3 years of age are at higher risk of seizure,” Dr. Payne says. “Once a patient is on EEG, within an hour we can see indications on the readout that tell us the level of risk that patient has for seizing down the line. For example, interictal discharges, or background activity that is slow or discontinuous, might go on to become seizure activity.”

In a study published in the May 2014 issue of Brain, Dr. Payne and colleagues found that above a maximum seizure burden threshold of 12 minutes an hour, the probability and magnitude of neurological decline rose sharply. Dr. Payne estimates that roughly 10 percent of children on ICU EEG monitoring at Mayo Clinic have status epilepticus; up to half of those children have entirely subclinical seizures. Clinical indications of seizure are especially difficult to discern in neonatal ICU patients.

“Without cEEG we would miss status epilepticus in a not insignificant proportion of our pediatric patients,” he says. “We don’t want to miss those high-risk kids.”

Quantitative trends analysis
Mayo Clinic uses quantitative EEG trends analysis, which compresses raw EEG readings to show as much as 16 hours of monitoring on a single page. “Within seconds you can identify where the seizures are probably happening,” Dr. Payne says. “It doesn’t replace the raw data because what might look like a seizure on the trend analysis can turn out to be an artifact when you check the raw reading. But having these trends posted right by the patient’s bedside is a very helpful tool.”

In a critical care setting, where other major organs are monitored, it is important to monitor the brain as well. “We have shown that prolonged seizure or status epilepticus is bad for the brain,” Dr. Payne says. “Seizures are just as bad if they’re subclinical, and they cannot be managed in the ICU without EEG.”

For more information

Figure. Imaging of an infant age 2 months with bacterial meningitis who presented with status epilepticus and underwent continuous electroencephalography (cEEG) in the pediatric ICU at Mayo Clinic. Frequent subclinical electrographic seizures were observed. On the left, 16-hour comprehensive quantitative trends analysis identifies seven focal seizures arising over the left hemisphere. 1. Amplitude integrated EEG. 2. Asymmetry relative spectrogram (blue streaks). 3. Right fast Fourier transform (FFT) spectrogram. 4. Left FFT spectrogram. 5. Left rhythmicity spectrogram identifies rhythmic seizure discharges at the same time. On the right, diffusion-weighted imaging and apparent diffusion coefficient imaging show areas of restricted diffusion primarily involving the corpus callosum, thalami, and left frontal-temporal-parietal and right frontal regions. Anti-seizure medications were administered, seizures were controlled, and after several weeks the patient was discharged home.
Deep brain stimulation (DBS) was first used by neurologic surgeons in the United States in 1997 at Mayo Clinic in Jacksonville, Florida. Since then, thousands of people with Parkinson’s disease, dystonia, essential tremor and other conditions have been successfully treated with DBS. Traditionally, patients are awake during the procedure, allowing them to respond to directives from surgical teams and help ensure correct placement of electrodes in the brain.

Patients at Mayo Clinic’s campus in Florida now have the option of treatment with DBS under general anesthesia. “Asleep DBS” uses intraoperative MRI to guide electrode placement, eliminating the need for microelectrode testing during surgery. As a result, asleep DBS is generally a shorter procedure than awake DBS — lasting only about three hours — and patients with Parkinson’s disease aren’t required to stop taking medications beforehand. The procedure is particularly suitable for patients with dystonia or Parkinson’s disease that isn’t tremor-predominant.

“There is no question that patients would prefer to be asleep during DBS, and the results of surgery are at least equal to the awake procedure,” says Robert E. Wharen Jr., M.D., chair of Neurologic Surgery at Mayo Clinic in Jacksonville, Florida.

**Intraoperative MRI guidance**

Asleep DBS is performed in Mayo Clinic’s intraoperative MRI operating room. The patient is given general anesthesia, and the head is secured to the operating table; a stereotactic frame isn’t necessary. An alignment grid is placed on the patient’s head and MRI is performed to pinpoint the target and trajectory for electrode placement. A small incision is made through the skull and a special alignment tower attached to the scalp (Figure 1). “All of this can be done through a 2-centimeter incision,” Dr. Wharen says.

MRI is repeated to ensure precise placement of the alignment apparatus. Electrodes are then placed down the trajectory, and MRI is used to verify alignment. Once the electrical lead is secured (Figure 2), the procedure is completed as awake DBS is, with implantation of the pulse generator in the chest.

“Asleep DBS eliminates the time and risk of hemorrhage from microelectrode recording,” Dr. Wharen says. “Trauma to the brain is minimized because you only ever make one pass. The intraoperative MRI means you’re watching throughout the procedure and confirms that you’re exactly where you want to be.”

Mayo Clinic brings to this new procedure its investment in intraoperative high-resolution MRI and the clinic’s highly experienced surgical team. “Our patients who have asleep DBS don’t experience the confusion and longer hospitalization we can see with patients after awake DBS,” Dr. Wharen notes. “While the long-term results for our patients with asleep DBS are at least as good as for those who have awake DBS, the short-term results are even better.”
Risk Prediction Model for MCI
Because patients with mild cognitive impairment (MCI) are at increased risk of dementia, it is important to understand the predictors of transition from normal cognition to MCI. Researchers at Mayo Clinic’s campuses in Minnesota and Arizona have developed a risk prediction model for MCI. The researchers used longitudinal data from residents of Olmsted County, Minnesota, ages 70 to 89 who are enrolled in the Mayo Clinic Study of Aging, a randomly selected, population-based study. At baseline and subsequent visits, study participants were evaluated for demographic, clinical and neuropsychological measures, and were diagnosed by consensus as having normal cognition, MCI or dementia. Using baseline demographic and clinical variables in proportional hazards models, the researchers derived sex-specific scores that predicted the risk of progressing from normal cognition to MCI. The basic model concentrated on information that could be obtained from a medical record before a clinic visit; augmented models added information that could be obtained at the clinic visit, including results of a blood test for APOE, the most common gene associated with late-onset Alzheimer’s disease. Of 1,449 participants with normal cognition, 401 (27.7 percent) developed MCI. Both men and women in the highest versus lowest sex-specific risk score quartiles of the augmented model had a roughly sevenfold higher risk of developing MCI. The Mayo Clinic models may be useful in identifying patients who might benefit from more-extensive diagnostic testing. (Mielke MM, et al. Predicting the risk of mild cognitive impairment in the Mayo Clinic Study of Aging. Presentation at: American Academy of Neurology Annual Meeting; 2015; Washington, D.C.)

STM Not Uncommon in NMOSD
Short transverse myelitis (STM) is considered uncharacteristic of neuromyelitis optica spectrum disorders (NMOSDs). However, Mayo Clinic researchers have found that short lesions are not uncommon in NMOSDs, and their occurrence might delay diagnosis and treatment, resulting in residual disability. The researchers identified patients seen for NMOSDs at Mayo Clinic from 1996 to 2014; 151 patients with initial longitudinally extensive transverse myelitis (LETM) were excluded. The remaining 25 patients included in the study had experienced a first transverse myelitis episode, were positive for AQP4-IgG antibodies, underwent MRI within 90 days of symptom onset and had a spinal cord T2-hyperintense lesion of less than three vertebral segments. A control group consisted of AQP4-IgG-negative patients with STM among a population-based cohort of inflammatory demyelinating central nervous system disorders in Olmsted County, Minnesota. In comparison with the excluded NMOSD patients whose initial myelitis episode was LETM, patients with short initial lesions had a greater delay in diagnosis and treatment. In 92 percent of AQP4-IgG-positive STM cases, subsequent myelitis episodes were longitudinally extensive. Attributes more common in AQP4-IgG-positive STM patients than in the control group included nonwhite race; tonic spasms; coexisting autoimmunity; MRI showing central cord lesions, T1 hypointensity and a brain inconsistent with multiple sclerosis; and lack of oligoclonal bands in cerebrospinal fluid. (Flanagan EP, et al. Short myelitis lesions in aquaporin-4-IgG-positive neuromyelitis optica spectrum disorders. Presentation at: American Academy of Neurology Annual Meeting; 2015; Washington, D.C.)

MSA: Clinical Characteristics and Survival
Reports of survival in multiple system atrophy (MSA) differ. It is also unclear whether parkinsonian or cerebellar motor subtype (MSA-P or MSA-C) and initial symptom type influence survival. Data from a study at Mayo Clinic in Rochester, Minnesota, suggest that median survival rates are similar for MSA-P and MSA-C and that age, stridor and autonomic dysfunction are important predictors of survival. The researchers reviewed all cases of MSA assessed with an autonomic reflex screen between January 1998 and December 2012 at Mayo Clinic’s campus in Minnesota. Patients were classified as MSA-P or MSA-C, and categorized by initial symptom type — motor, autonomic or combined. Demographic data, clinical variables, imaging findings and autonomic testing results were reviewed and assessed as survival variables. Among the 685 MSA patients identified, 593 had probable MSA and 92 had possible MSA. MSA-P was the predominant subtype in 430 patients (63 percent). Median disease duration from symptom onset to death in both subtypes was 7.5 years; time from diagnosis to death was 3.5 years. An initial motor symptom was found in 61 percent of cases; an initial autonomic symptom in 28 percent of cases; and combined motor and autonomic symptoms in 11 percent of cases. Initial symptoms didn’t differ by subtype and didn’t influence survival. However, men were more likely than women to have initial autonomic symptoms, while women were more likely to initially manifest motor symptoms. (Coon EA, et al. Clinical characteristics and survival in multiple system atrophy. Presentation at: American Academy of Neurology Annual Meeting; 2015; Washington, D.C.)

To read more about Mayo Clinic neurosciences research and patient care, visit www.MayoClinic.org/medicalprofs.
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Education 2015-2016 Neurology and Neurologic Surgery Continuing Medical Education Programs

2015 courses

September
Mayo Clinic 7th Annual Stroke and Cerebrovascular Disease Review 2015
Sept. 17-19, 2015
The Ritz-Carlton, Amelia Island, Fla.

November
Neuroradiology: Practice to Innovation Course 2015
Nov. 8-12, 2015
The Ritz-Carlton, Kapalua, Maui, Hawaii

December
International Dementia with Lewy Bodies Conference 2015
Dec. 1-4, 2015
Marriott Harbor Beach, Fort Lauderdale, Fla.

2016 course

April
Neurorehabilitation Summit: Regeneration, Recovery, Reintegration 2016
April 11-12, 2016
Leighton Auditorium, Mayo Clinic, Rochester, Minn.

Information and registration

Mayo Clinic in Rochester, Minnesota
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Email: cme@mayo.edu

Mayo Clinic in Jacksonville, Florida
Phone: 800-462-9633 (toll-free) or 904-953-0421
Email: cme-jax@mayo.edu

Mayo Clinic in Phoenix, Arizona
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