Stem cells for ALS

By Brian Moy, Staff Writer

Researchers from **Harvard University** and **Columbia University** reported in *Science* that induced pluripotent stem cells generated from an 82-year-old woman with amyotrophic lateral sclerosis (ALS) could be successfully differentiated into motor neurons and glia.[1] The finding someday could be useful for generating genetically matched healthy cells to replace diseased ones, such as damaged motor neurons, in patients with ALS. But as a more immediate application, the induced pluripotent stem cells could provide a better drug-screening tool than models currently used for ALS.

ALS is a neurodegenerative disorder in which motor neuron loss in the spinal cord and motor cortex leads to progressive paralysis and death.[2] Additionally, glia from ALS animal models have been shown to produce factors that are toxic to motor neurons.[3]

Current ALS screening tools include the G93A SOD1 mouse model, tests for a mutated version of the *superoxide dismutase 1* (*SOD1*) gene and primary rat spinal cord motor neuron cultures. Other screens include two immortalized cell lines: SY5Y, a neuroblastoma cell line used in neuroprotection assays, and NSC-34, an immortalized motor neuron cell line.

The graveyard of failed compounds for ALS includes multiple therapeutic strategies ranging from growth factors to gene therapy. Companies have not been deterred from the space, however, as ALS is an orphan indication with only one marketed drug: Rilutek riluzole, a glutamate release inhibitor from **sanofi-aventis Group** (see **Table 1, "Amyotrophic lateral sclerosis pipeline"**).

The researchers in the *Science* paper used skin cells from ALS patients and introduced transgenes encoding *Kruppel-like factor 4* (*KLF4*), *SRY-box containing gene 2* (*SOX2*), *POU domain, class 5, transcription factor 1* (*POU5F1*; *OCT4*) and *v-myc myelocytomatosis viral oncogene homolog* (*MYC*) into the fibroblasts using retroviruses. Exogenous expression of these four oncogenes has been shown to be sufficient for reprogramming human fibroblasts to a pluripotent state.[4] DNA fingerprinting analysis verified that the patient-specific induced pluripotent stem (iPS) cell lines were genetically matched to the donor.

iPS cells are reprogrammed adult cells with properties similar to human embryonic stem (ES) cells.

The researchers focused on characterizing three iPS cell lines derived from an 82-year-old subject diagnosed with classical ALS. Aggregates of cells formed from the patient's iPS cells, called embryoid bodies, were treated with signaling molecules known to differentiate mouse and human ES cells. Indeed, the signaling molecules caused the iPS cells to differentiate into spinal motor neurons and glia.

Table 1. Amyotrophic lateral sclerosis pipeline. Selected compounds in clinical development for amyotrophic lateral sclerosis (ALS).

Company	Product	Description	Status
Mitsubishi Tanabe Pharma Corp. (Tokyo:4508; Osaka:4508)	Edavarone (MCI-186)	Free radical scavenger	Phase III
Avicena Group Inc. (OTCBB:AVGO)	AL-02	Formulation of creatine	Start Phase III in 1Q09
Avicena	AL-08 plus celecoxib	Formulation of creatine plus celecoxib	Phase II
Knopp Neurosciences Inc.	KNS-760704	Optical enantiomer of dopamine agonist pramipexole	Phase II
CytRx Corp. (NASDAQ:CYTR)	Arimoclomol	Hydroxylamine derivative that induces heat shock protein expression	Phase II (on hold)
Ono Pharmaceuticals Co. Ltd. (Tokyo:4528; Osaka:4528)	Cereact capsule (ONO-2506PO)	Astrocyte modulator	Phase II
Teva Pharmaceutical Industries Ltd. (NASDAQ:TEVA)	Talampanel	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamate receptor blocker	Phase II
Sangamo BioSciences Inc. (NASDAQ:SGMO)	SB-509	Transcriptional activator of VEGFA	Start Phase II in 2008
Aeolus Pharmaceuticals Inc. (OTCBB:AOLS)	Manganese porphyrin (AEOL 10150)	Catalytic antioxidant	Phase I
Phytopharm plc (LSE:PYM)	Myogane	Nonpeptide neurotrophic factor inducer	Phase I
Trophos S.A.	TRO19622	Small molecule with cholesterol-like structure that interacts with the mitochondrial permeability transition pore (MPTP)	Phase I
Vasogen Inc. (TSX:VAS; NASDAQ:VSGN)	VP025	Anti-inflammatory compound that modulates cytokine levels	Phase I

iPS cell applications

Thane Kreiner, president and CEO of **iZumi Bio Inc.**, told *SciBX* that "the finding is a paradigm that can be applied to many different diseases. It will have a profound effect on drug discovery and regenerative medicine." iZumi is using iPS cells as an enabling technology for a variety of undisclosed applications in the fields of drug discovery and regenerative medicine.

In the long term, Kreiner said, "there is the potential for iPS cells to be used for cell-based therapy." Before that happens, however, he thinks the researchers will need to find a way to reprogram the iPS cells without using retroviruses.

Indeed, the authors of the *Science* article noted that "iPS-derived neurons will not be suitable for transplantation until the oncogenic genes and retroviruses used here are replaced with more controlled methods of reprogramming."

Because the neurons were derived from ALS patients, the authors said it will be important to fix the defects in the neurons that actually cause the disease before they can be put back into the body. "It likely will be necessary to understand and correct any intrinsic defects in the patient's neurons and glia before they can be rationally used as a basis for cell therapy," they wrote.

A more near-term application of the stem cells is for drug screening.

Deepak Srivastava, director of the Gladstone Institute of Cardiovascular Disease at the **University of California, San Francisco**, said "The iPS cells are a useful human cellular model for investigating the mechanism of disease and for screening new therapies to promote neuron survival. We have never had a human model of ALS."

Srivastava added that the novelty of the paper is that the researchers were able to generate iPS cells from an elderly individual. "It has been a concern because the older a person gets, the less likely it becomes that they will be able to generate iPS cells," he said.

In June, the Gladstone Institute and iZumi entered into a collaboration to develop the institute's iPS cells to treat cardiovascular disease.

However, "cell-replacement therapy is very far away," said John Dimos, a lead author on the *Science* paper.

"The importance of the paper is that we will be able to use a patient's actual motor neurons to screen for more efficacious drugs for treating ALS," said Dimos, a postdoctoral fellow in the Department of Stem Cell and Regenerative Biology at Harvard.

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> ***—Michael Bozik, Knopp Neurosciences Inc.***

Motor neurons derived directly from a diseased patient could allow for individually tailored screening of drug therapies. The *Science* authors wrote that "patient-specific iPS cells generated from individuals with sporadic disease would carry the precise constellation of genetic information associated with pathology in that person."

Dimos told *SciBX* that in "using stem cells, one has the benefits of an easily and rapidly growing cell line that is also indefinitely self-replenishing and can be used to generate the actual cell type affected in a disease."

Michael Bozik, president and CEO of **Knopp Neurosciences Inc.**, said "The use of this iPS cell line as a drug-screening tool and disease model could potentially accelerate the discovery of new medicines to treat ALS."

Bozik told *SciBX* that "motor neurons generated using iPS cells could shed insight into the pathophysiology behind a complex disease like ALS by enabling researchers to characterize the response of motor neurons derived from ALS subjects with those derived from healthy subjects."

Knopp's KNS-760704, an optical enantiomer of Mirapex pramipexole, is in Phase IIa testing to treat ALS. **Boehringer Ingelheim GmbH** markets Mirapex, a dopamine receptor agonist, to treat Parkinson's disease (PD) and restless legs syndrome (RLS).

REFERENCES

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COMPANIES AND INSTITUTIONS MENTIONED

Columbia University, New York, N.Y.
Boehringer Ingelheim GmbH, Ingelheim, Germany
Harvard University, Cambridge, Mass.
iZumi Bio Inc., San Francisco, Calif.
Knopp Neurosciences Inc., Pittsburgh, Pa.
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