

# Stem Cell Model of Spinal Muscular Atrophy

Allison D. Ebert, PhD; Clive N. Svendsen, PhD

**H**uman embryonic stem cells provide a useful source of material for studying basic human development and various disease states. However, ethical issues concerning their procurement limit their acceptance and possible clinical applicability. Recent advances in stem cell technology have provided an alternative source of pluripotent stem cells that does not require the use of an embryo. This review addresses the generation of induced pluripotent stem cells from skin fibroblasts taken from various patient populations, with a specific focus on the pediatric disorder spinal muscular atrophy. These patient-derived cells may help researchers devise more appropriate therapies through a greater understanding of the molecular mechanisms that underlie neuron dysfunction and death in a variety of diseases. Furthermore, they provide an ideal platform for small-molecule screening and subsequent drug development.

*Arch Neurol.* 2010;67(6):665-669

## PLURIPOTENT STEM CELLS

Since their isolation in 1998,<sup>1</sup> human embryonic stem cells (hESCs) have garnered much attention for a wide range of experimental and therapeutic applications. These cells are pluripotent because after injection into immunodeficient mice they form teratomas consisting of cell types from all 3 primitive germ layers (endoderm, mesoderm, and ectoderm), including muscle, heart, liver, and the central nervous system. For neurodegenerative diseases, hESCs can be further lineage restricted to generate very specific neural subtypes, including dopaminergic neurons, motor neurons, oligodendrocytes, and astrocytes, which display many of the neurochemical and electrical attributes of mature neurons (eg, neurotransmitters, transporters, and evoked action potentials).<sup>2</sup> Because of these attributes, hESCs offer a tremendous advantage to model diseases. However, ethical concerns surround their use

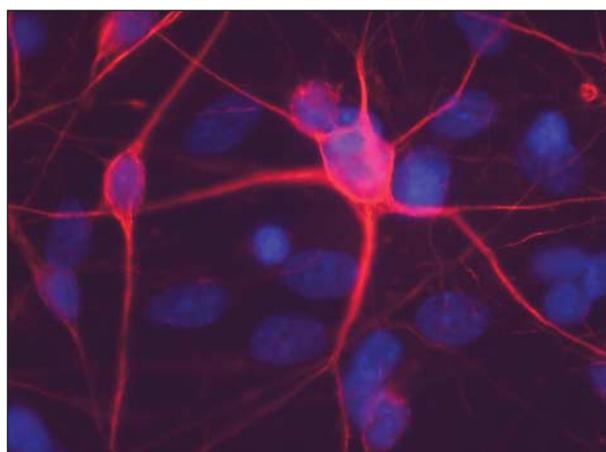
because the embryo is destroyed in the process of their procurement.

The groundbreaking discovery that mouse and human fibroblasts have the capacity to be reverted to an ESC fate through reprogramming has significantly advanced the field of stem cell and neurodegenerative disease research. Although performed independently and with some variation, these cells were all modified using DNA technology to overexpress important pluripotent stem cell genes in human neonatal or adult fibroblasts. These exogenously expressed genes reprogrammed the DNA of the fibroblast cells to make them display ESC-like morphologic features, exponential growth properties, and gene expression profiles. Similar to hESCs, the newly reprogrammed cells, termed *induced pluripotent stem cells* (iPSCs), can form teratomas and can be lineage restricted to the various cell types in the body.<sup>3</sup> Because these cells are derived from adult skin, 2 important implications arise. First, a fertilized embryo is not needed for the production of iPSCs, thus reducing some of the ethical concerns with their generation and use. Second, iPSCs can be derived from any pa-

**Author Affiliations:** Stem Cell and Regenerative Medicine Center (Drs Ebert and Svendsen), and Departments of Anatomy (Dr Svendsen) and Neurology (Drs Ebert and Svendsen), University of Wisconsin–Madison. Dr Svendsen is now with the Cedars-Sinai Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, California.

**Table. Spinal Muscular Atrophy Categories**

Classification	Age at Onset	Symptoms	Prognosis
Type I, Werdnig-Hoffman disease	Birth to 6 mo	Severe proximal muscle weakness; reduced muscle tone; inability to hold head up; possible skeletal deformities	Short life expectancy; death generally by age 2 y
Type II	6-18 mo	Moderate proximal muscle weakness; developmental motor delay; may be able to sit unaided	Reduced life span, <30 y
Type III, Kugelberg-Welander syndrome	>18 mo	Slow and mild proximal muscle weakness; may need assistance with standing and walking	Normal life span
Type IV, adult onset	>18 y	Slow and mild proximal muscle weakness; may need assistance with standing and walking	Normal life span



**Figure 1.** Induced pluripotent stem cells (iPSCs) can be lineage restricted to form motor neurons. Motor neurons were differentiated from iPSCs produced from an unaffected fibroblast sample and were stained for the neurofilament protein SMI-32 (red) (original magnification  $\times 100$ ). Cell nuclei appear in blue.

tient population,<sup>4,7</sup> which allows for analysis and modeling of the disease in the cell types particularly affected. This technique also provides the generation of a human model system for diseases with no known genetic component or for which few appropriate experimental models are available.

### SPINAL MUSCULAR ATROPHY

Spinal muscular atrophy (SMA) is an autosomal recessive disease that causes specific loss of alpha motor neurons in the spinal cord and is one of the leading genetic causes of infant mortality. Spinal muscular atrophy is caused by a mutation in the survival motor neuron gene (*SMN*; GenBank U18423) that leads to loss of SMN1 protein<sup>8</sup> and concomitant loss of motor neurons. Spinal muscular atrophy can be divided into 4 categories based on disease severity, with type I being the most severe and type IV being the least severe (**Table**). The *SMN* gene is located on chromosome 5, and approximately 95% of all patients with SMA are missing this gene. Humans are unique in that they have 2 versions of *SMN*: *SMN1* and *SMN2*.<sup>9</sup> *SMN1* produces a full-length protein found in the cytoplasm and the nucleus and is part of a large complex involved in a variety of RNA processes.<sup>10-12</sup> In contrast, a single C to T nucleotide transition in *SMN2* causes exon 7 to be excluded, generating low levels (approximately 10%-15%) of full-length SMN protein and high

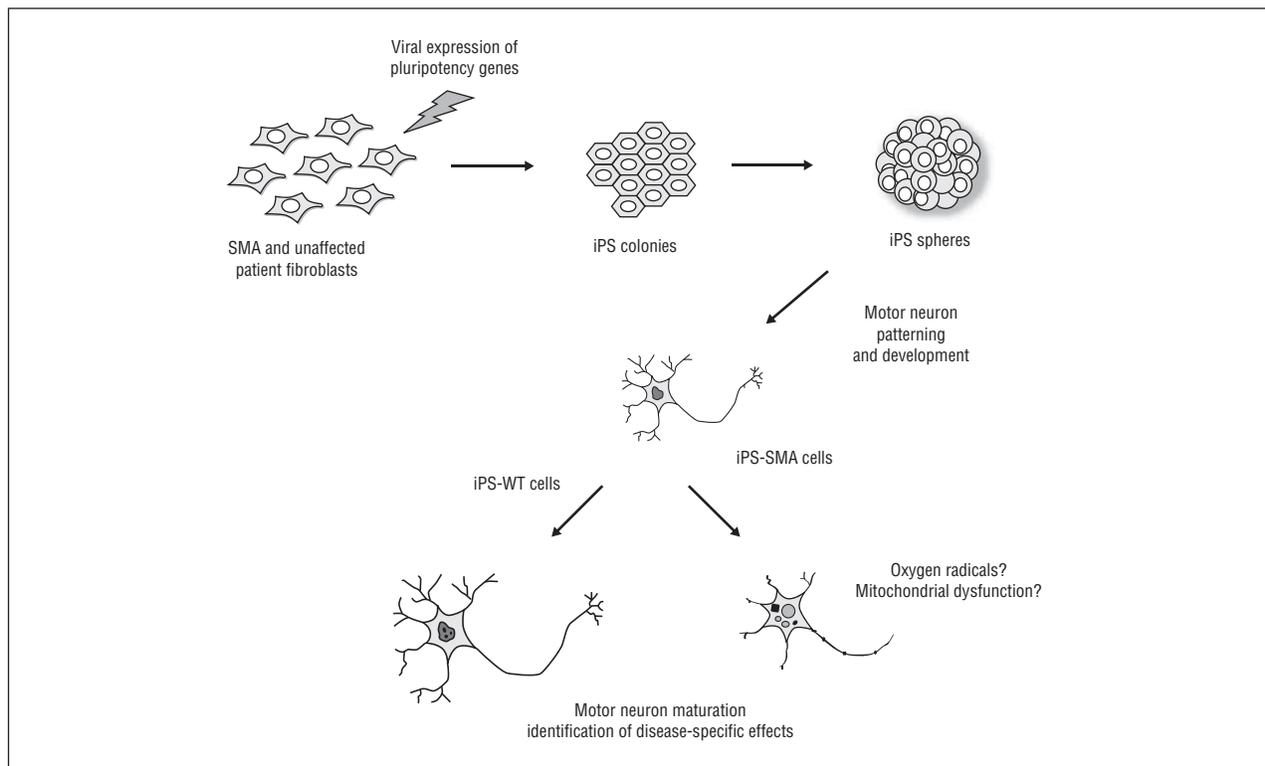
levels of an essentially nonfunctional, truncated SMN2 protein (*SMN $\Delta$ 7*).<sup>13,14</sup> Because *SMN2* can produce a small amount of full-length SMN protein, one study<sup>15</sup> has shown that disease severity is mitigated by how much full-length protein is produced. Therefore, many of the current experimental approaches use small-molecule induction or RNA manipulation to increase *SMN2* protein production to compensate for the loss of *SMN1* protein.<sup>16</sup>

### SMA MODEL SYSTEMS

Several experimental models using single and multiple cellular organisms have been used to study the molecular processes involved in SMA. Mice have become the most often used vertebrate model for mammalian genetic research because of the ability to manipulate the genome. Using homologous recombination technology and mating crosses, Schrank and colleagues<sup>17</sup> found that embryos that lack *SMN* die before uterine implantation, which underscores the importance of *SMN* during development in all cell types, not just motor neurons. Other mice have been developed that harbor the entire human *SMN2* locus or the *SMN $\Delta$ 7* mutation on the mouse *SMN* knockout background to better represent the human condition.<sup>18-20</sup> Despite providing invaluable protein and disease information, these animal models may not adequately represent the human condition because the physiologic and anatomical features of mice are radically different from those of humans, especially regarding the central nervous system.

Possibly some of the most useful cells studied in culture have been fibroblasts taken from patients with SMA. Fibroblasts are relatively easy to obtain from skin biopsy samples, are easy to grow and maintain in the culture dish, and have the added benefit of naturally lacking *SMN1*. One important weakness is that fibroblasts do not make motor neurons, astrocytes, or muscle. Owing to the selective loss of motor neurons and the importance of astrocytes and muscle on motor neuron health,<sup>21,22</sup> having a source of human cells that harbor the genetic mutation and that are capable of making these specific tissues would be highly beneficial. Patient-derived iPSCs can fulfill this need.

We generated iPSCs from commercially available fibroblast samples obtained from a 3-year-old boy with type I SMA and his unaffected (WT) mother.<sup>7</sup> To do this, we used lentiviral vectors to stably express repro-



**Figure 2.** Fibroblast cells can be converted to induced pluripotent stem cells (iPSCs) to model spinal muscular atrophy (SMA). Fibroblast cells were infected with viruses to express reprogramming genes. The iPSCs were cultured as flat colonies and then were lifted to form free-floating aggregate spheres in a nutrient medium. Next, using a series of chemical induction signals, iPSCs were instructed to form motor neurons, which the iPS-SMA and iPS-WT (from his unaffected mother) cells were able to do. Only after additional time in culture did we observe a specific deficit in the iPS-SMA–derived motor neurons. We speculate that this effect could be due to increased reactive oxygen species production or damaged mitochondria in the iPS-SMA cells.

gramming genes in the fibroblast samples. After a few weeks in culture, iPSCs formed, which are visually and functionally distinct from fibroblast cells, and expressed a range of pluripotency markers found in hESCs. We further showed that these cells were indeed pluripotent because, unlike the fibroblasts, they could generate teratomas. The iPS-SMA cells retained a lack of SMN1 expression compared with the iPS-WT cells.<sup>7</sup> Finally, given the appropriate culture conditions, the iPS-SMA and iPS-WT cells produced neural cells that expressed some of the molecular markers typical of motor neurons (**Figure 1**). Together, these characteristics represent a full conversion from the fibroblast state to a pluripotent stem cell.

Although SMN is present in all cell types, it is curious that such an essential and ubiquitously expressed cellular protein should cause such specific degeneration of motor neurons. In this regard, we analyzed motor neuron production in the iPS-SMA and iPS-WT cells. Early in the differentiation protocol, the affected and nonaffected iPSCs could form motor neurons of approximately the same number and size, which we used as an indication of neuron health. However, after a few more weeks of motor neuron development, we observed a selective reduction in the number and size of motor neurons derived from the iPS-SMA cells compared with nondiseased iPS-WT–derived motor neurons.<sup>7</sup> Additional data are needed to determine the mechanism that induces the motor neuron changes, but the present data suggest that an intrinsic property of the

SMA-derived motor neuron was leading to damaged (or dying) neurons rather than to a lack of motor neuron development (**Figure 2**). The SMN1 protein has been shown to have antioxidant properties, and it is possible that the metabolism and energy requirements in the SMA motor neurons generate more reactive oxygen species compared with other cell types. Oxidative stress can be initiated by a variety of processes, including mitochondrial dysfunction, that may lead to motor neuron dysfunction through activation of programmed cell death pathways. Answering these mechanistic questions hopefully will provide insight into the best method of therapeutic intervention.

#### DEVELOPING THERAPIES FOR SMA USING iPSCs

Despite the fact that gene therapy for SMA (eg, using RNA technology or oligonucleotides to modify SMN2 splicing)<sup>16</sup> is only recently becoming more widely used in research, experimental and clinical applications for gene therapy have been developed for other neurodegenerative diseases.<sup>23</sup> Using viral vectors to replace SMN1 was shown to decrease motor neuron death and increase life span in SMA mice.<sup>24,25</sup> Although this technique holds great clinical promise, the future of gene therapy may one day combine iPSC technology and cellular replacement with gene modification. In this regard, Hanna et al<sup>26</sup> reported an elegant study in which sickle cell anemia was corrected in a mouse by

generating iPSCs from this mouse, using gene-specific targeting to repair the hemoglobin allele, generating hematopoietic progenitor cells, and transplanting the repaired blood cells back into the same mouse. It is intriguing to consider the possibility of generating iPSCs from a patient with SMA, genetically elevating *SMN1* expression, deriving motor neurons and astrocytes that now express *SMN1*, and then transplanting these cells back to repopulate and repair the patient's spinal cord. Although there are major challenges associated with this approach, one study<sup>27</sup> reported that the growth of axons from transplanted motor neurons can reinnervate the muscle and have functional effects.

A more near-term use of iPS-SMA cells may be in the area of small-molecule drug discovery. The SMN protein is found in aggregate nuclear structures called *gems*, and the number of gems present is inversely correlated with disease severity<sup>15,28</sup>; therefore, therapeutic agents that increase protein output would be clinically valuable. Various compounds have been tested on fibroblast cells from patients with SMA and have been shown to increase SMN protein levels through stabilization or prevention of exon 7 skipping,<sup>29-32</sup> and one such compound, valproic acid, is currently being used in clinical trials for SMA.<sup>33</sup> Using 2 previously tested compounds (valproic acid and tobramycin), we found that the number of gems significantly increased in the iPS-SMA cells after treatment with each compound compared with untreated iPS-SMA cells,<sup>7</sup> suggesting that SMN protein production, stabilization, or both were enhanced. These results confirm that patient-derived iPSCs have similar molecular responses to drug treatment as do the routinely used fibroblast cells. We went on to identify gems in motor neurons, so the next step is to derive high-throughput screening assays assessing gem number specifically in motor neurons derived from patients with SMA. Further drug discovery investigation is crucial and, by using iPSCs, novel compounds can be tested in a human system on particularly vulnerable cell types, thereby increasing efficacy and the discovery of highly clinically relevant compounds.

In conclusion, SMA is a devastating and oftentimes fatal disease without an effective treatment. Much effort has been focused on determining the role of SMN in cellular function, on identifying the molecular processes involved in neuronal death, and on developing effective therapies. The ability to generate patient-specific iPSCs has opened new avenues of study for cell therapy and disease modeling. Spinal muscular atrophy may serve as a "proof of concept" that a specific neurologic abnormality can be mirrored in the culture dish. Armed with this model system, the hope is that new discoveries will be made that allow cell replacement and new drugs to be found to treat this disease.

Accepted for Publication: April 14, 2009.

Correspondence: Allison D. Ebert, PhD, Wisconsin Institutes for Medical Research, 1111 Highland Ave, Room 5033, Madison, WI 53705 (aebert@wisc.edu).

**Author Contributions:** Drs Ebert and Svendsen had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Drafting of the manuscript:* Ebert and Svendsen. *Critical revision of the manuscript for important intellectual content:* Ebert and Svendsen.

**Financial Disclosure:** None reported.

**Funding/Support:** This work was supported by the ALS Association (Dr Svendsen) and the National Institutes of Health (Dr Svendsen).

## REFERENCES

- Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts [published correction appears in *Science*. 1998;282(5395):1827]. *Science*. 1998;282(5391):1145-1147.
- Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell*. 2008;132(4):661-680.
- Müller LU, Daley GQ, Williams DA. Upping the ante: recent advances in direct reprogramming. *Mol Ther*. 2009;17(6):947-953.
- Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*. 2008;321(5893):1218-1221.
- Park IH, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. *Cell*. 2008;134(5):877-886.
- Soldner F, Hockemeyer D, Beard C, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell*. 2009;136(5):964-977.
- Ebert AD, Yu JY, Rose FF Jr, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*. 2009;457(7227):277-280.
- Lefebvre S, Bürglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*. 1995;80(1):155-165.
- Rochette CF, Gilbert N, Simard LR. *SMN* gene duplication and the emergence of the *SMN2* gene occurred in distinct hominids: *SMN2* is unique to *Homo sapiens*. *Hum Genet*. 2001;108(3):255-266.
- Pellizzoni L, Yong J, Dreyfuss G. Essential role for the SMN complex in the specificity of snRNP assembly. *Science*. 2002;298(5599):1775-1779.
- Fischer U, Liu Q, Dreyfuss G. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. *Cell*. 1997;90(6):1023-1029.
- Liu Q, Fischer U, Wang F, Dreyfuss G. The spinal muscular atrophy disease gene product, *SMN*, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell*. 1997;90(6):1013-1021.
- Monani UR, Lorson CL, Parsons DW, et al. A single nucleotide difference that alters splicing patterns distinguishes the *SMA* gene *SMN1* from the copy gene *SMN2*. *Hum Mol Genet*. 1999;8(7):1177-1183.
- Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the *SMN* gene regulates splicing and is responsible for spinal muscular atrophy. *Proc Natl Acad Sci U S A*. 1999;96(11):6307-6311.
- Lefebvre S, Burtlet P, Liu Q, et al. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet*. 1997;16(3):265-269.
- Baughan T, Shababi M, Coady TH, Dickson AM, Tullis GE, Lorson CL. Stimulating full-length *SMN2* expression by delivering bifunctional RNAs via a viral vector. *Mol Ther*. 2006;14(1):54-62.
- Schrank B, Götz R, Gunnarsen JM, et al. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. *Proc Natl Acad Sci U S A*. 1997;94(18):9920-9925.
- Hsieh-Li HM, Chang JG, Jong YJ, et al. A mouse model for spinal muscular atrophy. *Nat Genet*. 2000;24(1):66-70.
- Monani UR, Sendtner M, Coover DD, et al. The human centromeric survival motor neuron gene (*SMN2*) rescues embryonic lethality in *Smn(-/-)* mice and results in a mouse with spinal muscular atrophy [published correction appears in *Hum Mol Genet*. 2007;16(21):2648]. *Hum Mol Genet*. 2000;9(3):333-339.
- Le TT, Pham LT, Butchbach ME, et al. SMN Delta 7, the major product of the centromeric survival motor neuron (*SMN2*) gene, extends survival in mice with spinal muscular atrophy and associates with full-length *SMN*. *Hum Mol Genet*. 2005;14(6):845-857.
- Guettier-Sigrist S, Coupin G, Braun S, Warter JM, Poindron P. Muscle could be the therapeutic target in SMA treatment. *J Neurosci Res*. 1998;53(6):663-669.
- Van Damme P, Bogaert E, Dewil M, et al. Astrocytes regulate GluR2 expression

- in motor neurons and their vulnerability to excitotoxicity. *Proc Natl Acad Sci U S A*. 2007;104(37):14825-14830.
23. Björklund A, Kirik D, Rosenblad C, Georgievska B, Lundberg C, Mandel RJ. Towards a neuroprotective gene therapy for Parkinson's disease: use of adenovirus, AAV and lentivirus vectors for gene transfer of GDNF to the nigrostriatal system in the rat Parkinson model. *Brain Res*. 2000;886(1-2):82-98.
  24. Azzouz M, Le T, Ralph GS, et al. Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy. *J Clin Invest*. 2004;114(12):1726-1731.
  25. Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. *Nat Biotechnol*. 2010;28(3):271-274.
  26. Hanna J, Wernig M, Markoulaki S, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science*. 2007;318(5858):1920-1923.
  27. Deshpande DM, Kim YS, Martinez T, et al. Recovery from paralysis in adult rats using embryonic stem cells. *Ann Neurol*. 2006;60(1):32-44.
  28. Coovert DD, Le TT, McAndrew PE, et al. The survival motor neuron protein in spinal muscular atrophy. *Hum Mol Genet*. 1997;6(8):1205-1214.
  29. Brichta L, Hofmann Y, Hahnen E, et al. Valproic acid increases the SMN2 protein level: a well-known drug as a potential therapy for spinal muscular atrophy. *Hum Mol Genet*. 2003;12(19):2481-2489.
  30. Mattis VB, Rai R, Wang JH, Chang CW, Coady T, Lorson CL. Novel aminoglycosides increase SMN levels in spinal muscular atrophy fibroblasts. *Hum Genet*. 2006;120(4):589-601.
  31. Sumner CJ, Huynh TN, Markowitz JA, et al. Valproic acid increases SMN levels in spinal muscular atrophy patient cells. *Ann Neurol*. 2003;54(5):647-654.
  32. Wolstencroft EC, Mattis V, Bajaj AA, Young PJ, Lorson CL. A non-sequence-specific requirement for SMN protein activity: the role of aminoglycosides in inducing elevated SMN protein levels. *Hum Mol Genet*. 2005;14(9):1199-1210.
  33. Tsai LK, Yang CC, Hwu WL, Li H. Valproic acid treatment in six patients with spinal muscular atrophy. *Eur J Neurol*. 2007;14(12):e8-e9.

### Call for Papers

#### Archives Express

**ARCHIVES EXPRESS**

The *Archives* launched a new *Archives Express* section in the September 2000 issue. This section will enable the editors to publish highly selected papers within approximately 2 months of acceptance. We will consider only the most significant research, the top 1% of accepted papers, on new important insights into the pathogenesis of disease, brain function, and therapy. We encourage authors to send their most exceptional clinical or basic research, designating in the cover letter a request for expedited *Archives Express* review. We look forward to publishing your important new research in this accelerated manner.

Roger N. Rosenberg, MD