Advances in Translational Research in Neuromuscular Diseases

David Pleasure, MD

New therapies developed over the past 3 years for previously intractable diseases of skeletal muscle, neuromuscular junctions, peripheral nerves, and motor neurons are now being incorporated into our standard neuromuscular clinical practice. The past 3 years were also marked by important advances in our understanding of the pathogenesis and pathophysiology of inherited and acquired neuromuscular diseases; these advances were acquired by the use of high-throughput nucleotide and protein analytic methods, novel animal models, and human-induced pluripotent stem cell–derived “diseases in a dish.” Over the next decade, we can reasonably anticipate that these insights, coupled with advances in our ability to modulate immune mechanisms, to modify the activity of mutant genes, and to perform gene replacement therapies with enhanced viral vector–based and stem cell–based delivery systems, will revolutionize our management of neuromuscular diseases.

**Table 1** provides a sampling of recently tested therapies for neuromuscular disorders. Each has been reported to provide patients with significant symptomatic relief.

**GENE THERAPIES**

It is seductive to contemplate definitive reversal of genetic neuromuscular diseases by engineering permanent expression of the normal gene in the affected tissue. The potential of this approach was recently illustrated by the continued improvement in vision in patients with Leber hereditary optic atrophy 18 months after subretinal administration of an adeno-associated viral vector–encoded normal (REPRINTED) ARCH NEUROL/VOL 68 (NO. 4), APR 2011 WWW.ARCHNEUROL.COM

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The observation that adeno-associated viral vector–mediated transduction of α-sarcoglycan into extensor digitorum brevis in human limb-girdle muscular dystrophy type 2D restores the full sarcoglycan complex and increases muscle fiber size is also heartening. And the recent demonstration of the therapeutic efficacy of adeno-associated viral vector–mediated spinal cord SMN1 transduction in neonatal mice with mutant SMN1 spinal muscular atrophy argues that gene therapy could prove feasible for human motor neuron diseases as well.

**MODULATION OF GENE EXPRESSION**

Dystrophin and dysferlin, the latter mutated in limb-girdle muscular dystrophy type 2B and Miyoshi myopathy, are encoded by very large genes that cannot be packaged into present-day viral vectors. Although this delivery problem is potentially solvable (eg, by gene transfer in a human artificial chromosome), a promising alternative approach is to splice out mutated, nonessential, dystrophin or dysferlin exons by administration of antisense oligonucleotides, permitting translation of a near full-length, and functional, protein. Of the almost 900 known human dystrophin mutations, at least 60% are susceptible to mitigation by this exon-skipping approach, as are a substantial proportion of the more than 350 known dysferlin mutations. As are these mutations can also be rescued by aminoglycoside-induced, premature termination codon “read through,” a tactic that has yielded a substantial decrease in serum creatine kinase levels in the first Duchenne cohorts thus far treated. Further advances in design and delivery of exon-skipping and premature termination codon–skipping reagents may permit clinically significant mitigation of these muscular dystrophies. Oligonucleotide reagents can also be used to displace aberrant mutant RNA X protein interactions, an approach that has already shown promise in a mouse triple repeat expansion model of myotonic dystrophy type 1.

An alternate therapeutic approach to loss-of-function genetic neuromuscular diseases is to induce overexpression of a functionally overlapping gene. In mice with mutant SMN1 spinal muscular atrophy, for example, weakness is diminished, and survival enhanced, by antisense oligonucleotide–mediated SMN2 exon skip-ping to elevate SMN2 protein expression or by administering a histone deacetylase inhibitor to enhance SMN2 transcription. Although an initial phase II trial of the US Food and Drug Administration–approved histone deacetylase inhibitor valproate failed to show therapeutic efficacy in spinal muscular atrophy, results of a recent human trial showed that histone deacetylase inhibitor–mediated induction of ABCD2 can ameliorate the oxidative protein damage that is believed to contribute to neural inflammation in mutant ABCD1 adrenomyeloneuropathy, recent animal studies have suggested strategies to diminish downstream deleterious effects of mutant proteins, including administration of a cyclophilin inhibitor or a ryanodine receptor stabilizer to prevent calcium overload–induced myofiber necrosis in the muscular dystrophies, administration of cystamine to suppress polyalanine expansion toxicity in ocu-lopharyngeal muscular dystrophy, and administration of sialic acid metabolites to slow progression of distal myopathy with rimmed vacuoles–hereditary inclusion body myopathy.

**STEM CELL–BASED THERAPIES**

Stem cells can both self-renew and generate differentiated daughter cells. Skeletal muscle satellite cells are an example of tissue-specific stem cells. When stimulated to proliferate as a result of muscle injury, these cells generate myocytes that fuse to and repair damaged multinucleate myofibers. Animal studies have shown that transplantation of skeletal muscle satellite cells from a normal donor or satellite cell autografts from an affected individual after their engineering to express full-length or near full-length dystrophin can rescue Duchenne myofibers. Although generating sufficient numbers of fusion-competent myocytes to globally repair dystrophic muscle remains problematic, this might be accomplished by genetic manipulations that transiently induce terminally differentiated skeletal muscle to reenter the cell cycle and enhance the survival of the resulting myocytes.

Human embryonic stem cells (HESCs) and IPSCs may provide alternate sources for fusion-competent myocytes and can also be used to generate motor neurons or Schwann cells. The use of IPSCs avoids ethical concerns related to HESC harvesting and, because autografts could be derived by genetically engineering the patient’s own IPSCs, would diminish the need for long-term graft recipient immunosuppression.

Safety concerns need to be surmounted before HESC or IPSC transplants become integrated into the practice of neuromuscular medicine; these concerns include teratoma formation, which is an intrinsic property of stem cells, and malignant transformation as a consequence of alterations in the genome of the grafted cells. The efficacy of stem cell–derived motor neurons for ALS or spinal muscular atrophy will also require development of methods for broad dissemination to the central nervous system and for enhancing appropriate upstream and downstream synaptic interactions by the grafted cells. And, of course, stem cell–derived motor neuron transplants

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**Table 1. New Drugs and New Indications for Old Drugs in Human Neuromuscular Disorders**

<table>
<thead>
<tr>
<th>Disorder(s)</th>
<th>Therapy</th>
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<tr>
<td>Autoantibody-associated muscular dystrophy</td>
<td>Riluzole</td>
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<tr>
<td>RPE65 mutation</td>
<td>Rituximab, Etoposide, Trabectedin</td>
</tr>
<tr>
<td>Dystrophic myopathies</td>
<td>Pirenzepine, Cilnidipine, Lidocaine, Duloxetine</td>
</tr>
<tr>
<td>Dystrophinopathy</td>
<td>Etoracodine, Sotolol, Metyrosine</td>
</tr>
<tr>
<td>Stabilisation of muscle fibers</td>
<td>Endurance training</td>
</tr>
<tr>
<td>Late-onset Pompe disease</td>
<td>Alglucosidase alfa</td>
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*aLimited initial human trials of each of these therapies have yielded promising results.*
would be of limited utility in those motor neuron diseases in which, as in mutant SOD1 familial ALS, motor neuron loss is at least in part a consequence of a neuroglial defect.35,36

Although human stem cell–derived oligodendroglial progenitors have received approval from the US Food and Drug Administration and are already being tested in the United States for patients with spinal cord trauma and Pelizaeus-Merzbacher disease, efficacy trials of embryonic stem cell–derived or IPS-derived cell transplants for human neuromuscular diseases have not yet been initiated. However, HESC and IPS technologies have already proven their worth by permitting the development of human “disease in a dish” models for familial dysautonomia,37 spinal muscular atrophy,38 mutant SOD1 familial ALS,35,36 and Duchenne dystrophy13 (Table 2). These human cell–based models facilitate exploration of pathogenic mechanisms and can also be used for rapid screening of drug candidates30 (Figure).

### PATHOGENETIC AND PATHOPHYSIOLOGICAL ANALYSIS

Advances in sequencing of large chromosomal segments and in single-nucleotide polymorphism analysis have revolutionized the workup of familial neuromuscular diseases, permitting rapid identification of neuromuscular disease–causing gene mutations and revealing unexpected genetic relationships between phenotypically diverse syndromes. For example, single-nucleotide polymorphisms in what was previously considered a multiply repeated, untranscribed gene on chromosome 4q35 now appear to be the cause of facioscapulohumeral muscular dystrophy,39 and scapuloperoneal spinal muscular atrophy and Charcot-Marie-Tooth disease type 2C have proven to be allelic mutations of TRPV4, both of which increase TRPV4 channel calcium permeability.40 The potential importance of analyzing even very large genes (eg, dystrophin) for mutations susceptible to therapeutic remediation16 has already been mentioned. Even whole genome sequencing has now become economically feasible, permitting definitive diagnosis of neuromuscular disorders caused by the cumulative effects of several rare recessive mutations.41

Modern methods for nucleotide analysis can also reveal genetic susceptibilities to acquired neuromuscular disorders. Examples include the detection of mutations in a previously undiscovered potassium channel–encoding gene in patients with thyrotropic hypokalemic periodic paralysis42 and the detection of genetic polymorphisms that alter the rate of progression of sporadic ALS43 or modulate peripheral pain perception.44 Also, an important outcome of recognizing the many more rare mutations linked to familial ALS during the past 3 years has been the highlighting of metabolic pathways in which these genes participate and in which they may be perturbed in sporadic forms of ALS, including aberrant activation of nuclear factor κB45 and dysregulation of neural D-serine homeostasis.46 These pathways may provide opportunities for therapeutic intervention.

Our understanding of nongenetic neuromuscular diseases has also been enhanced during the past 3 years. To cite a few examples, protein microarray analysis, coupled with immunohistology, demonstrated a tight linkage between dermatomyositis and upregulation of the type 1 interferon-inducible protein ISG15,47 anti-GD1a antibodies were shown to induce electrophysiological dysfunction at motor nodes of Ranvier by activating calpain and complement,48 and combined epidemiological and immunological studies provided a link between an outbreak of polyradiculoneuropathy and workplace exposure to aerosolized porcine neural tissue.49

### Table 2. Human Embryonic Stem Cell Culture Model and Induced Pluripotent Stem Cell Culture Models of Neuromuscular Diseases

<table>
<thead>
<tr>
<th>Model</th>
<th>Comment(s)</th>
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<tbody>
<tr>
<td>Familial dysautonomia neural crest neurons (IPSC)</td>
<td>Tissue-specific mis-splicing of IKBKAP reduces neural crest IKBKAP expression and is associated with impaired neuroblast formation and migration; corrected by treatment with the plant hormone kinetin.37</td>
</tr>
<tr>
<td>Spinal muscular atrophy motor neurons (IPSC)</td>
<td>Autonomic defect in SMN mutant motor neurons; cultures useful for testing effects of exon-skipping reagents on SMN2 levels.36</td>
</tr>
<tr>
<td>Mutant SOD1 ALS, normal motor neurons (HESC)</td>
<td>Nonmotor neuron autonomous deleterious effect of G37R mutant SOD1 astroglia.35,36</td>
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<tr>
<td>Duchenne muscular dystrophy skeletal muscle (IPSC)</td>
<td>Correction of dystrophin deficiency by transfer of dystrophin-containing microchromosomes.76</td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; HESC, human embryonic stem cell; IPSC, induced pluripotent stem cell.

Figure. Human induced pluripotent stem cell colonies (original magnification ×4) stained in red (ie, vital stain) for the pluripotency marker TRA-1-60. This image was kindly provided by Bonnie Barrilleaux, PhD, and Paul Knoepfler, PhD, Institute for Pediatric Regenerative Medicine, Shriners Hospitals for Children Northern California, Sacramento.
CONCLUSIONS

Studies completed during the past 3 years have demonstrated the substantial benefits of new drugs (eg, rituximab for immune-mediated neuromuscular diseases) and new indications for old drugs (eg, ephedrine for mutant DOK7 congenital myasthenia). During the same period, the experimental infrastructure required for the design of human trials of gene replacement, RNA exon skipping and premature termination codon skipping, and stem cell transplantation has been strengthened, and advances in analytic technologies have vastly increased our ability to diagnose and evaluate the pathophysiology of neuromuscular diseases. However, many challenges remain: no new therapies for ALS have come on line since riluzole; we still lack effective treatments for most of the inherited polyneuropathies; and there have been no recent advances in our understanding of the most common of all neuromuscular diseases in developed countries (ie, diabetic polyneuropathy).

Accepted for Publication: August 31, 2011.

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Financial Disclosure: None reported.

Funding/Support: This review was supported by National Institutes of Health grant RO1 NS025044 and by the Shriners Hospitals for Children.

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