Myotonic Dystrophy: Therapeutic Strategies for the Future

Thurman M. Wheeler

Neuromuscular Disease Center, Department of Neurology, University of Rochester, Rochester, New York 14642

Summary: Myotonic dystrophy (DM) is a dominantly inherited neurodegenerative disorder for which there is no cure or effective treatment. Investigation of DM pathogenesis has identified a novel disease mechanism that requires development of innovative therapeutic strategies. It is now clear that DM is not caused by expression of a mutant protein. Instead, DM is the first recognized example of an RNA-mediated disease. Expression of the mutated gene gives rise to an expanded repeat RNA that is directly toxic to cells. The mutant RNA is retained in the nucleus, forming ribonuclear inclusions in affected tissue. A primary consequence of RNA toxicity in DM is dysfunction of two classes of RNA binding proteins, which leads to abnormal regulation of alternative splicing, or spliceopathy, of select genes. Spliceopathy now is known to cause myotonia and insulin resistance in DM. As our understanding of pathogenesis continues to improve, therapy targeted directly at the RNA disease mechanism will begin to replace the supportive care currently available. New pharmacologic approaches to treat myotonia and muscle wasting in DM type 1 are already in early clinical trials, and therapies designed to reverse the RNA toxicity have shown promise in preclinical models by correcting spliceopathy and eliminating myotonia. The well-defined ribonuclear inclusions may serve as convenient therapeutic targets to identify new agents that modify RNA toxicity. Continued development of appropriate model systems will allow testing of additional therapeutic strategies as they become available. Although DM is a decidedly complex disorder, its RNA-mediated disease mechanism may prove to be highly susceptible to therapy. **Key Words:** Myotonic dystrophy, myotonia, RNA disease, RNA toxicity, spliceopathy, MBNL1, CUGBP1.

INTRODUCTION

Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults, affecting approximately 1 in 7400.[1] Inheritance is autosomal dominant and results from a CTG repeat expansion in the 3′ untranslated region of the DM protein kinase gene (*DMPK*) on chromosome 19q.[2] The severity of disease is proportional to the size of the expansion. The number of repeats tends to increase from generation to generation, accounting for the genetic anticipation characteristic of this disease. As with several other types of muscular dystrophy, disease in DM1 is not limited to skeletal muscle. Instead it is a multisystemic disorder that includes myotonia, progressive weakness, muscle wasting, insulin resistance, cardiac conduction defects, neuropsychiatric symptoms, gonadal atrophy, and early cataracts.

A second form of myotonic dystrophy, DM type 2 (DM2), was discovered more recently. DM2 results from an unstable expansion of a CCTG repeat in intron 1 of the zinc finger protein 9 gene (*ZNF9*) gene on chromosome 3q.[3] DM2 shares the core features of DM1, including autosomal dominant inheritance, weakness, myotonia, and multisystem involvement. In contrast to DM1, however, weakness and myotonia affect proximal leg muscles at onset, and muscle atrophy generally is less severe.

There is no cure for DM. Current treatment for DM is limited to supportive care that partially alleviates signs and symptoms of the disease but does nothing to slow or halt disease progression. The ultimate goal in development of new therapies is reversal of the disease phenotype. Fortunately, we are at or near a stage where therapeutic intervention has begun to target specific biochemical pathways that are abnormal in DM. This review discusses new strategies already in clinical trials that target two clinical features of DM, myotonia and muscle wasting, as well as approaches that are designed to reverse the phenotype by directly targeting the RNA-mediated disease mechanism.

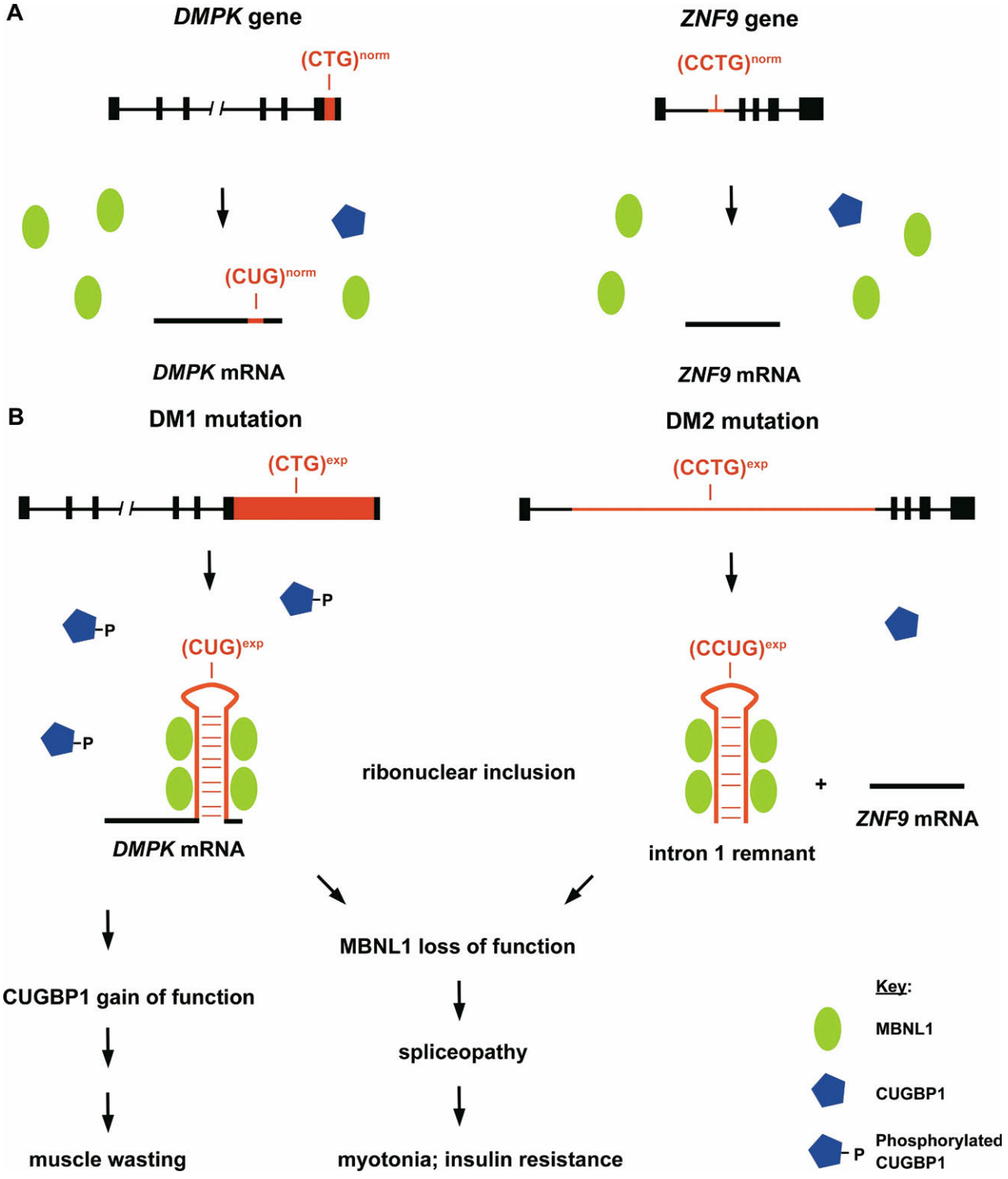
MOLECULAR PATHOGENESIS

Research on animal models of DM1 has uncovered a novel disease mechanism that is mediated directly by a toxic RNA, independent of the protein encoded (FIG. 1). In fact, because of the location of the repeat expansion mutations outside of the *DMPK* and *ZNF9* coding re-

Address correspondence and reprint requests to: Thurman M. Wheeler, M.D., Neuromuscular Disease Center, 601 Elmwood Ave., Box 673, Rochester, NY 14642. E-mail: thurman_wheeler@urmc.rochester.edu.

gions, the protein product in both types of DM is entirely normal. In DM1, the expanded CTG repeat is expressed as an expanded CUG repeat (CUG^{exp}) RNA, whereas in DM2 the expanded CCTG repeat is expressed as a $CCUG^{exp}$ RNA. In both conditions, the expanded repeat RNA is retained in nuclei of affected tissue, forming well-defined ribonuclear inclusions.[3,4]

Nuclear retention of the mutant RNA leads to dysfunction of at least two RNA-binding proteins: 1) muscleblind-like 1 (MBNL1) in both DM1 and DM2[5–7] and 2) CUG-binding protein 1 (CUGBP1) in DM1.[8,9] MBNL1 and CUGBP1 are antagonistic regulators of developmental alternative splicing of pre-mRNA.[7,10] Misregulated alternative splicing, or spliceopathy, currently is recognized



as the primary consequence of RNA toxicity in both types of DM. Myotonia and insulin resistance in DM result directly from spliceopathy of the muscle chloride channel ClC-1 and insulin receptor, respectively.[9,11–13] Although it is not clear which of the other core clinical features of DM, if any, are due to aberrant splicing, at least two dozen transcripts are misspliced in DM1, and it is likely that dozens if not hundreds more remain to be identified (see review by Osborne and Thornton[14]). The vast majority of these splicing changes are conserved in DM1 and DM2.[7] In each case, the spliceopathy involves a reversion to the fetal and early postnatal developmental splicing patterns.[7]

TREATMENT STRATEGIES THAT TARGET SPECIFIC ASPECTS OF DM

Myotonia reduction

A characteristic clinical feature of DM is delayed relaxation of muscle, due to repetitive action potentials. This phenomenon, called myotonia, is the symptom by which DM is recognized most often. Although not the most serious complication of DM, myotonia exacerbates disability, with preferential involvement of muscles that are the weakest, such as those in the hand and forearm. It is not known to what extent, if at all, myotonia contributes to the dystrophic process. The persistent muscle contractions induced by myotonia may increase susceptibility to muscle fiber dysfunction or damage, perhaps due to elevated calcium influx. If so, an additional advantage of myotonia reduction in DM may be improvement of muscle function.

Several drugs are used to treat myotonia. For example, mexiletine, an antiarrhythmic that acts on sodium channels, has been used off-label to treat myotonia in DM and in nondystrophic myotonias. In the absence of quality-controlled clinical trials, however, the safety and efficacy of antimyotonia agents in DM1 are unknown.[15] Recently, mexiletine has undergone closer investigation as an antimyotonia drug in DM1. Early results from two small 7-week, randomized, double-blind, placebo-controlled cross-over trials suggest that mexiletine is safe and effective at myotonia reduction in DM1.[16] Final results from data interpretation are forthcoming (R.T. Moxley, personal communication). If these preliminary results are confirmed, longer treatment trials will allow determination of whether myotonia reduction has any effect on muscle strength and function.

Although the effect of mexiletine on myotonia reduction in DM2 patients is predicted to be similar as in DM1, this will have to be determined in separate treatment trials. Compared to DM1, however, myotonia in DM2 patients tends to be qualitatively and quantitatively less severe,[17] suggesting that fewer DM2 patients may benefit from aggressive antimyotonia treatment.

Reversal of muscle wasting in DM1: IGF-1/IGFBP3

Progressive muscle wasting and weakness are hallmarks of DM1. Unlike other forms of muscular dystrophy, muscle wasting in DM1 does not result from extensive muscle necrosis, nor is it accompanied by conspicuous muscle fibrosis. Instead, evidence suggests that wasting in DM results from a defect in muscle anabolism. For example, rates of protein synthesis are reduced and the caliber of muscle fibers progressively diminishes, suggesting that muscle wasting in DM results from a defect in muscle anabolism.[18,19] These observations raise the possibility that a strong anabolic stimulus could reverse muscle wasting in DM1. Several anabolic agents already have been tested for their ability to improve muscle strength. Randomized trials of testosterone and creatine failed to improve muscle strength in DM patients.[20–23] A pilot study reported that dehydroepiandrosterone (DHEA) improved strength in 11 DM patients,[24] and a phase II/III trial testing two doses of oral DHEA in DM patients has been completed (NCT00167609 at http://www.clinicaltrials.gov), although final results of this study had not been published as of June 2008.

FIG. 1. RNA-mediated toxicity in myotonic dystrophy types 1 and 2 (DM1 and DM2). A, The normal dystrophia myotonica protein kinase gene (*DMPK*) contains a short CTG repeat (CTG^{norm}) in the 3′ untranslated region of exon 15. Expression of this gene produces a mRNA containing a short CUG repeat (CUG^{norm}). The normal zinc finger protein 9 gene (*ZNF9*) contains a CCTG ($CCTG^{norm}$) repeat in intron 1 and gives rise to a pre-mRNA containing a $CCUG^{norm}$ repeat. Because of removal of the introns, the $CCUG^{norm}$ is absent from the *ZNF9* mRNA. In the presence of nonexpanded CUG and CCUG repeats, activity of MBNL1 and CUGBP1 proteins is normal. B, DM1 is caused by an expansion of the CTG repeat (CTG^{exp}) in the *DMPK* gene. Expression of the mutated *DMPK* gene produces an mRNA containing a CUG expansion (CUG^{exp}). The CUG^{exp} RNA forms a double-stranded hairpin structure that is highly stable and retained in the nucleus. An expansion of the CCTG repeat ($CCTG^{exp}$) in the *ZNF9* gene causes DM2. As with the $CCUG^{norm}$ repeat, the $CCUG^{exp}$ repeat is removed from the pre-mRNA and absent from the *ZNF9* mRNA. However, the $CCUG^{exp}$ remnant of intron 1 is highly resistant to degradation, due to formation of a double-stranded hairpin structure similar to that adopted by CUG^{exp} RNA. In both DM1 and DM2, the pathogenic RNA is retained in the nucleus and recruits MBNL1 protein, forming ribonuclear inclusions. Sequestration of MBNL1 by the mutant RNA leads to loss of MBNL1 function, manifested by misregulated alternative splicing, or spliceopathy of at least two-dozen transcripts. Myotonia and insulin resistance in DM result from spliceopathy of ClC-1 and insulin receptor, respectively. It seems likely that additional clinical manifestations of DM1 and DM2 eventually will be linked to spliceopathy from MBNL1 loss-of-function. In DM1, hyperphosphorylation enhances stability of CUGBP1 protein, resulting in higher total protein levels. Differences in clinical manifestations, such as more prominent muscle wasting in DM1, may be related to CUGBP1 gain of function in DM1, which is absent in DM2. CUGBP1 gain of function may result from spliceopathy of transcripts regulated specifically by CUGBP1 but not by MBNL1.

Among hormones presently known to stimulate muscle anabolism, insulin-like growth factor-1 (IGF-1) has the strongest effect. Some hypothesize that decreased intracellular signaling by IGF-1 contributes to weakness or atrophy. For example, it can reverse age-related muscle wasting in animal models[25] and can ameliorate the degenerative effects of dystrophin deficiency in mice.[26] Furthermore, a preliminary study has suggested that recombinant human IGF-1 (rhIGF1) can improve muscle strength and function in adult patients with DM1.[27] The formulation of rhIGF1 used in the previous study has limitations, however. The preparation required twice-daily subcutaneous injections due to a short half-life. The short, circulating half-life of rhIGF1 may have limited the opportunity to observe a stronger therapeutic effect on skeletal muscle.

A longer acting preparation of rhIFG1 with fewer side effects has become available. The preparation is rhIGF1 complexed to recombinant human IGF binding protein-3 (rhIGFBP3). This novel compound is administered as a preformed complex as a once-daily subcutaneous injection, which can restore and maintain IGF-1 to physiologically relevant levels. IGF-1 serum concentrations are markedly increased by the rhIGF1:rhIGFBP3 complex (IPLEX; Insmed, Richmond, VA) over rhIGF1 therapy alone, and the incidence of hypoglycemia is much less. Preliminary studies in elderly women after hip fracture and type 2 diabetes indicated that rhIGF1:rhIGFBP3 is safe, well-tolerated, and effective.[28,28a] This suggests that rhIGF1:rhIGFBP3 is a good candidate for treatment of muscle atrophy in DM1.

The safety and tolerability of rhIGF1:rhIGFBP3 as a treatment for muscle wasting and weakness in DM1 patients is being tested in an open-label, dose escalation Phase II trial (NCT00233519 at http://www.clinicaltrials.gov). This study will also examine the effects on muscle mass and strength to obtain the data needed to plan a larger Phase II study of efficacy. This study is the first to examine the effects of rhIGF1:rhIGFBP3 complex in a human muscle disease. Preliminary results indicate that daily subcutaneous injection of rhIGF1:rhIGFBP3 is safe and well-tolerated by patients with DM1.[29] Based on these encouraging preliminary data, a randomized, double-blind, placebo-controlled, Phase II trial is now underway to evaluate tolerability, safety, and efficacy of rhIGF1:rhIGFBP3 in DM1 patients (NCT00577577 at http://www.clinicaltrials.gov).

Myostatin inhibition

Myostatin is a circulating protein produced and secreted by skeletal muscle that acts as a negative regulator of muscle growth and function. Mutations of myostatin lead to substantially increased muscle mass in cattle,[30] mice,[31] and humans,[32] and enhance athletic performance and muscle mass in whippet dogs.[33,34] Reduction of myostatin activity also has disease-modifying effects in muscular dystrophy. For example, loss of myostatin function, either by gene deletion or circulating antibodies against myostatin, increases muscle mass, improves function, and reduces dystrophic changes in a mouse model of dystrophin-deficient muscular dystrophy.[35,36] In addition, deacetylase inhibitors, which upregulate levels of the endogenous myostatin inhibitor follistatin, also improved muscle function in two forms of muscular dystrophy in mice.[37]

Myostatin is not known to be overactive in DM. Nonetheless, reduction of existing myostatin activity in DM1 may have beneficial effects on protein anabolism by acting through mechanisms independent of those that cause the disease. Inhibition of myostatin increases myofibrillar protein synthesis in adult muscle, enhances muscle regeneration by stimulating proliferation and differentiation of muscle precursor cells, and suppresses insulin resistance.[38–40] Defects of protein anabolism in DM1 may be related to one or more factors, including poor muscle maturation, impaired fiber regeneration, and insulin resistance, which suggests that myostatin inhibition may be a good approach for treatment of muscle wasting and weakness in these patients. Although a multicenter Phase I/II trial testing an antimyostatin antibody, MYO-029, failed to show efficacy in three types of adult muscular dystrophy, it was safe and well tolerated at the doses used.[41] No DM patients were included in the study, however. Due to the prominent and debilitating muscle wasting in DM1, a Phase I/II trial may be indicated to assess the potential of MYO-029 to reverse of muscle weakness and wasting in these patients.

Several additional approaches may be considered to reduce myostatin function in DM1, including deacetylase inhibitors,[37] blockade of myostatin receptors,[42] morpholino antisense oligonucleotides (Gene Tools, Philomath, OR) designed to block translation or skip exon 3 of myostatin[43] to produce a nonfunctional protein,[38] or viral overexpression of endogenous myostatin inhibitors.[44] A new mouse model of DM1 that features prominent muscle atrophy is now available[45] to test therapeutic strategies designed to reverse muscle wasting, including myostatin inhibition. Even if shown to be effective to reverse muscle wasting in DM, myostatin inhibition eventually would give way to a more specific treatment that targets the RNA-mediated disease mechanism.

Reduction of RNA-mediated toxicity

Reversal of spliceopathy: antisense oligonucleotide-induced exon skipping. Evidence now indicates that the physiological basis of myotonia in DM is due to dysfunction of the muscle-specific chloride channel ClC-1. Takahashi and Cannon[11] found that chloride conductance in muscle fibers from the HSA^{LR} transgenic mouse model of DM1 is dramatically reduced. This finding was correlated with identification of aberrant alternative splicing of the ClC-1 pre-mRNA and diminished

expression of chloride channel protein at the surface membrane in the HSA^{LR} transgenic mouse model and muscle biopsies from DM1 patients.[11] The splicing abnormality involves aberrant inclusion of exon 7a into the mature ClC-1 mRNA.[11,12] Inclusion of exon 7a leads to a premature termination codon downstream in exon 7, resulting in a truncated, poorly functional chloride channel protein. A recent study has confirmed ClC-1 channelopathy as responsible for development of myotonia in DM.[46]

The agents currently used to reduce myotonia typically provide only partial relief of myotonia in DM, probably because none act specifically to restore the ClC-1 function that is defective in DM. Direct targeting of the mechanism of myotonia is predicted to demonstrate greater efficacy of myotonia reduction. Recently this approach was tested in two mouse models of myotonic dystrophy: an antisense oligonucleotide (AON) was designed to skip the abnormally included exon of the muscle chloride channel, exon 7a.[47] By suppressing inclusion of exon 7a, the AON restored the reading frame and produced a normal ClC-1 mRNA. The end result is production of a full-length, fully functional chloride channel and elimination of myotonia for up to 8 weeks after a single treatment.

This was the first study to demonstrate that specific correction of spliceopathy by AONs can reverse phenotype in a DM1 model. The specificity, efficacy, and duration of effect suggest that this strategy could be a powerful method of myotonia reversal in DM. Unlike pharmacological agents such as mexiletine, which reduces myotonia indirectly by acting on the sodium channel and has disadvantages such as frequency of administration (TID) and the potential for cardiac complications, morpholino AONs are highly specific and effective for long-term myotonia reversal.

Although this approach is a powerful method to reduce myotonia in mouse models of DM, its use as a therapy in DM patients has limitations. First is delivery. To be effective in patients, the AONs would have to penetrate muscle tissue efficiently after systemic delivery. It is encouraging that systemic delivery of AONs already has proven effective to manipulate constitutive splicing in preclinical models of Duchenne muscular dystrophy (DMD).[48,49] AON-mediated exon skipping also has demonstrated safety and efficacy in DMD patients by intramuscular injection,[50] and a second Phase I/II study of morpholino AON-induced exon skipping in DMD patients is ongoing (NCT00159250 at http://www.clinicaltrials.gov). In dystrophin-deficient muscular dystrophy, the muscle tissue features segmental necrosis and leaky muscle membranes that enhance access of AONs to muscle fibers. In DM, however, the muscle fiber surface membrane is intact, greatly limiting uptake of AONs. To overcome an intact muscle surface membrane, application of AON-mediated exon skipping in DM will require use of special delivery moieties that enhance AON uptake in muscle fibers when delivered systemically. Two such moieties have shown early success in mouse models.[51,52]

A second limitation of this approach is that it targets only myotonia without addressing aspects of DM that result from spliceopathy of other transcripts. One possible solution includes combining multiple AONs designed to correct splicing of two or more transcripts and deliver as a cocktail. This strategy is being considered for treatment of DMD patients, in whom skipping of two or more exons is required to achieve an in-frame dystrophin transcript. Meaningful application of AON-mediated exon skipping for disease reversal in DM will depend on identifying additional aberrantly spliced exons that contribute to core features of the disease.

Upregulation of MBNL1 activity. Misregulated alternative splicing due to a loss of MBNL1 function or to an increase in CUGBP1 activity (or both) is recognized as the major consequence of RNA-mediated toxicity in DM.[6,8,9,12,53] Loss of MBNL1 protein function in DM is due to its depletion from the nucleoplasm by mutant RNA.[7] One strategy for reversing spliceopathy due to loss of MBNL1 function is to increase levels MBNL1 in affected tissue. This approach has been tested in a transgenic mouse model of DM1 by intramuscular injection with an adeno-associated virus (AAV) designed to overexpress MBNL1 protein.[54] In that study, a twofold upregulation of MBNL1 protein-saturated binding sites on CUG^{exp} RNA and restored localization of MBNL1 protein free in the nucleoplasm, effectively overcoming its sequestration by mutant RNA. The result was normalization of MBNL1 activity to a level sufficient to reverse myotonia and spliceopathy for up to several months after a single treatment. In a separate study, genetic overexpression of MBNL1 alleviated muscle degeneration in a fly model of DM1, providing further evidence that upregulation of MBNL1 activity can rescue phenotype due to CUG^{exp} RNA.[55]

AAV-mediated overexpression of MBNL1 may hold promise in reversing disease in DM patients; however, localized injection of AAV-MBNL1 is not practical for long-term clinical application. To be a viable therapy for DM patients, AAV-mediated overexpression of MBNL1 will require systemic delivery, such that muscle, and affected nonmuscle tissue can be treated throughout the body. Systemic AAV gene therapy has already proven very successful in restoring expression of a truncated dystrophin body-wide in skeletal muscle of preclinical models of DMD.[56] The feasibility of gene therapy for muscular dystrophy patients is being tested in separate, randomized, double-blind Phase I trials to investigate the safety and efficacy of AAV-mediated delivery of a mini-dystrophin gene in boys with DMD (NCT00428935 at

http://www.clinicaltrials.gov) and α-sarcoglycan in patients with limb girdle muscular dystrophy type 2D (NCT00494195 at http://www.clinicaltrials.gov) by intramuscular injection. If systemic gene therapy proves effective in preclinical models of DM, then Phase I trials can be designed to test safety and benefit in DM1 patients. Because of involvement of cortical neurons and anterior horn cells in DM1,[57,58] optimal delivery strategies for gene therapy in DM patients will include those that penetrate the CNS.

In DM2 patients, loss of MBNL1 function also leads to spliceopathy. Therefore, upregulation of MBNL1 activity by viral-mediated overexpression is predicted to show similar efficacy in DM2 as in DM1. Development of preclinical models of DM2 will allow testing of this hypothesis.

Downregulation of CUGBP1 activity. Steady-state CUGBP1 protein levels are increased in DM1 muscle,[9] but are normal in muscle from DM2 patients, despite spliceopathy of an almost identical subset of transcripts.[7] These observations, together with more recent evidence, suggest that elevated CUGBP1 activity may contribute to clinical features in DM1 that are distinct from DM2.

An exciting new transgenic mouse model that expresses 960 CUG repeats develops not only ribonuclear foci, MBNL1 sequestration, spliceopathy, and myotonia, but also important additional features of DM1 that are lacking in DM2: prominent muscle wasting, elevated CUGBP1 levels, and spliceopathy of newly identified transcripts that are regulated specifically by CUGBP1 but not by MBNL1.[45] A second new DM1 model that features cardiac-specific expression of CUG^{exp} RNA develops cardiac arrhythmias, cardiomyopathy, and spliceopathy that is associated with early elevation of CUGBP1 levels.[59] Together, these data suggest that overactivity of CUGBP1 may play an important role in the development of muscle wasting and/or cardiac disease in DM1, and that reducing CUGBP1 activity may be a strategy to ameliorate or even reverse these abnormalities.

Recently, CUGBP1 was shown to be hyperphosphorylated in DM1 by inappropriate activation of protein kinase C isoforms.[60] Hyperphosphorylation of CUGBP1 enhances its stability and accounts for the elevated steady-state levels in DM1.[60] To test the role of CUGBP1 hyperphosphorylation in development of muscle wasting and cardiac disease in DM1, these new transgenic models can be treated with protein kinase C inhibitors to downregulate, or prevent upregulation, of CUGBP1 activity by reducing its phosphorylation.

Neutralization or elimination of the mutant RNA. Clinical manifestations in both types of DM result from toxicity mediated directly by the mutant RNA. Some of the RNA-mediated toxicity is manifested as misregulated alternative splicing due to loss of MBNL1 function, gain of CUGBP1 activity, or both. It is not known, however, to what extent RNA toxicity in DM is the result of mechanisms distinct from spliceopathy. Therefore upregulation of MBNL1 function, downregulation of CUGBP1 activity, or both processes together may be insufficient to reverse all aspects of the disease.

An alternative, straightforward therapeutic strategy to reduce RNA toxicity in DM involves neutralization or elimination of the mutant RNA. Puymirat and colleagues[61] have tested the functional effects of CUG^{exp} RNA degradation by antisense gene therapy. In that study, human DM1 muscle cells in culture were transfected with a retrovirus expressing antisense RNA that was designed to bind and degrade the mutant *DMPK* allele. Expression of antisense RNA had the intended effect of preferential reduction of mutant *DMPK* RNA levels. In addition, muscle cell function was improved, manifested by more efficient differentiation and fusion, enhanced glucose uptake in response to insulin, and downregulation of CUGBP1 protein levels. In a separate study, the same research group also demonstrated that a ribozyme, a small catalytic RNA molecule capable of site-specific cleavage of RNA, can mediate degradation of mutant *DMPK* nuclear foci and partial resolution of insulin receptor splicing in DM1 myoblasts.[62]

Mahadevan et al.[63] provided further evidence that downregulation of CUG RNA can be sufficient to reverse the disease process by designing a transgenic mouse model of DM1 in which expression of CUG RNA could be turned on to precipitate development of disease manifestations and later turned off to follow resolution of the phenotype as levels of CUG RNA decreased. Nonviral approaches to reduce CUG^{exp} RNA include short interfering RNA (siRNA), which has demonstrated efficacy in DM1 fibroblasts.[64]

Together, these data support a role for degradation of the mutant RNA as a potential therapeutic strategy in DM1. Clinical application of these strategies necessitate development of efficient means of systemic delivery of viral-mediated antisense, siRNA, or both. High specificity for degradation of the mutant allele will be required to minimize nonspecific degradation of the normal allele and subsequent reduction of DMPK protein levels. Although these data are specific for DM1, similar methods can be tested for efficacy in DM2 cells to reduce $CCUG^{exp}$ RNA levels. In DM2, the concern for degradation of the normal *ZNF9* RNA by targeting $CCUG^{exp}$ RNA for degradation is low, because after RNA processing the *ZNF9* mRNAs from the normal and mutant allele are identical, due to removal of intron 1 that contains the mutant RNA.[65] The result is that the mutant RNA retained in DM2 nuclei consists entirely of $CCUG^{exp}$ RNA.

The presence of well-formed ribonuclear foci in DM may serve as convenient therapeutic targets for pharmacologic modification of RNA-mediated toxicity. Development of biochemical, genetic, or cell-based assays that

feature DM-like RNA toxicity will allow testing of this idea by high-throughput screening (HTS), in which hundreds or thousands of small molecule compounds are tested in parallel for their ability to initiate a desired outcome.[66] HTS of chemical libraries has identified several compounds that modify toxicity in cell and genetic models of polyglutamine disease.[67] The goal of high-throughput screening in DM is to facilitate the discovery of small molecules that modify RNA toxicity. Positive hits would include compounds that, for example, disrupt the mutant RNA-protein interaction by binding to CUG^{exp} or $CCUG^{exp}$ RNA, influence alternative splicing efficiencies, or downregulate total levels of mutant RNA.

In a recent report, screening of a chemical library consisting of 400 compounds identified 10 candidate drugs that modified RNA toxicity in a *Drosophila* model of DM1.[68] Screens of additional libraries are likely to identify new compounds that demonstrate similar effects. HTS of small libraries of biologically active compounds with low toxicity has been recommended as the most effective first step.[66] Considering that spliceopathy currently is recognized as the primary consequence of RNA toxicity in DM, a cell-based assay developed by Orengo et al.[69] that provides a quantitative readout of splicing outcomes, seems ideal for application of HTS to identify candidate drugs for DM. A new small molecule microarray platform that enables rapid screens for RNA–ligand interaction may facilitate identification of small molecules that bind to CUG^{exp} or $CCUG^{exp}$ RNA.[70]

CONCLUSION

Conventional approaches to treatment of DM are supportive and have failed to slow or halt disease progression. Now that it is clear that clinical manifestations of DM result directly from expression of a mutant expanded repeat RNA, the search for novel therapies for DM is underway. The ultimate goal for treatment of DM is reversal of the RNA-mediated toxicity. The mutant RNA itself and the pathways that lead to myotonia and abnormal alternative splicing in DM all represent therapeutic targets. Demonstration in animal models of DM1 that either antisense oligonucleotides or gene therapy can eliminate myotonia and spliceopathy, even after it is well established, is encouraging and suggests that other aspects of the disease such as weakness and muscle wasting may be reversible as well. The ideal approach for disease reversal probably involves neutralization or elimination of the mutant RNA transcripts. Once identified, candidate agents that demonstrate efficacy at reducing RNA toxicity in model systems should be extended to Phase I clinical trials. As development of targeted therapy continues to advance, DM may prove to be a highly treatable disorder.

REFERENCES

1. Harper PS. Myotonic dystrophy. 2nd ed. London, Philadelphia: W.B. Saunders, 1989.
2. Brook JD, McCurrach ME, Harley HG, et al. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member [Erratum in: Cell 1992;69:385]. Cell 1992;68:799–808.
3. Liquori CL, Ricker K, Moseley ML, et al. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of *ZNF9*. Science 2001;293:864–867.
4. Taneja KL, McCurrach M, Schalling M, Housman D, Singer RH. Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. J Cell Biol 1995;128:995–1002.
5. Miller JW, Urbinati CR, Teng-umnuay P, et al. Recruitment of human muscleblind proteins to $(CUG)_n$ expansions associated with myotonic dystrophy. EMBO J 2000;19:4439–4448.
6. Kanadia RN, Johnstone KA, Mankodi A, et al. A muscleblind knockout model for myotonic dystrophy. Science 2003;302:1978–1980.
7. Lin X, Miller JW, Mankodi A, et al. Failure of MBNL1-dependent post-natal splicing transitions in myotonic dystrophy. Hum Mol Genet 2006;15:2087–2097.
8. Philips AV, Timchenko LT, Cooper TA. Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. Science 1998;280:737–741.
9. Savkur RS, Philips AV, Cooper TA. Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. Nat Genet 2001;29:40–47.
10. Ho TH, Charlet-B N, Poulos MG, Singh G, Swanson MS, Cooper TA. Muscleblind proteins regulate alternative splicing. EMBO J 2004;23:3103–3112.
11. Mankodi A, Takahashi MP, Jiang H, et al. Expanded CUG repeats trigger aberrant splicing of ClC-1 chloride channel pre-mRNA and hyperexcitability of skeletal muscle in myotonic dystrophy. Mol Cell 2002;10:35–44.
12. Charlet-B N, Savkur RS, Singh G, Philips AV, Grice EA, Cooper TA. Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. Mol Cell 2002;10:45–53.
13. Savkur RS, Philips AV, Cooper TA, et al. Insulin receptor splicing alteration in myotonic dystrophy type 2. Am J Hum Genet 2004;74:1309–1313.
14. Osborne RJ, Thornton CA. RNA-dominant diseases. Hum Mol Genet 2006;15 Spec No 2:R162–R169.
15. Trip J, Drost G, van Engelen BG, Faber CG. Drug treatment for myotonia. Cochrane Database Syst Rev 2006;(1):CD004762.
16. Martens WB, Moxley RT 3rd, Logigian EL, et al. Mexiletine: effective antimyotonia treatment in myotonic dystrophy type 1. Neurology 2005;64:A413 (abstract).
17. Logigian EL, Ciafaloni E, Quinn LC, et al. Severity, type, and distribution of myotonic discharges are different in type 1 and type 2 myotonic dystrophy. Muscle Nerve 2007;35:479–485.
18. Halliday D, Ford GC, Edwards RH, Rennie MJ, Griggs RC. In vivo estimation of muscle protein synthesis in myotonic dystrophy. Ann Neurol 1985;17:65–69.
19. Griggs RC, Jozefowicz R, Kingston W, Nair KS, Herr BE, Halliday D. Mechanism of muscle wasting in myotonic dystrophy. Ann Neurol 1990;27:505–512.
20. Griggs RC, Pandya S, Florence JM, et al. Randomized controlled trial of testosterone in myotonic dystrophy. Neurology 1989;39:219–222.
21. Walter MC, Reilich P, Lochmüller H, et al. Creatine monohydrate in myotonic dystrophy: a double-blind, placebo-controlled clinical study. J Neurol 2002;249:1717–1722.
22. Schneider-Gold C, Beck M, Wessig C, et al. Creatine monohydrate in DM2/PROMM: a double-blind placebo-controlled clinical study. Neurology 2003;60:500–502.
23. Tarnopolsky M, Mahoney D, Thompson T, Naylor H, Doherty TJ. Creatine monohydrate supplementation does not increase muscle strength, lean body mass, or muscle phosphocreatine in patients with myotonic dystrophy type 1. Muscle Nerve 2004;29:51–58.

24. Sugino M, Ohsawa N, Ito T, et al. A pilot study of dehydroepiandrosterone sulfate in myotonic dystrophy. Neurology 1998;51: 586–589.
25. Musarò A, McCullagh K, Paul A, et al. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nat Genet 2001;27:195–200.
26. Barton ER, Morris L, Musaro A, Rosenthal N, Sweeney HL. Muscle-specific expression of insulin-like growth factor I counters muscle decline in *mdx* mice. J Cell Biol 2002;157:137–148.
27. Vlachopapadopoulou E, Zachwieja JJ, Gertner JM, et al. Metabolic and clinical response to recombinant human insulin-like growth factor I in myotonic dystrophy: a clinical research center study. J Clin Endocrinol Metab 1995;80:3715–3723.
28. Boonen S, Rosen C, Bouillon R, et al. Musculoskeletal effects of the recombinant human IGF-I/IGF binding protein-3 complex in osteoporotic patients with proximal femoral fracture: a double-blind, placebo-controlled pilot study. J Clin Endocrinol Metab 2002;87:1593–1599.

28a. Clemmons DR, Moses AC, Sommer A, et al. Rh/IGF-I/rhIGFBP-3 administration to patients with type 2 diabetes mellitus reduces insulin requirements while also lowering fasting glucose. Growth Horm IGF Res 2005;15:265–274.

29. Heatwole CR, Martens W, Quinn C, et al. Effects of IPLEX in myotonic dystrophy type 1. Neurology 2007;68:A404–A405 (abstract).
30. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. Proc Natl Acad Sci U S A 1997;94:12457–12461.
31. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature 1997; 387:83–90.
32. Schuelke M, Wagner KR, Stolz LE, et al. Myostatin mutation associated with gross muscle hypertrophy in a child. N Engl J Med 2004;350:2682–2688.
33. Mosher DS, Quignon P, Bustamante CD, et al. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. PLoS Genet 2007;3:e79.
34. Shelton GD, Engvall E. Gross muscle hypertrophy in whippet dogs is caused by a mutation in the myostatin gene. Neuromuscul Disord 2007;17:721–722.
35. Wagner KR, McPherron AC, Winik N, Lee SJ. Loss of myostatin attenuates severity of muscular dystrophy in *mdx* mice. Ann Neurol 2002;52:832–836.
36. Bogdanovich S, Krag TO, Barton ER, et al. Functional improvement of dystrophic muscle by myostatin blockade. Nature 2002; 420:418–421.
37. Minetti GC, Colussi C, Adami R, et al. Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. Nat Med 2006;12:1147–1150.
38. Welle S, Bhatt K, Pinkert CA. Myofibrillar protein synthesis in myostatin-deficient mice. Am J Physiol Endocrinol Metab 2006; 290:E409–E415.
39. Lee SJ. Regulation of muscle mass by myostatin. Annu Rev Cell Dev Biol 2004;20:61–86.
40. McPherron AC, Lee SJ. Suppression of body fat accumulation in myostatin-deficient mice. J Clin Invest 2002;109:595–601.
41. Wagner KR, Fleckenstein JL, Amato AA, et al. A phase I/II trial of MYO-029 in adult subjects with muscular dystrophy. Ann Neurol 2008;63:561–571.
42. Lachey J, Pullen A, Pearsall R, Seehra J. Novel myostatin inhibitors increase muscle mass in wild-type and *mdx* mice. Neuromuscul Disord 2007;17:785 (abstract).
43. Morcos PA. Achieving targeted and quantifiable alteration of mRNA splicing with Morpholino oligos. Biochem Biophys Res Commun 2007;358:521–527.
44. Haidet AM, Rizo L, Handy C, et al. Long-term enhancement of skeletal muscle mass and strength by single gene administration of myostatin inhibitors. Proc Natl Acad Sci U S A 2008;105:4318–4322.
45. Orengo JP, Chambon P, Metzger D, Mosier DR, Snipes GJ, Cooper TA. Expanded CTG repeats within the DMPK 3′ UTR causes severe skeletal muscle wasting in an inducible mouse model for myotonic dystrophy. Proc Natl Acad Sci U S A 2008;105: 2646–2651.
46. Lueck JD, Mankodi A, Swanson MS, Thornton CA, Dirksen RT. Muscle chloride channel dysfunction in two mouse models of myotonic dystrophy. J Gen Physiol 2007;129:79–94.
47. Wheeler TM, Lueck JD, Swanson MS, Dirksen RT, Thornton CA. Correction of ClC-1 splicing eliminates chloride channelopathy and myotonia in mouse models of myotonic dystrophy. J Clin Invest 2007;117:3952–3957.
48. Lu QL, Rabinowitz A, Chen YC, et al. Systemic delivery of antisense oligoribonucleotide restores dystrophin expression in body-wide skeletal muscles. Proc Natl Acad Sci U S A 2005;102: 198–203.
49. Alter J, Lou F, Rabinowitz A, et al. Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. Nat Med 2006;12:175–177.
50. van Deutekom JC, Janson AA, Ginjaar IB, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. N Engl J Med 2007;357:2677–2686.
51. Li YF, Morcos PA. Design and synthesis of dendritic molecular transporter that achieves efficient in vivo delivery of morpholino antisense oligo. Bioconjug Chem 2008;19:1464–1470.
52. Jearawiriyapaisarn N, Moulton HM, Buckley B, et al. Sustained dystrophin expression induced by peptide-conjugated morpholino oligomers in the muscles of *mdx* mice. Mol Ther 2008;16:1624–1629.
53. Dansithong W, Paul S, Comai L, Reddy S. MBNL1 is the primary determinant of focus formation and aberrant insulin receptor splicing in DM1 [Erratum in: J Biol Chem 2005;280:20176]. J Biol Chem 2005;280:5773–5780.
54. Kanadia RN, Shin J, Yuan Y, et al. Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly-(CUG) model for myotonic dystrophy. Proc Natl Acad Sci U S A 2006;103:11748–11753.
55. de Haro M, Al-Ramahi I, De Gouyon B, et al. MBNL1 and CUGBP1 modify expanded CUG-induced toxicity in a *Drosophila* model of myotonic dystrophy type 1. Hum Mol Genet 2006;15: 2138–2145.
56. Gregorevic P, Blankinship MJ, Allen JM, et al. Systemic delivery of genes to striated muscles using adeno-associated viral vectors. Nat Med 2004;10:828–834.
57. Jiang H, Mankodi A, Swanson MS, Moxley RT, Thornton CA. Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons. Hum Mol Genet 2004;13: 3079–3088.
58. Wheeler TM, Krym MC, Thornton CA. Ribonuclear foci at the neuromuscular junction in myotonic dystrophy type 1. Neuromuscul Disord 2007;17:242–247.
59. Wang GS, Kearney DL, De Biasi M, Taffet G, Cooper TA. Elevation of RNA-binding protein CUGBP1 is an early event in an inducible heart-specific mouse model of myotonic dystrophy. J Clin Invest 2007;117:2802–2811.
60. Kuyumcu-Martinez NM, Wang GS, Cooper TA. Increased steady-state levels of CUGBP1 in myotonic dystrophy 1 are due to PKC-mediated hyperphosphorylation. Mol Cell 2007;28:68–78.
61. Furling D, Doucet G, Langlois MA, et al. Viral vector producing antisense RNA restores myotonic dystrophy myoblast functions. Gene Ther 2003;10:795–802.
62. Langlois MA, Lee NS, Rossi JJ, Puymirat J. Hammerhead ribozyme-mediated destruction of nuclear foci in myotonic dystrophy myoblasts. Mol Ther 2003;7:670–680.
63. Mahadevan MS, Yadava RS, Yu Q, et al. Reversible model of RNA toxicity and cardiac conduction defects in myotonic dystrophy. Nat Genet 2006;38:1066–1070.
64. Krol J, Fiszer A, Mykowska A, Sobczak K, de Mezer M, Krzyzosiak WJ. Ribonuclease dicer cleaves triplet repeat hairpins into shorter repeats that silence specific targets. Mol Cell 2007;25:575–586.
65. Margolis JM, Schoser BG, Moseley ML, Day JW, Ranum LP. DM2 intronic expansions: evidence for CCUG accumulation without flanking sequence or effects on *ZNF9* mRNA processing or protein expression. Hum Mol Genet 2006;15:1808–1815.

66. Jones JO, Diamond MI. Design and implementation of cell-based assays to model human disease. ACS Chem Biol 2007; 2:718–724.
67. Desai UA, Pallos J, Ma AA, et al. Biologically active molecules that reduce polyglutamine aggregation and toxicity. Hum Mol Genet 2006;15:2114–2124.
68. Garcia-Lopez A, Monferrer L, Garcia-Alcover I, Vicente-Crespo M, Alvarez-Abril MC, Artero RD. Genetic and chemical modifiers of a CUG toxicity model in *Drosophila*. PLoS ONE 2008;3:e1595.
69. Orengo JP, Bundman D, Cooper TA. A bichromatic fluorescent reporter for cell-based screens of alternative splicing. Nucleic Acids Res 2006;34:e148.
70. Childs-Disney JL, Wu M, Pushechnikov A, Aminova O, Disney MD. A small molecule microarray platform to select RNA internal loop–ligand interactions. ACS Chem Biol 2007;2:745–754.