

STATE-OF-THE-ART REVIEW

Cardiovascular Disease in Duchenne Muscular Dystrophy

Overview and Insight Into Novel Therapeutic Targets



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HIGHLIGHTS

- **Cardiomyopathy is the leading cause of death in patients with DMD.**
- **DMD has no cure, and there is no current consensus for treatment of DMD cardiomyopathy.**
- **This review discusses therapeutic strategies to potentially reduce or prevent cardiac dysfunction in DMD patients.**
- **Additional studies are needed to firmly establish optimal treatment modalities for DMD cardiomyopathy.**

SUMMARY

Duchenne muscular dystrophy (DMD) is a devastating disease affecting approximately 1 in every 3,500 male births worldwide. Multiple mutations in the dystrophin gene have been implicated as underlying causes of DMD. However, there remains no cure for patients with DMD, and cardiomyopathy has become the most common cause of death in the affected population. Extensive research is under way investigating molecular mechanisms that highlight potential therapeutic targets for the development of pharmacotherapy for DMD cardiomyopathy. In this paper, the authors perform a literature review reporting on recent ongoing efforts to identify novel therapeutic strategies to reduce, prevent, or reverse progression of cardiac dysfunction in DMD. (J Am Coll Cardiol Basic Trans Science 2022;7:608-625) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Duchenne muscular dystrophy (DMD) is a progressive myopathic disorder caused by a recessive mutation in the dystrophin gene on the X chromosome. DMD affects roughly 1.3 to 2.1 per 10,000 live male births.¹ This devastating disease results in severe clinical symptoms. Progressive muscle weakness typically begins to affect young boys around the age of 2 or 3 years, eventually becoming wheelchair bound by the age of 12.

Orthopedic fractures commonly occur mainly due to frequent falls. Scoliosis often manifests in patients with DMD, increasing the possibility of respiratory failure.^{2,3} Severity of symptoms primarily correlate with the quantity of dystrophin in the muscle ranging from absent/minimal dystrophin to higher levels; the less dystrophin, the more severe the phenotype.⁴

Dystrophin, part of a large glycoprotein complex, is located on the cytoplasmic side of the plasma

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membrane of muscle fibers.¹ As part of this complex, known as the dystrophin-associated protein complex (DPC), dystrophin functions as mechanical reinforcement to the sarcolemma and to withstand contraction-induced injury, while also stabilizing the DPC, preventing its degradation. In the absence of dystrophin, the DPC degrades, resulting in several downstream detrimental effects including weakening of the sarcolemmal membrane, loss of membrane proteins, disrupted calcium homeostasis, up-regulation of inflammatory factors, and mitochondrial dysfunction, ultimately leading to degeneration of muscle fibers, necrosis, and cardiac fibrosis¹ (Figure 1).

With advances in respiratory and other therapies, the leading cause of death in the current era for DMD patients is cardiovascular disease (Central Illustration).^{2,5,6} DMD is associated with dilated cardiomyopathy and rhythm abnormalities, mainly supraventricular arrhythmias. The dilated cardiomyopathy seen in these patients is characterized by widespread fibrosis of the left ventricular (LV) free wall.⁷ Heart failure (HF) and arrhythmias will eventually develop as the disease progresses. Therefore, early diagnosis and management of cardiovascular disease is critical for the survival and/or improved quality of life for these patients. In addition, accumulating evidence over the last 2 decades has indicated that female carriers of DMD mutations are also at increased risk of developing cardiac disease.⁸⁻¹⁰

DMD is a devastating disease with early fatality. Current treatment options for cardiac management are aimed primarily at delaying development and progression of HF. Despite extensive research, there remains no cure. In this report, we review emerging potential therapeutic targets to reduce or prevent cardiac dysfunction in DMD (Table 1).

NONCARDIAC THERAPEUTIC STRATEGIES

Over the past several decades, several therapeutic advances have led to improved survival and quality of life for DMD patients. Long-term corticosteroids have been the mainstay of medical therapy over the past several decades. There is clear benefit to skeletal muscle function with early glucocorticoid use, depending on the type and frequency of dosing, evidenced by delay of loss of ambulation by an average of 1 to 3 years.¹¹ The cardiac benefits of chronic steroid use are less well-established. Acute glucocorticoid administration activates endothelial nitric oxide synthase and has potent anti-inflammatory and vasodilatory effects.^{12,13} Chronic use in DMD patients, particularly with deflazacort, has been associated

with longer preservation of LV systolic function.¹⁴ However, chronic steroid use also can increase the risk of obesity, dyslipidemia, hypertension, adrenal insufficiency, and osteopenia.^{15,16} There is also some evidence that pulsed corticosteroid regimens maintain many of the benefits of chronic use with less effects on bone density or adrenal function.¹⁷

Nonpharmacologic therapies have also improved morbidity and mortality for DMD patients. Impaired airway clearance increases risk for pneumonia and other respiratory infections that may accelerate the need for invasive ventilatory support. Cough assist devices, usually initiated when peak cough expiratory flow is <160 to 240 L/min, help to improve airway secretion clearance and delay initiation of invasive ventilation.¹⁸ Nocturnal noninvasive ventilatory (NIV) strategies, such as bilevel positive airway pressure ventilation, have had a tremendous effect on the care of this population. NIV improves survival compared with hypoventilatory DMD patients (RR: 0.62, 95% CI: 0.42 to 0.91; $P = 0.01$) and may delay need for chronic invasive ventilatory support.¹⁹ There may also be cardioprotective effects of NIV, with 1 study of DMD patients on long-term NIV (5 years), showing similar rates of cardiac events (acute HF, cardiac arrhythmia, and ischemic stroke) to patients on invasive ventilation and no difference between those with or without angiotensin-converting enzyme (ACE) inhibitor treatment.²⁰ Scoliosis occurs in up to 90% of untreated DMD boys as they become nonambulatory due to muscle weakness and pelvic imbalance.²¹ As scoliosis progresses, geometric changes in the thoracic cage can compromise cardiopulmonary function, including reducing lung capacity, increased risk of pulmonary infection, and direct cardiac impingement.²¹ Early spinal stabilization can reduce the incidence of surgical complications; however, the long-term benefits on cardiopulmonary function have been mixed.^{22,23}

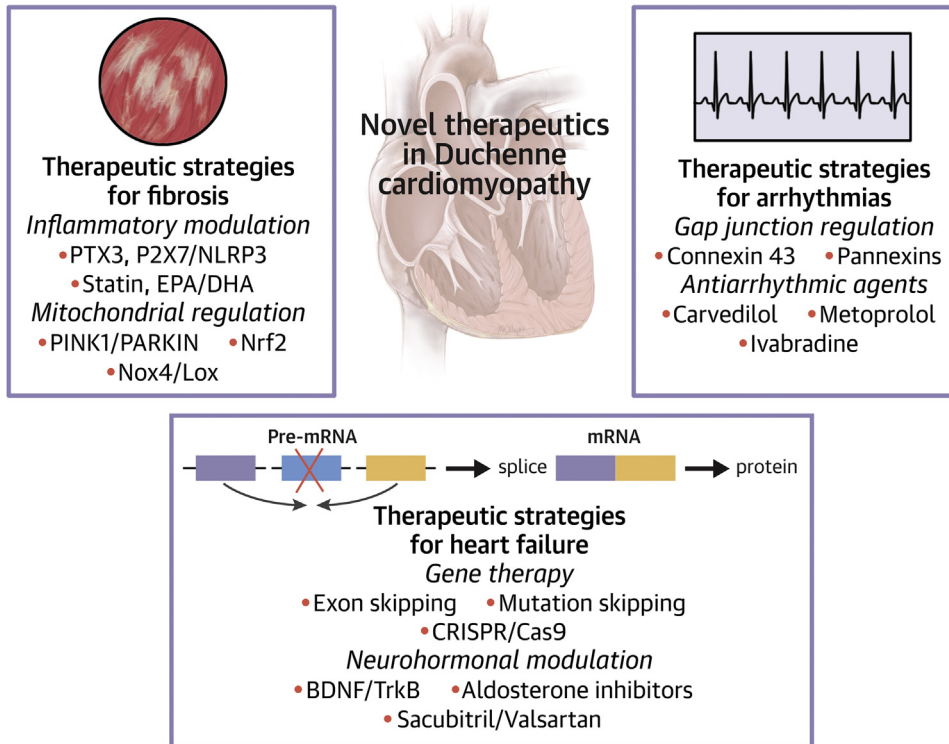
CARDIAC CHARACTERISTICS OF MOUSE MODELS OF DMD

Many of the preclinical studies discussed in this review have been performed in murine models of DMD. Although several mouse models have been developed, none of the current models has been able to

ABBREVIATIONS AND ACRONYMS

ACE	= angiotensin-converting enzyme
ApN	= adiponectin
ARB	= angiotensin receptor blocker
BB	= beta-blocker
BDNF	= brain-derived neurotrophic factor
CMR	= cardiac magnetic resonance imaging
Cx	= connexin
DMD	= Duchenne muscular dystrophy
DPC	= dystrophin-associated protein complex
FFA	= free fatty acid
HF	= heart failure
LNP	= lipid nanoparticle
LV	= left ventricular
LVEF	= left ventricular ejection fraction
miR	= microRNA
NIV	= noninvasive ventilation
Nrf2	= nuclear factor erythroid 2-related factor 2
PKA	= protein kinase A
PTX3	= pentraxin 3
Px	= pannexin
RNP	= ribonucleoprotein complexes
RT-qPCR	= reverse transcription-quantitative polymerase chain reaction
RyR2	= ryanodine receptor isoform 2
sgRNA	= single guide RNA
SR	= sarcoplasmic reticulum
TrkB	= tyrosine kinase B
TRPV2	= transient receptor potential cation channel, subfamily V, member 2

CENTRAL ILLUSTRATION Cardiomyopathy in Duchenne Muscular Dystrophy: Potential Therapeutic Targets



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Duchenne muscular dystrophy (DMD) has several systemic effects, including cardiomyopathy. DMD cardiomyopathy is characterized by cardiac fibrosis, arrhythmias, and heart failure. Inflammatory modulation and mitochondrial regulation could reduce cardiac fibrosis associated with DMD. In addition, gap junction regulation and therapy with antiarrhythmic agents could reduce incidence of arrhythmia in DMD. Furthermore, gene therapy and neurohormonal modulation could be beneficial in reducing heart failure in DMD. BDNF = brain-derived neurotrophic factor; Cas9 = CRISPR associated protein 9; CRISPR = clustered regularly interspaced short palindromic repeats; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; Lox = lysyl oxidase; NLRP3 = NOD-LRR-and pyrin domain-containing protein3; Nox4 = NADPH oxidase 4; Nrf2 = nuclear factor-erythroid factor 2-related factor; PARKIN = E3 ubiquitin ligase; PINK1 = PTEN-induced kinase; PTX3 = pentraxin 3; TrkB = tropomyosin receptor kinase B.

completely imitate the natural history of disease progression seen in humans (Table 2). Cardiac phenotypes can vary substantially, and care should be taken when selecting a model or interpreting results depending on the variables being assessed in the study. The original murine DMD model, C57BL/10ScSn-Dmdmdx/J (henceforth mdx), does not accurately recapitulate the cardiac phenotype seen in human DMD patients with fibrosis and dysfunction occurring very late, if at all.²⁴ The inbred D2.B10-Dmdmdx/J (D2mdx) strain has an accelerated cardiac phenotype with early fibrosis and mild-to-moderate dysfunction by echocardiography typically peaking at approximately 6 months.^{25,26} However, there is evidence that there is some

recovery that occurs in older animals as well as an increase in fibrosis seen in older wild-type (DBA/2J) control animals,²⁷ making it potentially less reliable for longer-term studies of cardiac function. Dystrophin/telomerase RNA double deficient (mdx/mTR) models have a severe cardiac phenotype including fibrosis, mitochondrial fragmentation, and development of dilated cardiomyopathy.^{28,29} Unlike most other murine models, and similar to human DMD patients, early death from HF is common.

GENE THERAPY

An ideal, potentially curative therapy for DMD in patients would be to correct the genetic defect in the

TABLE 1 Current Experimental Investigations for DMD Cardiomyopathy

Drug/Class	Target/Mechanism	Stage of Development	Advantages	Disadvantages
Exon skipping gene therapy				
Casimersen	DMD exon 45	FDA approved under accelerated review	Specifically targets the causative defect, produces at least somewhat functional dystrophins	Require regular infusions, only available for specific mutations, cardiac benefits unclear
Eteplirsen	DMD exon 51	FDA approved		
Golodirsen	DMD exon 53	FDA approved under accelerated review		
Vitolarsen	DMD exon 53	FDA approved		
Nonsense mutation suppression				
Ataluren	Release factor inhibition	Approved in EU, orphan status with FDA	Specifically targets the causative defect, produces at least somewhat functional dystrophin	Only effective for patients with a relatively small subset of DMD mutation types (nonsense)
G418 sulfate	Binds 80s ribosome, increased near-cognate tRNA mispairing	Preclinical for DMD		
Direct CRISP/Cas9 editing	Specific mutation correction	Preclinical for DMD	Potentially curative	May result in permanent side effects
KT5720	Selective PKA inhibition	Preclinical for DMD		
Tranilast	TRPV2 inhibition	Phase 1 and 2 clinical investigation	Side-effect profile known through its use as antiallergic medication	
Gap19	Cx43 hemichannel-specific inhibition	Preclinical for DMD		
Gap26	Cx43 gap junction channel-specific inhibition	Preclinical for DMD		
Probenecid	Px channel inhibition, TRPV2 agonist, inhibits renal tubular urate resorption	FDA approved for gout, Phase 2 investigation for HF	Well-established safety profile	Several potential mechanisms, may be less effective than more specific agents
Aldosterone inhibitors	Aldosterone inhibition, Px channel inhibition (?)	Phase 3 clinical investigation, spironolactone FDA approved for HF	Evidence of improvement in subclinical HF in DMD population	Mild diuretic effect, risk of hyperkalemia and gynecomastia
ACEI inhibitors	Inhibit Ang II formation and bradykinin metabolism	FDA approved for HF		Risk of angioedema and chronic cough, hypotension and hyperkalemia
Angiotensin receptor blockers	Competitive inhibition of Ang II binding to the angiotensin 1 receptor	FDA approved for HF	Less angioedema and cough than ACE inhibitors	Potentially increased risk of hypotension and hyperkalemia compared to ACE inhibitors
β -blockers	Nonselective or selective inhibition of β adrenergic receptors	FDA approved for HF and arrhythmia		Risk of hypotension and bradycardia
Sacubitril	Neprilysin inhibition	FDA approved for HF	May be superior to ACE inhibitors in reducing risk hospitalization and death in symptomatic HF	Incidence of hypotension and hyperkalemia may be more common than with ACE inhibitors
Eicosapentaenoic acid, docosahexaenoic acid	Inflammatory pathway inhibition	FDA approved for risk reduction in major cardiovascular events	Minimal side effect profile, may improve lipid profile in patients with concomitant dyslipidemia	
Zidovudine	Reverse transcriptase inhibition, P2X7 receptor antagonism	FDA approved for HIV, preclinical for DMD/HF		Reports of cardiomyopathy and myopathy (particularly at higher doses), class 2B carcinogenic risk
Ivabradine	I _f inhibition	FDA approved for HF	Improves outcomes in symptomatic HF with reduced LVEF and persistent heart rate ≥ 70 beats/min, HR reduction with low risk of hypotension	Risk of bradycardia and/or atrial fibrillation
Sulforaphane	Nrf2-mediated TGF- β /Smad signaling, NLRP3 inhibition (?)	Preclinical for DMD, Phase 1 for other indications		

ACE = angiotensin-converting enzyme; DMD = Duchenne muscular dystrophy; EU = European Union; FDA = Food and Drug Administration; HF = heart failure; LVEF = left ventricular ejection fraction.

affected cells, leading to functional dystrophin protein and preventing or greatly mitigating symptomatic involvement. However, there are currently several hurdles to achieving this goal. First, dystrophin is the largest known human gene, with 79 exons and roughly 2,200 kb, which makes packaging the gene into traditional viral vectors challenging. Additionally, the size of the gene also leads to hundreds of documented mutations of various types, including deletions, duplications, and missense mutations.^{30,31} DMD typically results from a deletion of more than 1 exon causing a premature stop codon, which in turn leads to a lack of dystrophin.³² Because cardiac dysfunction associated with DMD is quite variable, studies have investigated whether the underlying genetic mutations that cause DMD could predict the severity of cardiac dysfunction in DMD patients. Studies have suggested that dystrophin gene mutations in exons 48-49 have been associated with earlier onset of cardiomyopathy whereas mutations in exons 51-52 could be cardioprotective.^{33,34} However, the genotype/phenotype correlation is also complicated by emerging evidence that polymorphic variation in other genes, such as β 2-adrenergic receptors,³⁵ secreted phosphoprotein 1,³⁶ and brain-derived neurotrophic factor (BDNF),³⁷ may affect functional, respiratory, and cardiac outcomes. Timing of initiation of therapy is also an important consideration, because the primary benefit is in preventing functional decline and not with recovery of lost functional parameters, which may limit the utility in older patients. Despite these hurdles, there are several promising gene therapy strategies that have received approval for use in patients.

EXON SKIPPING. Exon skipping involves using specific synthetic antisense oligonucleotides vector-targeted to skip out-of-frame mutations at the level of the messenger RNA. This results in re-establishment of the correct reading frame and the generation of functional micro- or mini-dystrophins. An estimated 80% of all DMD mutations are potentially amenable to exon skipping. It is important to note that these truncated dystrophins are still abnormal proteins, and functional dystrophin expression in treated patients is estimated at approximately 0.3% of normal levels, and as such, it is not necessarily a curative therapy.³⁸⁻⁴⁰ The most common regions in the DMD gene for mutations to occur are between exons 45-55, with the deletion of exon 50 being most frequent.⁴¹ Clinically, there are currently 4 exon skipping therapies approved for human use: eteplirsen (exon 51), casimersen (exon 45), golodirsen (exon 53), and viltolarsen (exon 53).

Approximately 30% of DMD patients have mutations that would be amenable to treatment with 1 of these drugs. Although initial data regarding skeletal muscle function are encouraging for these therapies, the cardiac benefits require ongoing evaluation. To this end, it is not a foregone conclusion that skeletal muscle benefit will extend to cardiac muscle. Pre-clinical studies in DMD mouse models have had mixed results in terms of expression of microdystrophins in cardiac tissue.^{42,43}

NONSENSE MUTATION SUPPRESSION. Up to 25% of DMD patients have mutations resulting in premature stop codons. DMD mutations that result in premature stop codons may be targeted using small molecule nonsense suppressors, also known as translational read-through-inducing drugs, which attempt to bypass the premature stop codon and produce functional dystrophin. The 2 most well-characterized translational read-through-inducing drugs are ataluren (currently approved for use in DMD patients in Europe) and the aminoglycoside G418. Ataluren acts by inhibiting release factor activity and thus preventing the termination of translation when a stop codon is encountered.⁴⁴ By contrast, G418 binds tightly to the ribosome and increases functional near-cognate tRNA (single nucleotide) mispairing with the premature stop codon, resulting in continuation of translation past the stop codon.⁴⁴ The long-term cardiovascular effects of these medications remain unexplored, however, and further data will be necessary as their use becomes more widespread.

CRISPR/CAS9. Clustered regularly interspaced short palindromic repeats/CRISPR-associated 9 (CRISPR/Cas9) has emerged as a powerful genome editing tool that relies on delivery of ribonucleoprotein complexes (RNPs). There have been reported delivery options into cells such as DNA nanoclews,⁴⁵ cationic lipid nanoparticles (LNPs),⁴⁶⁻⁴⁸ lipoplexes, gold-based nanoparticles,⁴⁹⁻⁵¹ and zeolitic imidazole frameworks.⁵² Despite the various approaches there are still numerous problems that present themselves such as controlling the size, uniformity, and stability of the formulations, limiting in vivo application of CRISPR-Cas9 technology. One study was completed to further investigate a more applicable delivery system of CRISPR-Cas9.⁵³ The investigators successfully developed an approach designed to preserve RNP integrity through inclusion of a permanently cationic lipid in ionized LNP formulation. This was applicable to different classes of LNPs such as dendrimer LNPs, stable nucleic acid lipid particles, and lipid-like nanoparticles. Furthermore, they successfully demonstrated that modified dendrimer LNPs

TABLE 2 Cardiac and Noncardiac Phenotypes of Established DMD Mouse Models

First Author, Year, Ref. #	Model	Mutation	Background Strain	Lifespan	Histopathologic Changes (Onset)
Dystrophin-deficient models					
Bulfield et al, 1984 ²⁴	Mdx	Exon 23 point mutation	C57BL/10	2 y	Mild (≥ 10 mo)
Krivov et al, 2009 ¹³⁰	Albino Mdx	Exon 23 point mutation	Albino	2 y	Mild (≥ 10 mo)
Schmidt et al, 2011 ¹³¹	Mdx/BALB/c	Exon 23 point mutation	BALB/c		
Duan et al, unpublished data	Mdx/BL6	Exon 23 point mutation	C57BL/6		
Schmidt et al, 2011 ¹³¹	Mdx/C3H	Exon 23 point mutation	C3H		
Fukada et al, 2010 ²⁵	Mdx/DBA2	Exon 23 point mutation	DBA2	1.5-2 y	Severe (≥ 8 wk)
Wasala et al, 2015 ¹³²	Mdx/FVB	Exon 23 point mutation	FVB		
Chapman et al, 1989 ¹³³	Mdx2cv	Intron 42 point mutation	C57BL/6	2 y	Mild
Chapman et al, 1989 ¹³³	Mdx3cv	Intron 65 point mutation	C57BL/6	2 y	
Chapman et al, 1989 ¹³³	Mdx4cv	Exon 53 point mutation	C57BL/6	2 y	
Chapman et al, 1989 ¹³⁴	Mdx5cv	Exon 10 point mutation	C57BL/6	2 y	None
Araki et al, 1997 ¹³⁴	Mdx52	Exon 52 deletion	C57BL/6	2 y	
Wertz and Füchtbauer, 1998 ¹³⁶	Mdx β geo	Insertion of gene trap vector (ROSA β geo) in exon 63 along with LacZ reporter	C57BL/10	2 y	Mild (≥ 10 mo)
Kudoh et al, 2005 ¹³⁷	DMD-null	Cre-loxP mediated deletion of entire DMD gene		2 y	None
Double knockout models					
Guo et al, 2006 ¹³⁷	mdx/ $\alpha 7^{-/-}$	$\alpha 7$ -Integrin/dystrophin double deficient	mdx	≤ 4 wk	Mild (≥ 20 days)
Deconinck et al, 1997 ¹³⁸	Mdx/Utr $^{-/-}$ (Deconinck strain)	Utrophin/dystrophin double deficient	mdx	20 wk	Moderate (≥ 8 wk)
Grady et al, 1997 ¹³⁹	Mdx/Utr $^{-/-}$ (Grady strain)	Utrophin/dystrophin double deficient	mdx	20 wk	Moderate (≥ 8 wk)
Megeney et al, 1996 ¹⁴⁰	Mdx/Myod1	MyoD/dystrophin double deficient		12 mo	Severe (≥ 5 mo)
Chandrasekharan et al, 2010 ¹⁴¹	Mdx/Cmah	Cmah/dystrophin double deficient		11 mo	Moderate/severe (≥ 3 mo)
Sacco et al, 2010 ²⁸	Mdx/mTR ^{G2}	Telomerase RNA/dystrophin double deficient	mdx/BL6	4-18 mo	Severe (≥ 32 wk)
Sacco et al, 2010 ²⁸ , Mourkioti et al, 2013 ²⁹	Mdx ^{4cv} /mTR ^{G2}	Telomerase RNA/dystrophin double deficient	mdx4cv	4-18 mo	Severe (≥ 32 wk)
Grady et al, 1999 ¹⁴²	Mdx/Dtna $^{-/-}$	α -Dystrobrevin/dystrophin double deficient		8-10 mo	Moderate/severe (≥ 4 wk)
Li et al, 2009 ¹⁴³	Mdx/Sgcd $^{-/-}$	δ -Sarcoglycan/dystrophin double deficient	mdx/BL6	10-14 mo	Moderate/severe (≥ 8 wk)

DCM = dilated cardiomyopathy; ECG = electrocardiogram; HCM = hypertrophic cardiomyopathy; LV = left ventricular.

Continued on the next page

TABLE 2 Continued

Cardiac Dysfunction (Onset)	Cardiac Phenotype Comments	Other Comments
Mild/none (≥ 10 mo)	Frequent ECG abnormalities, DCM in females and HCM in males	Most widely used model, available through Jackson Labs (C57BL/10ScSn-Dmdmdx/J, stock #001801)
Mild/none (≥ 10 mo)	Same as mdx	
Mild/moderate (≥ 10 wk)	Frequent ECG abnormalities, normalization of fractional shortening reported at 1 year	More severe dystrophic phenotype (polymorphism in LTBP4 gene), increased fibrosis and fat accumulation, calcifications seen in both dystrophic and wild type strains, available through Jackson Labs (D2.B10-Dmdmdx/J, stock #013141)
None		Chemically induced mutation, fewer revertant fibers, available through Jackson Labs (B6Ros.Cg-Dmdmdx-2Cv/J, stock #002388)
		Chemically induced mutation, full-length dystrophin expressed at ~5% wild-type levels with all other isoforms eliminated, available through Jackson Labs (B6Ros.Cg-Dmdmdx-3Cv/J)
None	No significant cardiac phenotype observed	Chemically induced mutation, fewer revertant fibers, available through Jackson Labs (B6Ros.Cg-Dmdmdx-4Cv/J, stock #002378)
		Chemically induced mutation, more severe skeletal muscle disease, available through Jackson Labs (B6Ros.Cg-Dmdmdx-5Cv/J, stock #002379)
Mild/none (≥ 10 mo)		Targeted inactivation of hotspot (between exons 45-55), fewer revertant fibers
		All dystrophin isoforms affected, LacZ reporter replaces CR and CT domains
None	No significant cardiac phenotype observed	No revertant fibers
None	Ultrastructural changes seen by electron microscopy including necrosis and cardiomyocyte and mitochondrial disarray	
Moderate (≥ 8 wk)	Cardiomyocyte fragility and necrosis early then fibrosis, LV dilation, and reduced functional parameters late, frequent ECG abnormalities	Largest utrophin isoform is inactivated by targeted mutation at utrophin exon 7 (other isoforms are active), severe dystrophic phenotype, available through Jackson Labs (Utrntm1Ked/Dmdmdx/J, stock #014563)
Moderate (≥ 8 wk)	Cardiomyocyte fragility and necrosis early then fibrosis, LV dilation, and reduced functional parameters late, frequent ECG abnormalities	All utrophin isoforms are inactivated by targeted mutation at utrophin CR domain, severe dystrophic phenotype, available through Jackson Labs (stock #016622)
Mild/moderate (≥ 6 mo)	DCM occurs after 5-6 mo, fibrosis occurs by 10 mo, epicardial involvement of LV similar to human DMD cardiomyopathy	Severe dystrophic phenotype, MyoD only expressed in skeletal muscle
None	Cardiomyocyte necrosis early, no overt DCM	Humanized model of cytidine monophosphate-N-acetylneuraminic acid hydroxylase-like protein deletion, severe dystrophic phenotype, available from the Jackson Laboratory (stock #017929)
Severe (≥ 32 wk)	DCM occurs by 8 mo	Severe dystrophic phenotype, available through Jackson Labs (stock #018915).
Severe (≥ 32 wk)	DCM occurs by 8 mo	Severe dystrophic phenotype, available through Jackson Labs (stock #023535)
none	No overt dilation/hypertrophy but increased nuclear cell infiltration and necrosis, increased susceptibility to stress-induced injury	Pronounced skeletal muscle phenotype but less severe than mdx/Utr ^{-/-} , available through Jackson Labs (B6.Cg-Terctm1Rdp Dmdmdx-4Cv/BlauJ, stock #023535)
	Frequent ECG abnormalities, DCM ≥ 8 wk, increased risk of spontaneous death at 6 mo	Severe phenotype, knockdown-targeted replacement of Sgcd exon 2 leading to loss of whole sarcoglycan complex and sarcospan

delivered RNPs to restore dystrophin expression in DMD mice with a deletion of exon 44. In this study, mice were injected with LNPs into the tibialis anterior muscles weekly (3 injections, 1 mg/kg single guide RNA [sgRNA]) and detected the expression of dystrophin protein in tibialis anterior muscles 3 weeks after the last injection. This was determined through immunofluorescence and Western blot analysis.⁵³

The use of CRISPR/Cas9 has the promise to potentially correct DMD mutations, thereby serving as a potential for curative therapeutic strategies. One pre-clinical study generated a mouse model using CRISPR/Cas9 that resulted in a deletion of exon 50.⁴¹ These mice displayed severe muscle dysfunction including a depletion of dystrophin in the cardiac muscle tissue. Through the use of CRISPR/Cas9, the authors used a sgRNA that created reading-frame mutations that allowed for skipping of exon 51. The results from this study showed a significant recovery of dystrophin in the cardiac tissue, establishing a new mouse model of DMD.⁴¹ Additionally, findings from this study demonstrate that systemic delivery of AAV9 encoding a sgRNA directed toward exon 51 recovered the expression of dystrophin thereby preventing the onset of DMD.⁴¹ Recently, a 2-virus system with separate AAV9 constructs for the sgRNA and Cas9 (associated with a creatine kinase cassette to target muscle cells) was successful in repairing the exon 50 deletion in the deltaE50-MD canine model.⁵⁴ The promise of gene editing with a CRISPR/Cas9 approach is that the result would be permanent and would not require periodic treatments as with the other approaches mentioned in the preceding text. However, this permanency is also a reason for caution, because it cannot easily be reversed should adverse side effects become apparent in the long term. In addition, toxicity has been observed with systems such as AAV9-mediated gene delivery. An article recently published discusses the severe toxicity observed in nonhuman primates and piglets that received high-dose intravenous administration of adeno-associated virus vectors.⁵⁵ Examples include systemic and sensory neuron toxicity, with hepatic toxicity potentially manifesting as disseminated intravascular coagulation and liver necrosis.

INFLAMMATORY MODULATORS

Cardiac dysfunction in DMD has been associated with up-regulation of inflammatory pathways,⁵⁶ which in turn likely contribute to increased cardiac fibrosis.⁵⁷ Numerous studies have therefore examined the roles of inflammatory modulators in cardiac dysfunction in DMD to help identify potential therapeutic targets.

PENTRAXIN 3. Given the inflammatory processes occurring in DMD, numerous studies have further examined the roles of inflammatory modulators as potential targets for therapies. The protein pentraxin 3 (PTX3) is an inflammatory mediator that plays a critical role in the inflammatory process by contributing to activation of the complement pathway and coordinating macrophage function.⁵⁸ PTX3 is induced by myeloid differentiation primary response protein 88 (MYD88) in cardiac tissue, leading to downstream effects on critical inflammatory pathways involving Toll-like receptors and interleukins.⁵⁹ PTX3 has been found to be present in high concentrations in DMD animal models⁶⁰ and overexpressed in cardiac tissue of mdx mice in an age-dependent manner.⁶¹ Results from the latter study demonstrated that dystrophic expression of PTX3 was correlated with inflammatory and fibrotic pathways in cardiac tissue in mdx mice (C57BL/6/10ScSn-DMDmdx/J).⁶¹ Expression of PTX3 was slightly up-regulated in 9 month old mice and significantly increased as the mice approached 18 months of age. Results were determined through reverse transcription-quantitative polymerase chain reaction (RT-qPCR), immunohistochemistry, and Western blot analysis. These findings suggest that PTX3 could serve as potentially a prognostic marker, as well as a target for cardiac therapies in DMD patients.

POLYUNSATURATED FATTY ACIDS. Eicosapentaenoic acid and docosahexaenoic acid are polyunsaturated fatty acids that have anti-inflammatory effects and have shown benefit in improving inflammatory processes in cardiac disease and rheumatoid arthritis.⁶² One study further investigated the involvement of free fatty acid (FFA) receptors in the anti-inflammatory role of eicosapentaenoic and docosahexaenoic acid in dystrophic muscles.⁶³ Mdx mice (C57BL/10-Dmdmdx/PasUnib) were treated with fish oil capsules alone or with FFA1 or FFA4 blockers. Following 1 month of treatment, results showed that in the group receiving the blockers of FFA1 and FFA4, the anti-inflammatory effects of fish oil were inhibited due to a significant increase in the pro-inflammatory markers tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), which was determined through Western blot analysis. These findings indicate that the anti-inflammatory effects of fish oil in the dystrophic heart are mediated through FFA1 and FFA4, offering a mechanistic view of omega-3 in DMD. Furthermore, these results suggest that modulation of key inflammatory markers such as TNF- α and IL-1 β could be potential therapeutic targets to improve cardiac function in DMD.

STATINS. Statins are well known to be beneficial in cardiovascular disease such as lowering cholesterol, improved endothelial function, increase stability of atherosclerotic plaques, and reducing inflammation. However, data in DMD models thus far have been mixed. One study examined whether simvastatin could halt or rescue cardiac function in male dystrophin-deficient (mdx) mouse model. Results from this study determined that simvastatin administration significantly improves LV diastolic and systolic function and prevents cardiac fibrosis based on echocardiography, electrocardiography, and Western blot analysis.⁶⁴ In this study, simvastatin was administered at 40 mg/kg or 80 mg/kg for short-term (8 weeks) or long-term (12 months) study intervals. Another study was completed to further investigate the effect of rosuvastatin and its potential effects in both male and female mdx mice (C57BL/10-DMD^{mdx}/PasUnib).⁶⁵ Rosuvastatin was administered for 30 days beginning at the time of weaning, before muscle degeneration had occurred. Results of histopathological and morphometric analysis, immunofluorescence, and Western blot analysis showed no changes in serum creatine kinase or in markers of inflammation such as TNF- α , NF- κ B, or 4-HNE in mdx cardiac tissue. In addition, there was no significant reduction in cardiac fibrosis in mdx mice treated with rosuvastatin.⁶⁵ Taken together, these results suggest that rosuvastatin may not have the beneficial impact on dystrophy seen in mdx mice. Additional studies are necessary to better delineate the specific role of statins on cardiac function in DMD. Interestingly, statins also have a common adverse side effect of drug-induced myopathy.⁶⁶ The risk of myopathy or rhabdomyolysis is particularly concerning in patients with underlying muscle disease such as DMD, and thus statin use in this population is not routine.

ADIPONECTIN. Adiponectin (ApN), a hormone physiologically secreted by adipocytes, is an inflammatory regulator in a variety of muscle tissues, playing a role in the NLRP3 inflammasome pathway.^{67,68} NLRP3 is a highly characterized inflammasome pathway, and its specific involvement in the pathogenesis and progression of DMD continues to be investigated.^{69,70} Results from 1 study using NLRP3-KO, mdx, and mdx/NLRP3-KO mouse models determined that NLRP3 was expressed in skeletal muscle, and that ApN down-regulates NLRP3 expression through the inflammatory mediator, microRNA (miR)-711.⁷¹ These findings confirm that the NLRP3 inflammasome plays a key role in DMD, and ApN-mediated down-regulation of the NLRP3 inflammasome could serve as a potential therapeutic target for inhibiting the inflammatory process occurring in DMD.

GHRELIN. Ghrelin, a hormone produced by the stomach, plays a key role in regulating appetite and growth hormone release. Ghrelin also exerts anti-inflammatory effects in numerous inflammatory states including cardiovascular disease; however, its effects in DMD are not fully understood.⁷²⁻⁷⁴ One study investigated the effect and potential mechanism of ghrelin on muscle morphology and function in male mdx mice (C57BL/10ScSn-Dmdmdx/NJU).⁷⁵ Mdx mice were injected with ghrelin (100 μ g/kg) or saline for 4 weeks. Results determined that ghrelin significantly improved motor performance and decreased inflammatory cell infiltration. Additionally, ghrelin administration inhibited NLRP3 inflammasome activation, partly through suppression of JAK2-STAT3 and p38 mitogen-activated protein kinase (MAPK) pathway. Results were determined through histologic analysis, immunohistochemistry, immunofluorescence, grip strength test, and RT-qPCR. These findings suggest the ghrelin could serve as a potential therapy to decrease muscle inflammation occurring in DMD, slowing disease progression and decreasing symptoms.

PURINERGIC RECEPTORS. Purinergic receptors have been implicated in inflammation and fibroproliferation in different tissues, including the heart.^{76,77} In pressure overload-induced fibrosis in murine models, the G-protein coupled P2Y6 receptor activation by extracellular purine stimulation leads to cardiac fibrosis.^{78,79} In mdx mouse models of DMD, increased purine metabolism has been noted with involvement of G-protein coupled (P2Y2) and non-G-protein coupled (P2X7) purinergic receptors.⁸⁰ P2X7 contributes to up-regulation of IL-1 β and NLRP3 inflammasome activation.⁸¹ Activation of P2X7 pathways in DMD result in muscle cell damage and death along with increase inflammation.^{82,83} One study was completed in male mdx mice to determine the outcome of suppressing P2X7 through a known allosteric inhibitor, zidovudine.⁸¹ Results indicated that administration of 4 weeks of zidovudine led to a significant decrease in CD68 macrophage markers, as well as a significant decrease in TNF- α and inducible nitric oxide synthase (iNOS) transcript levels in murine cardiac tissue. In addition, although not statistically significant, it was also observed that the myocardial area affected by increased inflammation was reduced with zidovudine administration. Results were determined through histochemical analysis, immunofluorescence, Western blot analysis, and RT-qPCR. These findings suggest that P2X7 could be a potential therapeutic target for DMD cardiomyopathy. Interestingly, purinergic receptor activation may be tied to ATP release through pannexin (Px)

channels, which are found in close proximity within the plasma membrane and may represent an important mechanism for crosstalk between cardiomyocytes and inflammatory cells.⁷⁶

PROTEOGLYCANS. Proteomic studies have shown the correlation of the deficiency in dystrophin and the secondary changes that occur in cardiac tissue, including a recent study that was the first to successfully identify members of the cardiac dystrophin-glycoprotein complex through whole-tissue proteomics in mdx-4c mice.⁸⁴ Results from this study identified changes in various proteins such as the extracellular matrix protein laminin, the Ca²⁺ binding protein sarcalumenin, the matricellular protein periostin, the proteoglycans asporin and lumican, the cardiac-specific myosin light chain kinase, heat shock proteins, and a large number of mitochondrial and glycolytic enzymes. Fibrotic changes were observed through the presence of the matricellular protein, periostin, and other extracellular matrix proteins, such as the proteoglycans lumican and asporin, which are markers of reactive myofibrosis. These results could potentially contribute to the development of future therapeutic strategies targeted toward the progressive fibrosis that occurs in DMD.

REDOX/MITOCHONDRIAL TARGETS

Mitochondrial dysfunction has been identified in mouse models of DMD specifically in cardiac, diaphragmatic, and skeletal muscle, although the specific mechanisms remain unclear.⁸⁵⁻⁸⁹ It is believed that microtubules regulate mitochondrial bioenergetics through binding of the outer mitochondrial membrane voltage-dependent anion channel and influencing ADP/ATP cycling. The mechanism of action in cardiac dysfunction in DMD remains under investigation. One study examined mitochondrial bioenergetics involved in cardiac function focusing on the ability of creatine to facilitate mitochondrial-cytoplasmic phosphate shuttling and LV mitochondrial responsiveness to ADP in the D2-mdx/2J mouse model of DMD.⁸⁵ At 4 weeks of age, impairments in ADP-stimulated respiration and ADP attenuation of H₂O₂ emission were noted. The ability of creatine to increase ADP's control of mitochondrial bioenergetics was also decreased, whereas mitochondrial H₂O₂ emission was elevated.⁸⁵ By using echocardiography, Western blot analysis, mitochondrial respiration, H₂O₂ emission, and calcium retention capacity of mitochondria, findings showed that selective mitochondria dysfunction precedes the onset of

cardiomyopathy in DMD mice. These findings indicate mitochondrial bioenergetic modulators, including ADP and creatine phosphate shuttle, could be potential therapeutic targets to improve cardiac function in DMD.

Dystrophic cardiomyopathy is associated with increased oxidative stress and defective intracellular Ca²⁺ signaling.⁹⁰⁻⁹² One study aimed to determine whether mitochondrial damage or loss of function were due to impaired autophagic/mitophagic mechanisms in a mouse model of DMD (C57BL/10ScSn-Dmd^{mdx}/J).⁹³ Mitochondrial structure and function were assessed along with autophagy and mitophagy through techniques such as Western blot analysis, RT-PCR, and confocal and electron microscopy. Structural damage of mitochondria, a significant decrease in ATP production, and increased autophagy were observed in mdx mice, but interestingly, mitophagy was suppressed.⁹³ Down-regulation of several proteins in the PINK1/PARKIN pathway of mitophagy were identified, suggesting that reduced mitophagy could be contributing to the worsened mitochondrial impact in DMD. Targeting improved mitophagic mechanisms by modulation of the PINK1/PARKIN pathway could be a potential therapeutic strategy to improve cardiac function in DMD.

LEUCINE ZIPPER. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a basic leucine zipper that regulates antioxidant pathways to reduce negative effects of inflammation.⁹⁴ Sulforaphane (SNF) is an activator of Nrf2, protecting against fibrosis in the liver and lung;⁹⁵ however, its effects on dystrophic cardiac muscle are unknown. Results from 1 study in a mouse model of DMD (C57BL/10ScSn/Dmdmdx/NJU) showed that SNF administration from 3 to 6 months of age significantly decreased cardiac muscle fibrosis through Nrf2-mediated inhibition of TGF- β /Smad signaling, as determined through RT-qPCR, histologic and morphometric analysis, immunohistochemistry, and immunofluorescence.⁹⁶ Notably, Nrf2 is also known to inhibit activation of the NLRP3 inflammasome in cardiac and other tissue types.^{97,98} These findings suggest that modulation of Nrf2 is a potential target to promote antifibrotic effects in DMD.

In addition to Nrf2, other genes implicated in the fibrotic process have been studied in DMD, namely Nox4 and Lox. A study in a mouse model of DMD (C57BL/10ScSn-Dmd^{mdx}/J) used echocardiography, histomorphometry, and gene expression analysis to identify overexpression of the profibrotic genes Nox4 and Lox in LV tissue associated with significant LV thickening.⁹⁹ Interestingly, Lox expression was increased from the 3-month time point, and Nox4 was

increased in cardiac tissue, but not skeletal tissue. These findings suggest that Nox4 and Lox may be contributing to the development of cardiac fibrosis and may represent cardiac-specific targets in DMD.

CALCIUM CHANNEL TARGETS

Dystrophin is not only important in force transduction, but also serves as an essential scaffolding protein in muscle cells for several signaling proteins near the cell membrane, including sarcoglycans and matrix metalloproteins, among others. As a result of the lack of dystrophin in DMD, the mechanisms of normal calcium handling are disrupted including at the sarcoplasmic reticulum (SR). Similar to other forms of HF, the calcium sensitivity of the ryanodine receptor isoform 2 (RyR2) in the SR is increased in dystrophic cardiomyocytes. However, unlike in other forms of HF, there is a hypersensitivity to excitation-contraction coupling in dystrophic cardiomyocytes instead of a decrease. This creates the possibility that targeting RYR2 may offer a unique benefit in DMD cardiomyopathy.

One such possible target in DMD cardiomyopathy may be protein kinase A (PKA). Genetic or pharmacologic (using the selective PKA inhibitor KT5720) inhibition of PKA-mediated phosphorylation of RyR2 prevents dystrophic cardiomyopathy by reducing SR Ca²⁺ leak.¹⁰⁰

Another calcium channel target is the stretch-sensitive transient receptor potential cation channel, subfamily V, member 2 (TRPV2). Overexpression of TRPV2 has been observed in the sarcolemmal membrane of DMD patients, as well as in the cytoplasmic membrane of mdx mouse skeletal and cardiac cells.¹⁰¹ A recent study examined a small group of DMD patients with advanced HF and treated them with the antiallergy medication tranilast, which has anti-TRPV2 activity in addition to inhibitory effects on cytokine release and NLRP3 inflammasome activation. Tranilast led to reduction in HF biomarkers such as B-type natriuretic peptide, as well as a reduction in TRPV2 cytoplasmic membrane of mononuclear cells. Tranilast also attenuated the increase in circulating levels of miR-208a-5p, a known regulator of cardiac hypertrophy, and miR-223-3p, a marker of heart disease.¹⁰²

GAP JUNCTION PROTEIN TARGETS

Gap junctions are collections of intracellular proteins that connect adjacent cells and allow for direct cell-to-cell transfer of ions and small molecules. The

family of structurally similar proteins includes connexins (Cx) and Pxs. Recently, these proteins have been implicated as important disease modulators in animal models of DMD and represent potential therapeutic targets for DMD patients.

CONNEXINS. Cxs are 6-subunit hemichannels that form functional channels after docking end-to-end at the plasma membranes between adjacent cells. The primary isoforms expressed in cardiac tissue are Cx43 (ventricle) and Cx40 (atria), and they have been shown to be stretch-activated.¹⁰³ Recent studies in dystrophic mice and humans have demonstrated a role for Cx43 in DMD cardiomyopathy.^{104,105} In these models, Cx43 is up-regulated and becomes lateralized beyond the borders of the cardiomyocyte gap junctions, thus creating the potential for ionic “leak” and triggered arrhythmias. High-dose isoproterenol-induced severe ventricular arrhythmias in mdx and mdx:utr mouse models of DMD were prevented by treatment with Cx43 mimetic peptides targeted to inhibit hemichannel (Gap19) and gap junction channel (Gap26) opening.¹⁰⁴ Additionally, genetic mdx models ablating Cx43 (mdxCx^{-/-})¹⁰⁵ or altering the phosphorylation of Cx43 by replacing the serine triplet with phospho-mimicking glutamic acids (mdxS3E)¹⁰⁶ resulted in improvement in calcium handling, reduced cardiac fibrosis, less inducible arrhythmia, and overall improved survival. There is also a link between cellular oxidation stress and Cx activity. Treatment of mdx mice with the NADPH oxidase inhibitor apocynin (40 mg/kg/day over 1 month) resulted in decreased lateralization of Cx43, reduction in hyper-S-nitrosylation of Cx43, and an overall reduction in apoptosis in the dystrophic heart.¹⁰⁷

PANNEXINS. Unlike Cxs, Pxs are not isolated to gap junctions and have larger conductance (300-500 pS).^{108,109} They have been shown to be important in apoptosis signaling through release of ATP through their large pore region. Like Cxs, they are also stimulated by stretch but are additionally activated by elevated intracellular calcium and directly by ATP. Recent studies have shown increased Px expression in mdx mouse skeletal¹¹⁰ and cardiac¹¹¹ muscle. In isolated mdx hearts, infusion with ATP stimulates ventricular ectopy, which is suppressed by coinfusion with carbenoxolone at concentrations selective for Px channels.¹¹¹ Recently, the aldosterone inhibitor spironolactone was found to have Px inhibitory properties in endothelial cells,¹¹² allowing for a possible dual action of this pharmacologic class.

NEUROHORMONAL MODULATORS

Neurohormonal modulators have also been implicated in DMD cardiomyopathy. Specifically, BDNF, aldosterone inhibitors, and sacubitril/valsartan have been studied in the context of DMD cardiomyopathy.

BDNF/TYROSINE KINASE B. BDNF is a neuronal growth and survival factor, and plasma levels have been shown to correlate with cardiovascular risk in general¹¹³ and specifically LV ejection fraction (LVEF) in DMD patients.^{114,115} In DMD animal models, polymorphisms within the BDNF gene lead to reduced BDNF metabolism into the active form, alteration in cardiac-specific gene expression, and reduced cardiomyocyte contractility.³⁷ BDNF acts primarily by binding to the tyrosine kinase B (TrkB) receptor, leading to autophosphorylation of the receptor and downstream activation of several signaling pathways, including MAPK, the phospholipase C-gamma (PLC- γ), and the phosphatidylinositol 3-kinase (PI3K). Dystrophic mice with genetic ablation of neutral sphingomyelinase 2/sphingomyelin phosphodiesterase 3, important enzymes in the signaling cascades of inflammation and apoptosis, showed recovery of BDNF levels.¹¹⁶ Recently, it has also been demonstrated that acute inhibition of TrkB in wild-type mice leads to reduced heart rate and LVEF,³⁷ further reinforcing the potential therapeutic benefit of this target in DMD cardiomyopathy.

ALDOSTERONE INHIBITORS. Early mineralocorticoid receptor antagonist therapy with spironolactone in combination with ACE inhibitors has been shown to preserve normal circumferential strain and prevent cardiomyocyte damage in a utrophin haploinsufficient mdx mouse model of DMD,¹¹⁷ although concurrent prednisolone therapy may attenuate these effects.¹¹⁸ Low-dose eplerenone stabilizes left ventricular strain in DMD boys with detectable myocardial damage but preserved ejection fraction.¹¹⁹ Spironolactone appears to be equally effective as eplerenone in DMD patients.¹²⁰ Concurrent use of aldosterone inhibitors with traditional modulators of cardiac remodeling, including ACE inhibitors/angiotensin receptor blockers (ARBs) or beta-blockade,¹²¹ is currently recommended to be started by age 12 in asymptomatic DMD boys or sooner if evidence of late gadolinium enhancement is seen on cardiac magnetic resonance imaging (CMR).

SACUBITRIL/VALSARTAN. Sacubitril is a prodrug that is metabolized to sacubitrilat, a potent inhibitor of the enzyme neprilysin, which is responsible for the

degradation of atrial and brain natriuretic peptides. Combined with an ARB (valsartan), sacubitril has been recommended for patients with acquired symptomatic HF with reduced ejection fraction and has demonstrated partial recovery of LVEF in these patients.¹²² The PARADIGM-HF (Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) trial demonstrated a 20% reduction in mortality and a reduction in HF admission with sacubitril/valsartan compared with standard treatment with an ACE inhibitor.¹²³ More recently, clinical experience has been increasing with the use of sacubitril/valsartan in DMD patients. Initial reports suggest that sacubitril/valsartan may improve cardiac remodeling and LVEF in DMD patients compared with ACE inhibitor/ARB alone.¹²⁴

ANTIARRHYTHMIC AGENTS

There has been growing evidence that antiarrhythmic agents, particularly beta-blockers (BBs), may have a beneficial role in improving cardiac function in DMD. One study examined the effects of adrenergic agonists and BB treatments in DMD patient-specific human induced pluripotent stem cell-derived cardiomyocytes.¹²⁵ Results from this study determined that BB therapy in vitro and in vivo decreased the incidence of arrhythmogenesis and rescued lethality in mdx mice after beta-adrenergic stimulation.

DMD patients treated with the BB carvedilol over 5 years demonstrated significantly higher survival rate free from primary endpoints, which included all-cause death, advanced HF, and severe arrhythmia, compared with the non-BB group.¹²⁶ No significant differences in LVEF were identified between the BB group and non-BB group. These results suggest that BBs, specifically carvedilol, may be a safe and effective approach to reduce cardiac events in DMD patients. Despite the benefit seen with carvedilol, not all BBs have similar efficacy in DMD. One study in male mdx mice was completed, testing the effects of the BB metoprolol (2.5 mg/kg/day) on ventricular function and myocardial calcium influx.¹²⁷ Results from this study concluded that administration of metoprolol at an early stage of cardiomyopathy lead to worsening right ventricular ejection fraction though with no effect on myocardial calcium influx. These results were determined through CMR and in vivo myocardial calcium influx with manganese enhanced CMR. Given the conflicting impact of different BBs on left or right ventricular function, taken together, these findings suggest that more studies are needed to better

elucidate the specific effect of BBs on cardiomyopathy in DMD.

Recently, the sinoatrial pacemaker or “funny” current inhibitor, ivabradine, has also been investigated as a possible treatment for end-stage DMD cardiomyopathy. In a study of 20 teenage DMD patients with LVEF <40% and on long-term ACE inhibitor therapy, patients who underwent a heart rate reduction therapy with BB alone or with ivabradine had significantly lower major acute cardiac events compared with those who did not have aggressive rate control.¹²⁸ Additionally, it has been suggested that ivabradine has an independent effect on cardiac remodeling and thus may have a role in DMD cardiomyopathy treatment in combination with standard therapy.¹²⁹

CONCLUSIONS

DMD is a devastating disease with an ultimately poor prognosis and no cure. In recent years, cardiovascular disease has become the leading cause of death in patients with DMD. Research has identified therapeutic strategies that could help delay the progression of cardiac disease in patients with DMD including genetic manipulation, antiarrhythmic therapy, mitochondrial regulation, and modulation of inflammatory and neurohormonal factors. Gene therapy approaches such as CRISPR/Cas9 technology and exon skipping offer cautious optimism for the development of novel pharmacologic therapies for DMD. Although genetic strategies to correct the dystrophin mutation have shown promising results for skeletal muscles in DMD, their impact on cardiac dysfunction in DMD remains unclear. Furthermore, improved skeletal function in DMD may even cause decline in cardiac function in these patients due to earlier unmasking of underlying cardiac dysfunction facilitated by improved exertional capacity. As gene therapy becomes more widespread, some of these questions will begin to be answered; however, further research is necessary to firmly establish specific therapeutic approaches in DMD cardiomyopathy and to establish guidelines for early implementation of cardioprotective therapies and medications in these patients.

Preclinical evaluation of potential treatments for DMD cardiomyopathy has been limited by the phenotypic variation of currently available animal models. As has been discussed earlier, there is no 1 murine model that ideally mimics the cardiac and skeletal muscle DMD phenotype seen in humans. Experiments in larger animals, such as canine or porcine models, may more faithfully recapitulate the DMD cardiac

phenotype; however, their utility is limited by the inability to easily control for genetic background variation, the length of time to maturity, and the sample sizes needed to power studies depending on the effect size of the outcome variables. In addition, ethical and cost concerns make widespread use of these models prohibitive in all but the most select studies. Advances in human pluripotent stem cell technology has made studying cellular mechanisms in human cells more reliable. These systems have the advantage of being able to have human background genetic variations and, through the use of CRISPR/Cas9 systems, the ability to create isogenic control cell lines. Initial assessment of cellular toxicities can potentially be addressed in these systems as well, making it easier to move forward with the most promising therapies in humans. However, such *in vitro* studies cannot yet address complex interactions beyond monolayers or differential cell-cell interfaces.

There are several impediments to performing cardiac-specific randomized controlled trials in DMD patients. The relative rarity of DMD and the prevalence of established therapies such as glucocorticoids make study design more complicated, particularly for recruiting proper control patients. Additionally, many treatments are justifiably focused on mitigating the skeletal muscle effects of dystrophinopathies; however, until relatively recently, cardiac outcomes were either not evaluated or only evaluated as secondary outcomes. This has led to several multi-institutional efforts to pool outcomes and natural history data that can be used as control data and also for increasing the power of studies investigating newer therapies. Consortia such as the Advanced Cardiac Therapies Improving Outcomes Network (ACTION) and Parent Project Muscular Dystrophy (PPMD) have developed patient registries that collect extensive data on DMD patients, including advanced imaging parameters (echocardiographic and CMR), biomarkers, and medication adherence. As they continue to grow, these extensive data networks will allow for more streamlined and consistent evaluation of newer cardiac therapies in this population.

Based on the studies presented here, of particular promise are treatments that modulate the calcium regulation and the ongoing activation of the inflammatory cascade. Across several pharmaceutical classes, preclinical experiments that normalize intracellular calcium have a significant phenotype-mitigating effect. Additionally, better understanding of the mechanisms behind the efficacy of aldosterone

inhibitors and combination sacubitril/valsartan in DMD may lead to more targeted therapies. More human trials are needed, however, in order to establish long-term cardiovascular efficacy. Even as newer therapies become more standard for the treatment of skeletal muscle disease, it is likely that cardiac care will continue to be an important factor in monitoring DMD patients. It would not be surprising if cardiac-specific medications are still necessary even as skeletal muscles involvement improves and earlier initiation of cardioprotective therapies may soon become standard of care in this setting.

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