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PRINCIPAL INVESTIGATOR: Dongsheng Duan

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Dongsheng Duan

E-Mail: duand@missouri.edu

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The Curators of the University of Missouri
Sponsored Programs Administration
601 Turner Avenue
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14. ABSTRACT

The goal of this project is to test adeno-associated virus (AAV)-mediated gene therapy with the Dwarf open reading frame (DWORF). Abnormal cytosolic calcium overloading is a critical pathogenic mechanism in Duchenne muscular dystrophy (DMD). DWORF is a micropeptide that enhances cytosolic calcium uptake by sarco/endoplasmic reticulum (SR) calcium ATPase (SERCA). We hypothesize that AAV-mediated DWORF expression can increase SR calcium uptake and attenuate muscle disease in the mouse model of DMD. We further hypothesize that the combined DWORF/microdystrophin gene therapy will lead to better therapy than either micro-dystrophin or DWORF therapy alone. In this funding period, we tested whether AAV DWORF therapy can attenuate Duchenne cardiomyopathy, a leading cause of morbidity and mortality in DMD patients. We first showed that DWORF expression was significantly reduced at both the transcript level and the protein level in the heart of mdx mice, the most used mouse DMD model. We then delivered an AAV.DWORF vector by tail vein injection to mdx mice. We found that AAV.DWORF vector significantly increased DWORF expression but did not alter the expression of other calcium-regulating proteins. Importantly, we found AAV DWORF expression significantly enhanced SR calcium uptake, reduced myocardial fibrosis, improved cardiac electrophysiology, and enhanced heart hemodynamics. Unexpectedly, the left ventricular relaxation time constant Tau was not altered, suggesting AAV DWORF expression may impair atrium-ventricle coupling. In summary, our results suggest that AAV DWORF holds promise to treat DMD cardiomyopathy. Disruption of atrium-ventricle coupling suggests DWORF overexpression may have potential risks. Given the importance of toxicity in gene therapy, we will determine whether AAV-mediated DWORF expression is a real safety concern in the next funding period. If we confirm that AAV DWORF therapy is safe, we will test combined DWORF and microdystrophin gene therapy to determine whether the combinatory therapy is superior to the single therapy.

15. SUBJECT TERMS

None listed.

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1. Introduction

Duchenne muscular dystrophy (DMD) is a progressive muscle-wasting disease caused by the loss of dystrophin. Recent studies suggest that calcium overload in the cytosol represents a major underlying pathogenic mechanism in DMD. SERCA is a calcium pump that accounts for 70-90% of calcium removal from the cytosol in muscle cells. SERCA activity is significantly reduced in DMD muscle. DWORF is a positive regulator of SERCA. We have recently published proof-of-principle data suggesting that SERCA overexpression by adeno-associated virus (AAV) may ameliorate muscle disease. We now propose to evaluate AAV DWORF gene therapy in the murine DMD model.

2. Keywords

Duchenne muscular dystrophy, DMD, dystrophin, adeno-associated virus, AAV, cardiomyopathy, heart, calcium, sarco/endoplasmic reticulum (SR) calcium ATPase, SERCA, Dwarf open reading frame, DWORF, microdystrophin

3. Accomplishments

Major goal. We have two specific aims. Our first aim is to test systemic AAV DWORF therapy in the mouse model. Our second aim is to test if combined AAV microdystrophin-DWORF therapy is superior to microdystrophin therapy alone or DWORF therapy alone.

Accomplishments.

DWORF expression is significantly reduced in the mdx mouse model of DMD. We compared DWORF expression between normal mice and dystrophin-deficient mdx mice. At 6 months of age, we found DWORF expression was significantly reduced in the heart of mdx mice **(Figure 1)**. Further, we found DWORF expression was significantly reduced in soleus muscle of mdx mice **(Figure 2)**. We also examined DWORF expression in the heart of young (6-week-old) and aged (18-month-old) normal and mdx mice and found consistent DWORF reduction in mdx mice at these ages **(Figure 3)**.

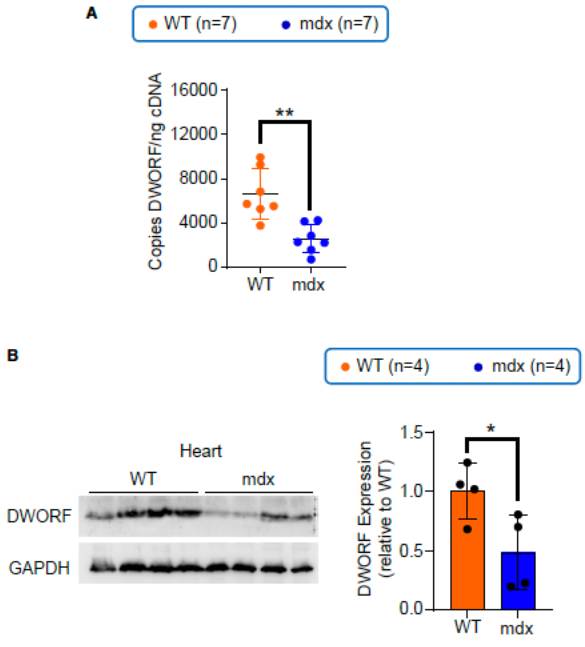


Figure 1. DWORF expression was decreased in mdx mouse hearts. A, Quantification of DWORF transcript in the ventricle of 6-month-old wild-type BL10 (WT) and mdx mice. **B,** Quantification of DWORF protein expression in the ventricles of 6-month-old WT and mdx mice by western blot. The left panel shows western blot images, and the right panel shows densitometry results. Sample size refers to the number of mice used in the study. *p<0.05; **p<0.01.

Figure 2. DWORF expression was reduced in the soleus muscle of mdx. Quantification of DWORF protein expression in the soleus of 6-month-old wild-type and mdx mice by western blot. The left panel shows western blot images, and the right panel shows densitometry results. Sample size refers to the number of mice used in the study. Data are presented as mean ± S.E.M. *p<0.001.

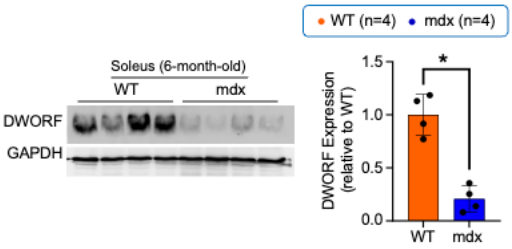
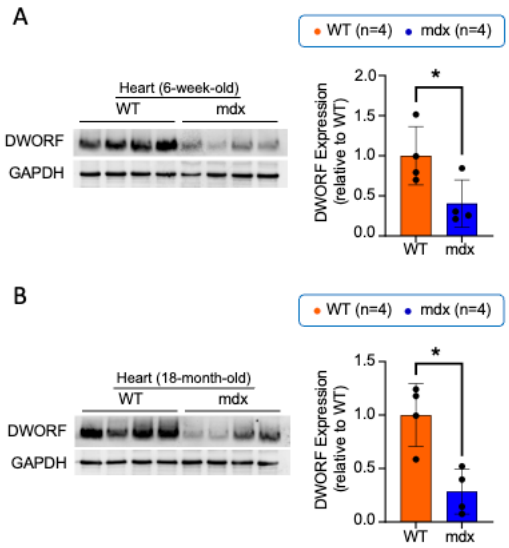


Figure 3. DWORF expression was reduced in the ventricles of mdx mice irrespective of age. A, Quantification of DWORF protein expression in the ventricles of 6-week-old WT and mdx mice by western blot. The left panel shows western blot images, and the right panel shows densitometry results. **B,** Quantification of DWORF protein expression in the ventricles of 18-month-old WT and mdx mice by western blot. The left panel shows western blot images, and the right panel shows densitometry results. **C,** Uncropped western blot image for panels A and B. Sample size refers to the number of mice used in the study. Data are presented as mean ± S.E.M. *p<0.05; **p<0.01; **p<0.001.



AAV.DWORF delivery increased DWORF expression and enhanced SR calcium uptake in the mdx mouse heart. To develop a DWORF gene therapy approach, we engineered an AAV9.DWORF vector **(Figure 4A)**. In this vector, a codon-optimized mouse DWORF cDNA was expressed from the ubiquitous CAG promoter. To facilitate the detection of AAV transduction, we also introduced the enhanced green fluorescence protein (EGFP) cDNA under the control of an internal ribosomal entry site (IRES). The AAV genome was packaged in myocardial-tropic AAV9 capsids. We injected the AAV9.DWORF vector to 6-week-old mdx mice at the dose of 6 x 10^{12} vector genome particles (vg)/mouse via the tail vein and examined DWORF expression in the ventricle when mice reached 6 months of age **(Figure 4B)**. Supra-physiological levels of DWORF expression were detected in the AAV-injected mdx mouse hearts (~ 50-fold higher than those of un-injected mdx mouse hearts). For reasons yet unknown, we failed to detect EGFP expression **(Figure 5)**. Despite the dramatic increase of DWORF expression in AAV-injected mdx mice, no significant changes were detected in the transcript level of the endogenous DWORF gene **(Figure 6)**, nor were protein levels of SERCA2a (SERCA isoform expressed in the heart), calsequestrin (CSQ), sarcolipin (SLN), or phospholamban (PLN) altered **(Figure 7)**. Next, we analyzed SR calcium uptake in the ventricular extracts **(Figure 8)**. As expected, SR calcium uptake was reduced in mdx hearts compared to WT controls. AAV9.DWORF delivery significantly enhanced SR calcium uptake in the mdx heart. The maximum velocity of calcium uptake (Vmax) was also significantly increased. Nonetheless, improvements did not reach the levels of WT mouse hearts.

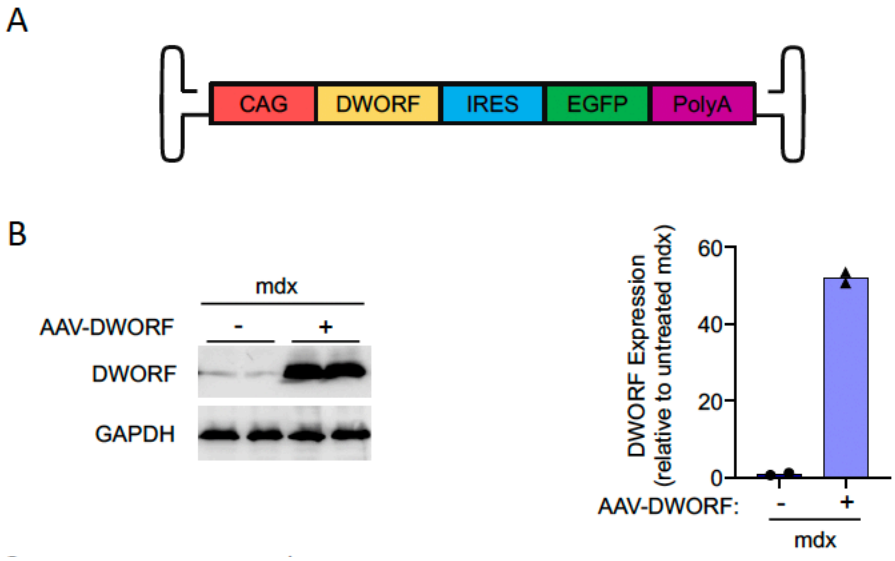


Figure 4. AAV.DWORF delivery at 6 weeks of age increased DWORF expression in mdx hearts at 18 months of age. **A,** Schematic drawing of the AAV.DWORF vector. **B,** Quantification of DWORF protein expression in the ventricles by western blot. The left panel shows western blot images, and the right panel shows densitometry results.

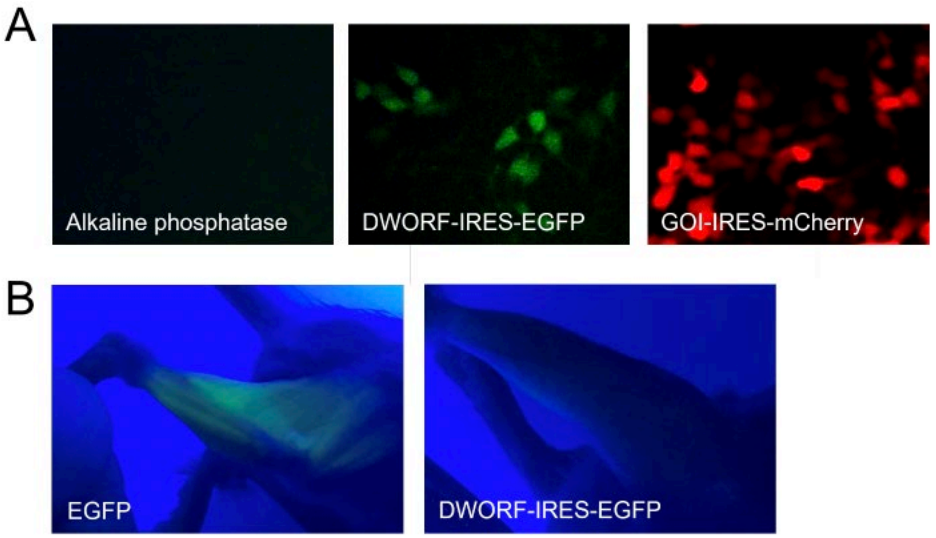


Figure 5. Unexpected observation of protein expression from the internal ribosomal entry site (IRES) used in the study. **A,** IRES resulted in efficient transgene expression in 293 cells. Three independent plasmids were used in transfection. One plasmid expressed non-fluorescent reporter alkaline phosphatase from the RSV promoter (left panel). The second plasmid (the plasmid used in the current study) expressed EGFP from the IRES (middle panel). The third plasmid expressed mCherry from the IRES (right panel). GOI, gene of interest. **B,** IRES failed to mediate EGFP expression in mouse muscle in vivo. EGFP expression was readily visualized in leg muscles 4 weeks following systemic injection of an AAV9.CMV.EGFP vector (6 x 10^{12} vg particles/mouse) in 6-week-old mdx mice (left panel). EGFP expression was not detected in leg muscles 4 weeks following systemic injection of the AAV9.CAG.DWORF-IRES-EGFP vector (6 x 10^{12} vg particles/mouse) in 6-week-old mdx mice (right panel).

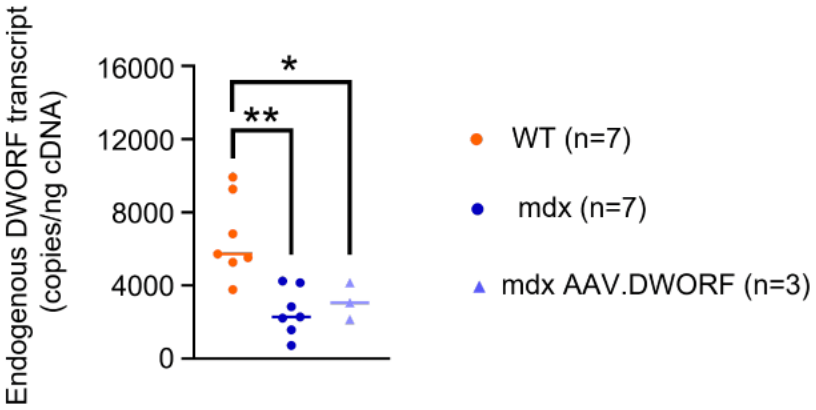


Figure 6. AAV-mediated DWORF expression did not alter the level of the endogenous DWORF transcripts. Quantification of endogenous DWORF transcript by digital droplet PCR. Sample size refers to the number of mice used in the study. Data are presented as mean ± S.E.M. *p<0.05; **p<0.01.

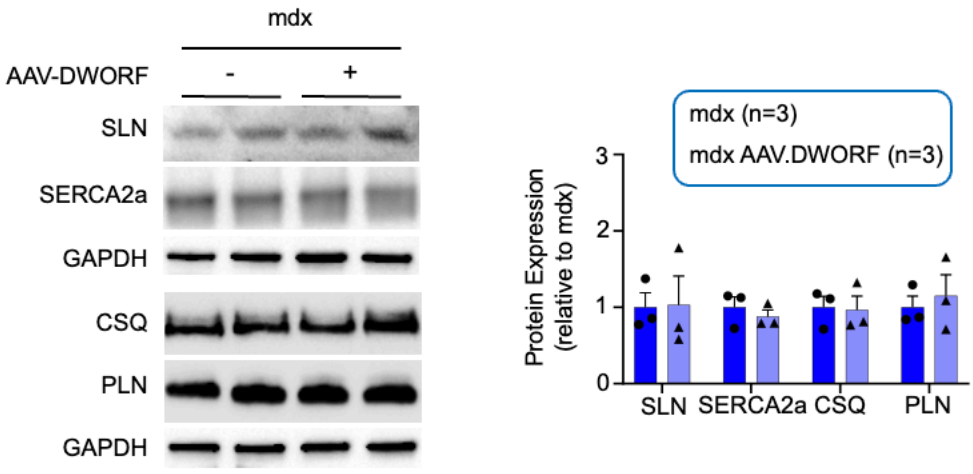


Figure 7. AAV-mediated DWORF expression did not alter the expression of SERCA2a and negative SERCA regulators (sarcolipin SLN and phospholamban PLN) and SR calcium regulation protein (calsequestrin CSQ). Quantification of the expression of various calcium-handling proteins in the ventricles. The left panel shows representative western blot images, and the right panel shows densitometry quantification results. Please note SERCA2a and sarcolipin (SLN) blots were run with one set of lysates while calsequestrin (CSQ) and phospholamban (PLN) were run with another.

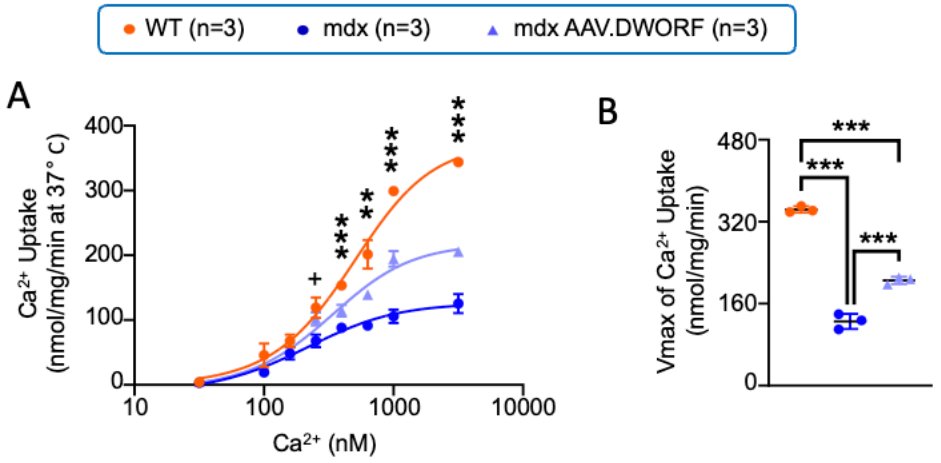


Figure 8. AAV-mediated DWORF expression enhanced sarcoendoplasmic reticulum (SR) calcium uptake. A, Heart SR calcium-dependent calcium uptake curve. **B,** Maximum rate of calcium uptake in the heart (V_{max}). Sample size refers to the number of mice used in the study. **p<0.01 between all compared groups, ***p<0.001 between all compared groups, +p<0.05 between mdx and all other groups but there is no statistically significant difference between wild-type BL10 (WT) and AAV.DWORF-treated mdx mice.

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AAV.DWORF delivery did not change heart mass but reduced myocardial fibrosis in aged mdx mice. We evaluated the therapeutic effect of the AAV9.DWORF vector in mdx mice (6 x 10^{12} vg/mouse, tail vein injection at 6 weeks of age, examination at 18 months). We opted to study 18-month-old mice because this is the age at which mdx mice display dilated cardiomyopathy. On anatomic examination, we did not detect significant differences in the heart weight, ventricular weight, and weight ratios between DWORF-treated and saline-injected control mdx mice **(Table 1)**. On histological examination, we performed Masson trichrome staining and quantified fibrosis **(Figure 9)**. DWORF gene therapy significantly reduced myocardial fibrosis compared to saline-injected mdx mice.

Table 1. Weights and weight ratios

	Wild type	mdx	mdx AAV.DWORF
Sample Size (N)	15	14	11
BW (g)	28.24 ± 0.52	22.95± 0.37*	22.67 ± 0.65*
HW (mg)	98.87 ± 1.95	104.45 ± 2.15	104.60 ± 3.61
VW (mg)	94.11 ± 2.00	100.59 ± 2.08	101.18 ± 3.44
TW (mg)	33.07 ± 0.75	28.69 ± 1.04*	27.29 ± 1.01*
Tibia Length (mm)	18.17 ± 0.06	18.72 ± 0.07*	18.57 ± 0.09*
HW/BW (mg/g)	3.52 ± 0.09	4.56 ± 0.10*	4.61 ± 0.09*
HW/ Tibia Length	5.44 ± 0.11	5.58 ± 0.12	5.63 ± 0.18
TW/BW (mg/g)	1.18 ± 0.04	1.25 ± 0.04	1.20 ± 0.03
HW/TW (mg/g)	3.00 ± 0.06	3.69 ± 0.13*	3.85 ± 0.10*
VW/BW (mg/g)	3.35 ± 0.08	4.39 ± 0.09*	4.46 ± 0.09*
VW/ Tibia Length	5.18 ± 0.11	5.38 ± 0.11	5.44 ± 0.17
VW/TW (mg/g)	2.86 ± 0.05	3.56 ± 0.12*	3.73 ± 0.10*

*, significantly different from WT

Abbreviations: ***BW****, body weight;* ***HW****, heart weight;* ***TL****, tibia length;* ***TW****, tibialis muscle weight;* ***VW****, ventricle weight.*

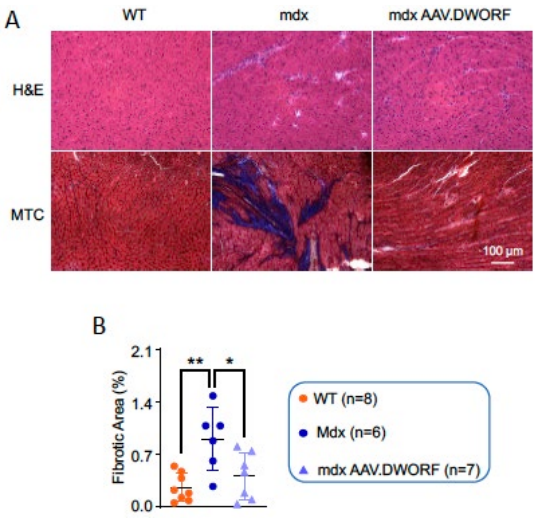


Figure 9. AAV-mediated DWORF expression enhanced sarcoendoplasmic reticulum (SR) calcium uptake. A, Heart SR calcium-dependent calcium uptake curve. **B,** Maximum rate of calcium uptake in the heart (V_{max}). Sample size refers to the number of mice used in the study. **p<0.01 between all compared groups, ***p<0.001 between all compared groups, +p<0.05 between mdx and all other groups but there is no statistically significant difference between wild-type BL10 (WT) and AAV.DWORF-treated mdx mice.

AAV.DWORF delivery ameliorated mdx ECG defects.

To determine whether DWORF gene therapy can improve cardiac electrophysiology, we performed ECG **(Figures 10** and **11)**. All parameters showed expected changes in mdx mice compared to WT mice. DWORF therapy normalized the heart rate, QTc interval, and cardiomyopathic index in mdx mice. Other ECG parameters (PR interval, QRS duration, and Q amplitude) showed a trend of improvement, though not statistically significant.

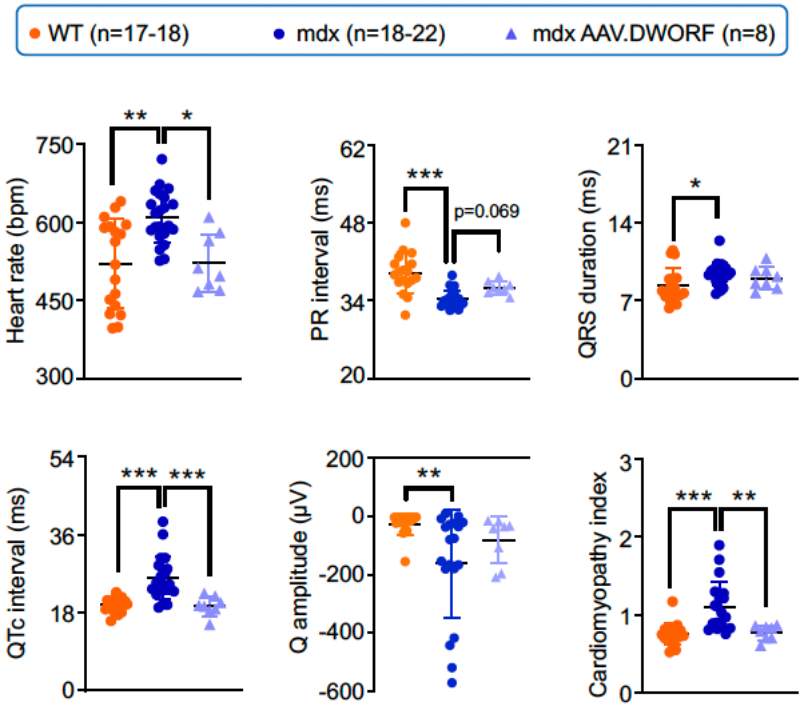


Figure 10. AAV.DWORF delivery at 6 weeks of age improved ECG at 18 months of age. ECG evaluation of heart rate, PR interval, QRS duration, QTc interval, Q amplitude, and cardiomyopathy index. Sample size refers to the number of mice used in the study. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

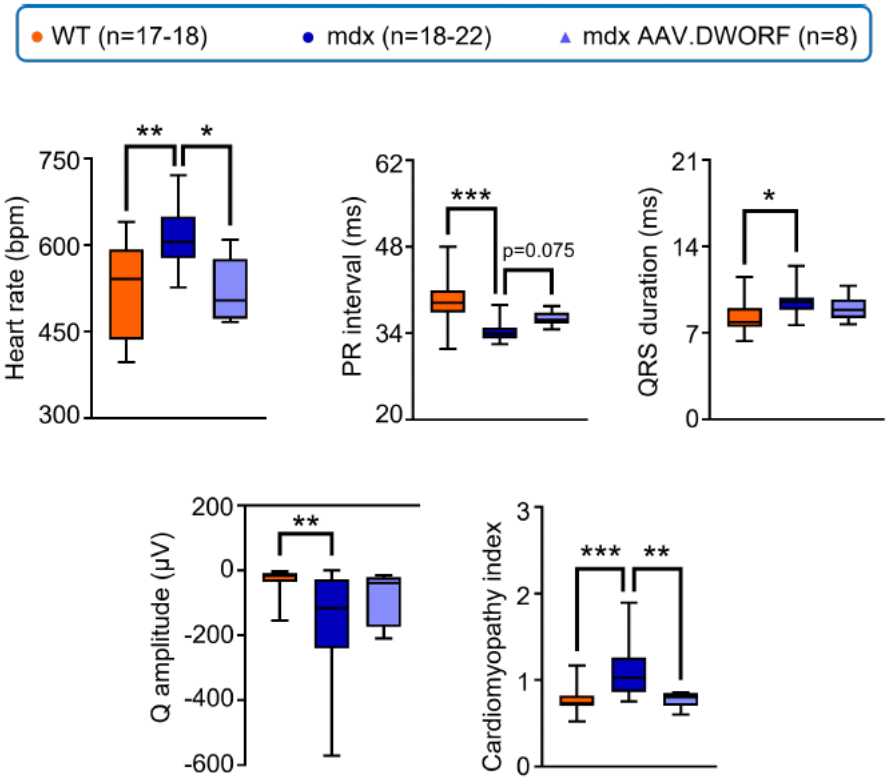
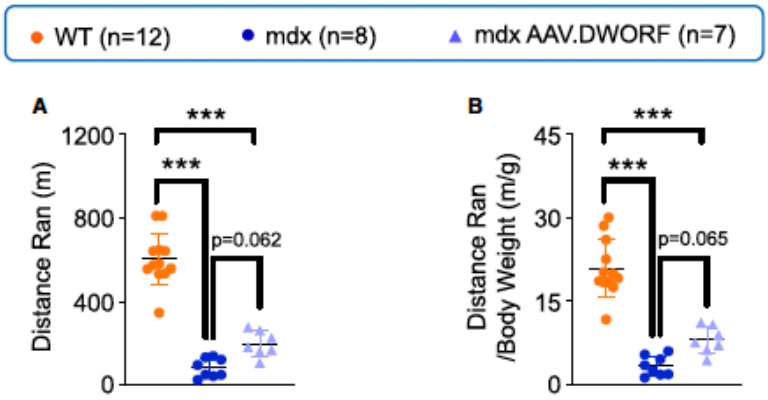


Figure 11. AAV.DWORF therapy improved ECG performance. ECG Results from the heart rate, PR interval, QRS duration, Q amplitude, and cardiomyopathy index were analyzed by the Kruskal-Wallis test. Data are presented as median (25% IQR, 75% IQR). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

AAV.DWORF delivery improved uphill treadmill running. Uphill treadmill running is frequently used to evaluate mouse heart function. Following 5 days of acclimation, we quantified mouse running distance on a 7° uphill treadmill. Mdx mice ran an average of 82 m or 3.3 meters/g body weight (m/g). WT mice ran an average of 603m or 20.9 m/g **(Figure 12)**. DWORF gene therapy increased treadmill performance (p=0.065). Treated mice reached an average of 198 m or 8.0 m/g **(Figure 12)**.

Figure 12. AAV.DWORF delivery at 6 weeks of age improved uphill treadmill running at 18 months of age. A, Total distance ran on a 7° uphill treadmill. **B,** Total distance ran on a 7° uphill treadmill normalized by mouse body weight. Sample size refers to the number of mice used in the study. ***p<0.001.



AAV.DWORF delivery enhanced left ventricular hemodynamic function in mdx mice. Cardiac pump function was evaluated using a closed-chest catheterization assay. Consistent with the literature, saline-injected mdx mice showed a classic profile of dilated cardiomyopathy **(Figures 13, Table 2)**. Specifically, end-systolic and end-diastolic chamber volumes were significantly enlarged while the ejection fraction, maximum pressure, and rates of pressure change during contraction (dP/dt max) and relaxation (dP/dt min) were significantly decreased. DWORF gene therapy normalized maximum pressure and ejection fraction in mdx mice. A trend of improvement was also detected in dP/dt max and dP/dt min. Intriguingly, end-systolic and end-diastolic volumes of the AAV9.DWORF-treated mdx mice were smaller than those of WT mice, though statistical significance between WT mice and DWORF-treated mdx mice was only observed for the end-diastolic volume.

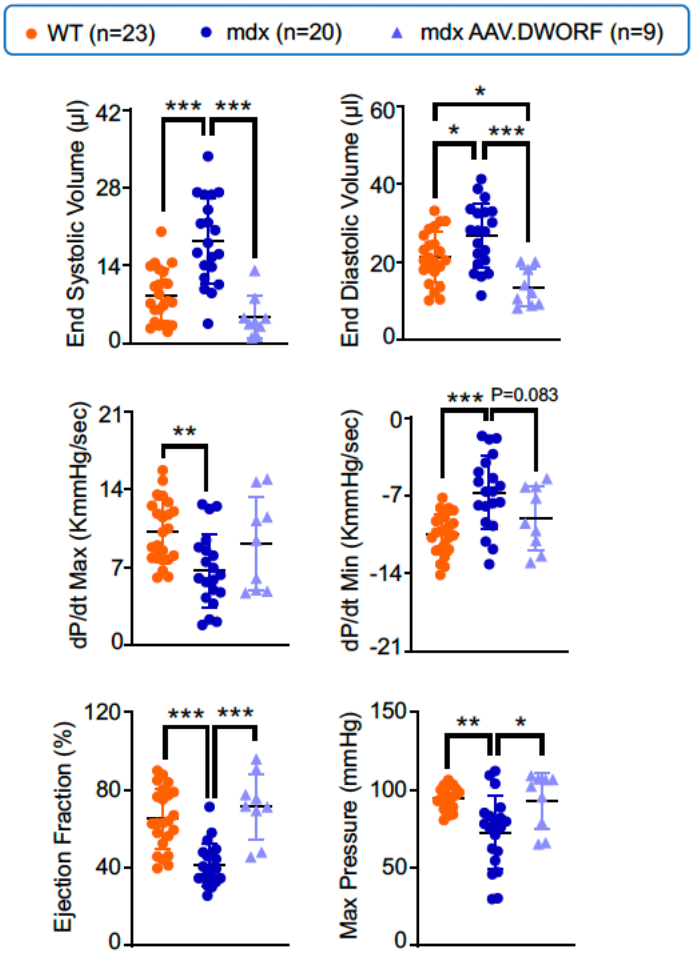


Figure 13. AAV.DWORF delivery at 6 weeks of age improved left ventricular hemodynamics at 18 months of age. Evaluation of left ventricular hemodynamics, including the end-systolic volume, end-diastolic volume, maximum pressure, ejection fraction, dP/dt max, and dP/dt min. Sample size refers to the number of mice used in the study. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Table 2. Left ventricle hemodynamic parameters.

	Wild type	mdx	mdx AAV.DWORF
Sample Size (N)	23	20	9
Body Surface Area (cm^2)	93.50 ± 1.91 [a,*]	79.77 ± 1.06 [+]	77.67 ± 1.68
Stroke Volume Index (mL/m^2)	1.51 ± 0.13 [*]	1.34 ± 0.10 [+]	1.38 ± 0.17
Cardiac Index ($L/min/m^2$)	0.86 ± 0.09 [*]	0.72 ± 0.06 [+]	0.72 ± 0.11
Min P (mmHg)	2.32 ± 0.62	3.15 ± 0.79	6.30 ± 2.37
End Systolic Pressure (mmHg)	89.07 ± 1.39	70.32 ± 5.19 [b]	86.53 ± 6.44
End Diastolic Pressure (mmHg)	5.08 ± 0.77	5.79 ± 1.00	8.97 ± 2.32
Stroke Work (KmmHgµL)	1118.04 ± 101.51	591.70 ± 62.28 [b]	780.78 ± 156.62
Arterial Elastance (mmHg/µL)	6.78 ± 0.52	6.78 ± 0.63	8.76 ± 1.01
dV/dt Max (µL/sec)	671.83 ± 59.21	631.30 ± 51.08	422.60 ± 35.78 [b]
dV/dt Min (µL/sec)	-779.91 ± 62.33	-708.40 ± 59.42	-582.00 ± 76.95
P@dV/dt Max (mmHg)	26.01 ± 4.97	20.91 ± 4.43	24.26 ± 9.05
P@dP/dt Max (mmHg)	60.11 ± 1.30	41.51 ± 3.74 [a]	62.52 ± 4.77 [c]
V@dP/dt Max (µL)	21.88 ± 1.27	26.83 ± 1.73 [a]	14.60 ± 1.50 [c]
V@dP/dt Min (µL)	8.84 ± 0.89	17.55 ± 1.59 [a]	4.96 ± 1.19 [c]
Max Power (mWatts)	6.54 ± 0.66 [#]	5.06 ± 0.64 [†]	6.41 ± 1.31
Preload Adjusted Max Power ($mWatts/\mu L^2$)	244.42 ± 37.59 [#]	145.85 ± 26.04 [†]	463.44 ± 132.01 [c]

[a], significantly different from other two groups
[b], significantly different from WT
[c], significantly different from mdx
[*], N=18; [+], N=13; [#], N=14; [†], N=9

AAV.DWORF delivery may have impaired atrium-ventricle coupling. Despite significant improvement in multiple electrophysiological and hemodynamic parameters, the left ventricular relaxation time constant Tau, the most established index for left ventricular diastolic function, was not improved, suggesting diastolic dysfunction **(Figure 14A)**. This is further confirmed in the diastolic filling segment of the pressure-volume loop in some treated mice **(Figure 14B)**. These results suggests

that AAV DWORF expression may have affected atrium-ventricle coupling and impaired relaxation. It should be pointed out that such condition has been shown to contribute heart failure.

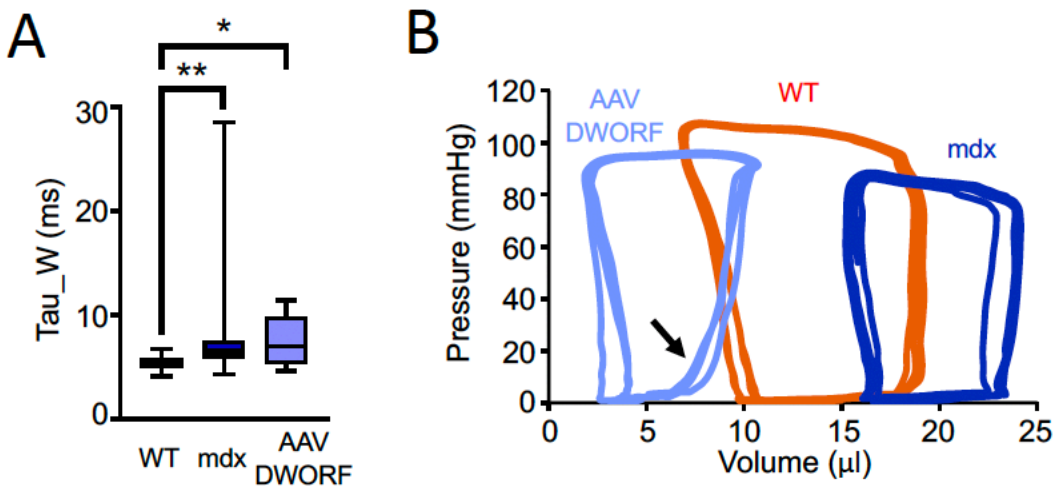


Figure 14. AAV DWORF treated mdx mice displayed impaired cardiac relaxation. A, Time constant of left ventricular relaxation (Tau) in wild type (WT), mdx, and AAV DWORF treated mdx mice. Data are presented as median (25% IQR, 75% IQR). *, $p < 0.05$; **, $p < 0.01$. **B,** Representative pressure-volume loops from wild-type BL10 (WT), mdx, and AAV.DWORF-treated mdx mice. Arrow, diastolic filling segment in an AAV DWORF treated mdx mice.

In summary, our results suggest that AAV DWORF holds promise to treat DMD cardiomyopathy. Disruption of atrium-ventricle coupling suggests DWORF overexpression may have potential risks. Given the importance of toxicity in gene therapy, we will determine whether AAV-mediated DWORF expression is a real safety concern in the next funding period. If we confirm that AAV DWORF therapy is safe, we will test combined DWORF and microdystrophin gene therapy to determine whether the combinatory therapy is superior to the single therapy.

Training and professional development opportunities. Nothing to report.

Dissemination of the results. Above mentioned study results have been published in a peer-reviewed scientific journal.

Plan for the next reporting period.

In this funding period, we made two critical observations. First, we found that DWORF expression is reduced in the mdx mouse model of DMD and the AAV DWORF therapy improved cytosolic calcium removal, reduced myocardial fibrosis, enhanced treadmill running, improved ECG and several hemodynamic defects in aged mdx mice. Second, we found that AAV DWORF expression may impair atrium-ventricle coupling and cause diastolic dysfunction. This is a significant safety concern. In light of this new finding, we will first determine if AAV DWORF delivery is safe in normal mice in the next funding period. If we confirm the AAV DWORF delivery causes toxicity, we will stop pursuing this line of research as a potential gene therapy for DMD. If we find there is no

safety concerns in normal mice, we will test if combined AAV microdystrophin and DWORF therapy can yield the best therapeutic effect.

4. Impact. Nothing to report.

5. Changes/Problems. Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them.
We do not anticipate significant technical hurdles.

Changes that had a significant impact on expenditures.
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animal, biohazards and/or selected agents.
Nothing to report.

6. Products

6.1. Peer-reviewed publications

Morales ED, Yue Y, Watkins TB, Han J, Pan X, Gibson AM, HB, B-E O, Yao G, Makarewich CA, Babu GJ, **Duan D**. *Dwarf open reading frame (DWORF) gene therapy ameliorated Duchenne muscular dystrophy cardiomyopathy in aged mdx mice*. **Journal of American Heart Association** 12:e027480, **2023**.

7. Participants/collaborating organizations*:*

What individuals have worked on the project?

Name: Dongsheng Duan
Project Role: PI
Research Identifier: ORCID#: 0000-0003-4109-1132
Nearest person month worked: 1
Contribution to project: Dr. Duan has overseen all aspects of the project.

Name: Gang Yao
Project Role: co-I
Research Identifier: N/A
Nearest person month worked: 1
Contribution to project: Dr. Yao assisted Dr. Duan in overseeing the research aspects of the project.

Name: Xiufang Pan
Project Role: Post-doc
Research Identifier: N/A

Nearest person month worked: 2
Contribution to project: Dr. Pan led AAV engineering and molecular analysis, and co-led cardiac function assay.

Name: Yongping Yue
Project Role: Lead Research Specialist
Research Identifier: N/A
Nearest person month worked: 2
Contribution to project: Ms. Yue was responsible for AAV production, purification, titer determination, and quality control. Ms. Yue also co-led cardiac function assay and assisted histology analysis.

Name: Jin Han
Project Role: Technician
Research Identifier: N/A
Nearest person month worked: 2
Contribution to project: Ms. Han was responsible for histology analysis. Ms. Han also assisted with molecular analysis.

Changes in the active other support of the PI and key personnel since the last reporting period.

Dongsheng Duan, PI

Previous Active Grants That Have Closed

Whole body single AAV microgene therapy in canine DMD
PI, 20% effort
NIH, NINDS R01 NS090634
National Institutes of Health; 6701 Rockledge Drive; Suite 1040, MSC 7710; Bethesda, MD 20817
09/01/2015-07/31/2022 (ncte)
(direct costs/current year)
In this study, we will test whether a newly developed canine Y731F AAV-9 micro-dystrophin vector gene therapy can lead to clinically meaningful improvement in dystrophic dogs.
Specific aim 1 is to test regional therapy in the hope of applying it to improve life quality in late-stage patients and aim 2 is to test systemic therapy in the hope of achieving bodywide improvement in young patients.
(There is no scientific/budget overlap with the current proposal.)

Treatment of Duchenne muscular dystrophy with the muscle calcium pump
PI, 20% effort
NIH/NIAMS (R01 AR070517-01)
National Institutes of Health; 6701 Rockledge Drive, Suite 1040, MSC 7710; Bethesda, MD 20817
07/01/2016-08/31/2022 (ncte)
(direct costs/current year)
Goal: Elevation of cytosolic calcium is a pivotal pathogenic event in Duchenne muscular dystrophy (DMD). We found that sarco/endoplasmic reticulum calcium ATPase 2a (SERCA2a) therapy can reduce

muscle disease and improve muscle function in the mouse DMD model. In the proposed study, we will test whether this therapy can treat symptomatic DMD dogs and our results will lay the foundation for a future clinical trial.
The specific aims are: (1) to test whether regional AAV SERCA2a therapy can ameliorate limb muscle disease and improve function and (2) to test whether systemic AAV SERCA2a therapy can lead to bodywide improvement in affected dogs.
(There is no scientific/budget overlap with the current proposal.)

Maintenance of the DMD canine colony for testing CRISPR therapy
4% effort, PI
NIH/NIAMS (R56 AR081544)
National Institutes of Health; 6701 Rockledge Drive, Suite 1040, MSC 7710; Bethesda, MD 20817
09/01/2022-08/31/2023
(direct costs/current year)
Goal: The goal is to maintain the DMD dog colony at the University of Missouri for testing AAV CRISRP therapy.
The specific aim is to maintain a healthy and robust colony for one year in preparation for the upcoming study to test CRISPR therapy in the canine DMD model.
(There is no scientific/budget overlap with the current proposal.)

AAV micro-dystrophin gene therapy for aging-associated atrial fibrillation
Co-I (PI: Timothy Domeier), 2% effort
TRIUMPH award – University of Missouri, School of Medicine
Debbie Taylor, MA204 Medical Sciences Building, University of Missouri, Columbia, MO 65212
05/01/2021-04/30/2024 ncte
(direct costs/current year)
The major goal is to determine whether systemic micro-dystrophin delivery can treat atrial fibrillation in aged mice.
(There is no scientific/budget overlap with the current proposal.)

A pilot study to evaluate ASO-mediated miR128 inhibition therapy in the canine DMD model
1% effort, PI
Project Number: N/A
Elenae Therapeutics
11/15/2022-02/14/2023
(direct costs/current year)
Goal: The goal is to test an antisense oligonucleotide-mediated inhibition of miR128 in dystrophic dogs.
The specific aim is to test an antisense oligonucleotide-mediated inhibition of miR128 in dystrophic dogs.
(There is no scientific/budget overlap with the current proposal.)

Current Research Grants

Mechanism of immune response to muscle-directed AAV gene transfer
15% effort, PI
NIH/NIAID (R01 AI177600)
National Institutes of Health; 6701 Rockledge Drive; Suite 1040, MSC 7710; Bethesda, MD 20817
05/19/2023-04/30/2028
(direct costs/current year)
Goal: The goal of this study is to investigate molecular and cellular mechanisms underlying AAV gene delivery in muscle.
Specific aim 1 is to define the mechanisms that link innate immune sensing to adaptive immune responses in AAV muscle gene transfer. Aim 2 is to prevent deleterious immune responses against transduced/gene-edited muscle following systemic AAV vector delivery. Aim 3 is to develop a novel protocol for re-administration of systemic AAV delivery.
(There is no scientific/budget overlap with the current proposal).

CRISPR editing therapy for Duchenne muscular dystrophy
25% effort, PI
NIH/NINDS (R01 NS131406)
National Institutes of Health; 6701 Rockledge Drive; Suite 1040, MSC 7710; Bethesda, MD 20817
07/01/2023-06/30/2028
(direct costs/current year)
Goal: The major goal of this project is to study the immune response to Cas9 following local and systemic delivery in animal models of Duchenne muscular dystrophy.
Specific Aims: Aim 1. Reducing the Cas9-induced CTL response by vector genome engineering. Aim 2. Reducing the Cas9-induced CTL response by shortening Cas9 expression. Aim 3. Reducing the Cas9-induced CTL response with combinatorial approaches..
(There is no scientific/budget overlap with the current proposal).

Development of optimized AAVrh74 vectors for gene therapy of muscular dystrophies
2.5% effort, MPI
NIH/NINDS (R01 NS131406)
National Institutes of Health; 6701 Rockledge Drive; Suite 1040, MSC 7710; Bethesda, MD 20817
01/25/2023 to 12/31/2023
(direct costs/current year)
Goal: The major goal of this project is to engineer a novel Opt AAVrh74 micro-dystrophin vector (Opt74-uDys).

Specific Aims include (1) Development of capsid+genome-modified, liver de-targeted Opt AAVrh74 vectors for high-efficiency transduction of primary human skeletal muscle cells in vitro, and in a mouse model in vivo; (2) Development of novel Opt AAVrh74 vectors with muscle-specific enhancer elements to augment transgene expression in muscle at further reduced doses in a mouse model in vivo; (3) Development of a novel Opt AAVrh74-micro-dystrophin vector (Opt74-μDys) for systemic gene therapy of DMD in a mouse model in vivo.
(There is no scientific/budget overlap with the current proposal).

Aging, calorie restriction and insulin sensitivity
5% effort, co-I (PI: Gregory Cartee)
NIH/NIA (R01 AG010026)
NIH - University of Michigan (Duan subcontract) Khaled J. Eid, MSF – Contract Administrator Lead Sponsored Programs – OCA, 5065 Wolverine Tower, 3003 S. State, St., Ann Arbor, MI 48109
05/01/2019-02/28/2024 (no cost extension)
(direct costs/current year)
Goal: Duan lab will oversee recombinant adeno-associated viral vector (AAV) production, purification and characterization. The AAV vector will be used by Dr. Cartee's lab to perform in vivo study in experimental subjects. Dr. Duan will also participate in the design of AAV related studies, data analysis and trouble shooting.
Specific Aims are Aim 1. Reducing the Cas9-induced CTL response by vector genome engineering. Aim 2. Reducing the Cas9-induced CTL response by shortening Cas9 expression. Aim 3. Reducing the Cas9-induced CTL response with combinatorial approaches.
(There is no scientific/budget overlap with the current proposal.)

Super AAV for DMD gene therapy in human patients
5% effort, PI
Grant number: N/A
Jesse's Journey: The Foundation for Gene & Cell Therapy
Lisa Hoffman; PO Box 51 Station B; London, ON NGA 4V3; CANADA
04/01/2020-05/30/2024 (no cost extension)
(direct costs/current year)
Goal: We will develop super-capsids that can outperform the ones currently in use in DMD patients. Our super- capsids will greatly reduce safety concerns and AAV manufacture burden of the current AAV DMD therapy.
Specific Aim is to engineer and test super AAV in human muscle
(There is no scientific/budget overlap with the current proposal.)

Novel AAV for DMD gene therapy in human patients
0.3% effort, PI
Grant number: N/A
Ryan's Quest
David Shultz; PO Box 2544; Hamilton, NJ 08690
04/01/2020-05/30/2024 (no cost extension)

(direct costs/current year)
Ryan's Quest is one of two foundations (Michael's Cause) that have agreed to fund this project as a start-up with the Jesse's Journey project "Super AAV for DMD gene therapy in human muscle". (There is no scientific/budget overlap with the current proposal.)

Novel AAV for DMD gene therapy in human patients
0.3% effort, PI
Grant number: N/A
Michael's Cause
Robert Capolongo; PO Box 120323; Staten Island, NY 10312
04/01/2020-05/30/2024 (no cost extension)
(direct costs/current year)
Michael's Cause is one of two foundations (Ryan's Quest) that have agreed to fund this project as a start-up with the Jesse's Journey project "Super AAV for DMD gene therapy in human muscle". (There is no scientific/budget overlap with the current proposal.)

DWORF gene therapy for DMD
10% effort, PI
W81XWH2210808
Dept. of Defense
Danielle L. Reckley, Grants Officer, 820 Chandler St, Fort Detrick, MD 21702 09/30/2022-09/29/2024
(direct costs/current year)
09/01/2022-08/30/2024
Major Goals: The major goal of this project is to engineer and test DWORF gene therapy in murine DMD model.
Specific Aim: (1) To evaluate systemic AAV.DWORF delivery in normal and mdx mice; and (2) To compare therapeutic efficacy of systemic AAV.mDys, AAV.DWORF, and AAV.mDys-DWORF therapy in mdx mice.
(There is the current proposal.)

Other organizations: Nothing to report.

8. Special reporting requirements: N/A

9. Appendix:
One peer-reviewed publication