Presented virtually at the Muscular Dystrophy Association Clinical & Scientific Conference, March 13–16, 2022, Nashville, TN

TIMP-1 and CKM as Novel Noninvasive Serum Biomarkers of Therapeutic Response for Exon-Skipping Therapies in Duchenne Muscular Dystrophy

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Objective

To assess the utility of TIMP-1 and CKM as serum biomarkers of therapeutic response to PPMO in the *mdx* mouse

Key Takeaway

TIMP-1 and CKM may serve as potential noninvasive biomarkers of therapeutic response for exon-skipping therapies, which may have implications for patients with DMD

BACKGROUND

- Clinical development of peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) and other treatments for Duchenne muscular dystrophy (DMD) is challenged by a lack of noninvasive methods for assessing therapeutic response
- Levels of tissue inhibitor of metalloproteinases-1 (TIMP-1) and creatine kinase muscle-type (CKM) are elevated in serum of patients with DMD and in the *mdx* mouse model of DMD and correlate with disease severity[1,2]
- Previous work found that although matrix metalloproteinase-9 (MMP-9) levels are elevated in patients with DMD, they are not reflective of treatment response to antisense therapy[3]

CONCLUSIONS

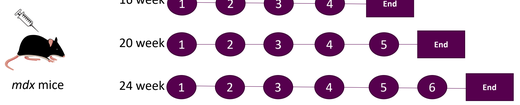
- In the *mdx* mouse, TIMP-1 and CKM are measurable serum biomarkers of muscle disease that decrease significantly after exon-skipping therapy
  - —Biomarker response is specific to exon-skipping therapy as shown by lack of reduction in MMP-9 serum levels
- Decreases in TIMP-1 and CKM serum concentrations are sustained while on therapy through 6 months
- Decreases in TIMP-1 and CKM correlate with exon skipping, dystrophin production, and improved muscle function

METHODS

Methods: RC-1001 dosing in *mdx* mice every 4 weeks (Q4W) for 24 weeks

Groups and number of doses

RC-1001 (PPMO targeting *DMD* exon 23 skipping, the *mdx* mutation)



mdx mice

Experimental design:

- *mdx* mice (n=6 per dose) received vehicle or RC-1001 20 mg/kg, 40 mg/kg, or 80 mg/kg intravenously
- Tissues were collected 4 weeks after the last dose in each group

Assessments:

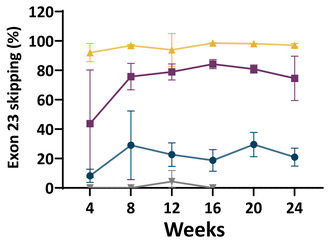
- Exon 23 skipping (ddPCR)[a]
- Dystrophin production (western blot)[a]
- Grip strength
- Serum levels of TIMP-1, CKM, and MMP-9[b]

[a]Exon 23 skipping and dystrophin protein expression assessed from biceps tissue; [b]Serum levels of TIMP-1 and MMP-9 were measured by enzyme-linked immunosorbent assay, and serum levels of CKM were measured by Meso Scale Discovery electrochemiluminescence assay.
CKM=creatine kinase muscle-type; ddPCR=droplet digital polymerase chain reaction; DMD=Duchenne muscular dystrophy; MMP-9=matrix metallopeptidase 9; PPMO=peptide-conjugated phosphorodiamidate morpholino oligomer; TIMP-1=tissue inhibitor of metalloproteinases-1.

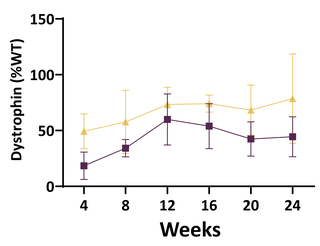
RESULTS

RC-1001 led to dose-dependent increases in exon 23 skipping, dystrophin protein, and grip strength after a single dose, sustained through the 24-week study with Q4W dosing

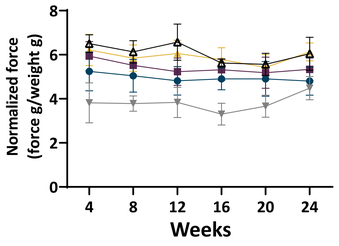
Exon skipping[a]



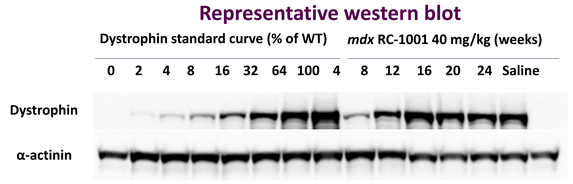
Dystrophin protein production[a]



Grip strength



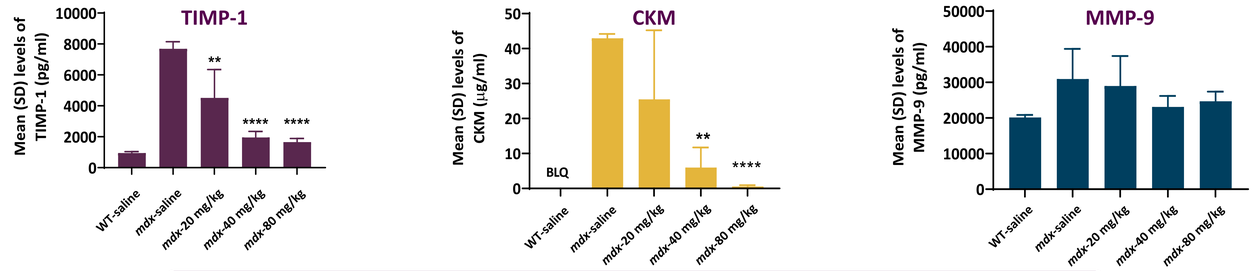
Representative western blot



mdx-saline
mdx-20 mg/kg
mdx-40 mg/kg
mdx-80 mg/kg
WT-saline

*Exon skipping and protein data are from bicep tissue. Q4W=once every 4 weeks; WT=wild-type.

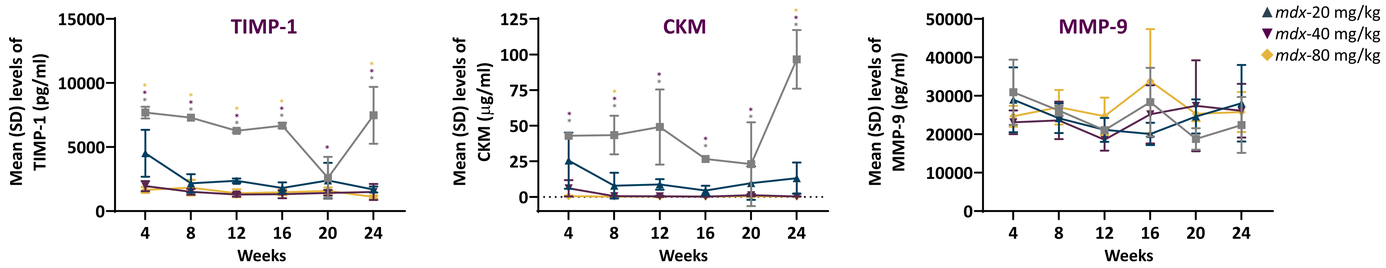
Serum biomarkers of muscle injury following single RC-1001 dosing at week 4 in *mdx* mice



Significant dose-dependent decreases in mean serum levels of TIMP-1 and CKM after 1 dose of RC-1001, but not in MMP-9 levels, demonstrate specificity of the biomarker response

**P<0.01; ****P<0.0001 (2-way analysis of variance with Dunnett's multiple comparison test to compare with the *mdx* saline control group). CKM=creatine kinase muscle-type; MMP-9=matrix metallopeptidase 9; SD=standard deviation; TIMP-1=tissue inhibitor of metalloproteinases-1; WT=wild-type.

Serum biomarkers of muscle injury following RC-1001 Q4W dosing in *mdx* mice

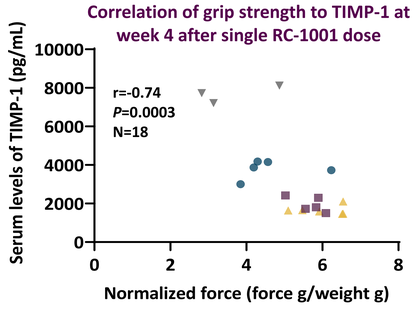


There were sustained decreases in serum levels of TIMP-1 and CKM, but not MMP-9, in RC-1001–treated *mdx* mice throughout study, demonstrating the specificity of the biomarker response

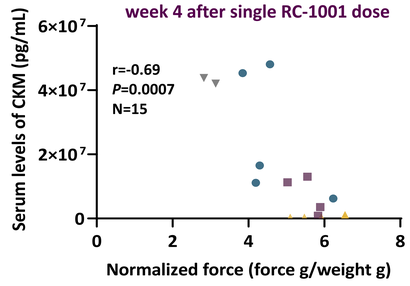
*P<0.05 versus *mdx*-saline. Dunnett's test was used to carry out multiple comparison analyses of biomarker concentration between the control and treatment groups at each time point. CKM=creatine kinase muscle-type; MMP-9= matrix metallopeptidase 9; Q4W=once every 4 weeks; SD=standard deviation; TIMP-1=tissue inhibitor of metalloproteinases-1.

Correlation of muscle function with biomarker serum levels

Correlation of grip strength to TIMP-1 at week 4 after single RC-1001 dose



Correlation of grip strength to CKM at week 4 after single RC-1001 dose



mdx-saline
mdx-20 mg/kg
mdx-40 mg/kg
mdx-80 mg/kg

Increase in grip strength after single dose of RC-1001 correlated in a dose-dependent manner with decreased TIMP-1 and CKM levels, but not MMP-9 levels (r=-0.42; *P*=0.07), as early as after 1 dose of the PPMO[a]

[a]Pearson correlation was performed between grip strength and biomarker concentration. CKM=creatine kinase muscle-type; MMP-9=matrix metallopeptidase 9; PPMO=peptide-conjugated phosphorodiamidate morpholino oligomer; TIMP-1=tissue inhibitor of metalloproteinases-1.

REFERENCES

1. Burch P, et al. *J Neuromuscul Dis*. 2015;2:241-55. 2. Nadarajah V, et al. *Neuromuscul Disord*. 2011;21:569-78. 3. Lourbakos A, et al. *Sci Rep*. 2017;7(1):17888.

ACKNOWLEDGMENTS & DISCLOSURES

This study was sponsored by Sarepta Therapeutics, Inc. Editorial support was provided by Paraskevi Briassouli, PhD, of Eloquent Scientific Solutions, and funded by Sarepta Therapeutics, Inc. All authors are employees or former employees of Sarepta Therapeutics, Inc. and may own stock in the company. Products are investigational only. Previously presented at the 17th Annual Meeting of the Oligonucleotide Therapeutics Society: Virtual Conference, September 26–29, 2021.