Selective PPARδ modulator MA-0211 improves disease phenotype in DMD muscle cells and mdx mice

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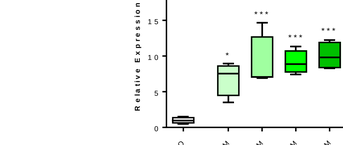
Summary

Mitochondrial dysfunction is detected in Duchenne Muscular Dystrophy (DMD) patient tissues and cultured cells as well as animal models, and may represent a critical early defect contributing to muscle fiber pathology. MA-0211 (MTB-1, A0367) is a novel, potent and selective modulator that increases muscle fatty acid oxidation (FAO) and mitochondrial biogenesis. The findings summarized here support the hypothesis that mitochondrial dysfunction may be a key component of the dystrophin deficient phenotype and suggest that MA-0211 improves cellular, histological and functional measures of skeletal and cardiac muscle health.

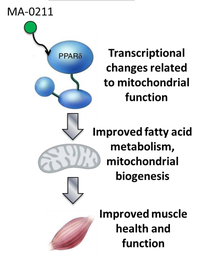
Background

DMD Muscle fuel utilization is unbalanced



- DMD Muscle fat utilization is reduced
- Protein is shunted to rebuild damaged muscle
- Reliance on Carbohydrates leads to poor endurance & inflammation

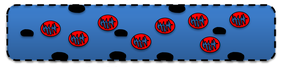
Hypothesis



Introduction

❖ **Mitochondrial deficits are an early and critical facet of DMD.**

1. Genetic loss of Dystrophin
2. Plasma membrane instability
3. Persistent Ca++ leak into fibers generates mitochondrial stress
4. Mitochondrial Stress/Dysfunction
   - **Reduced fatty acid oxidation**
   - Fewer mitochondria for cellular repair
   - Sensitization to apoptosis/necrosis
5. **Manifestations**
   - **Exercise intolerance/fatigue**
   - **Muscle loss**
   - **Persistent inflammation**



DMD muscle fiber

Plasma membrane (sarcolemma)
Nuclei
Dysfunctional Mitochondria

"DMD is characterised by a systemic mitochondrial impairment that is central to disease aetiology rather than a secondary pathophysiological consequence of dystrophin-deficiency" (*Timpani et al. 2015).*

❖ **Mitochondrial dysfunction in DMD leads to poor utilization of fatty acids by cells, a crucial fuel source for skeletal and cardiac muscle.**

❖ **PPARδ is a transcription factor that can increase cellular capacity for fatty acid oxidation.**

❖ **MA-0211 is a potent, highly-selective orally-available small molecule modulator of PPARδ.**

MA-0211 increases PPARδ Target Gene expression in DMD Myotubes

Figure 1: qPCR gene expression of DMC myotubes treated for 24 hours demonstrates potent engagement of PPARδ target genes in DMD myotubes with MA-0211.

MA-0211 improves mitochondrial function in DMD Myotubes

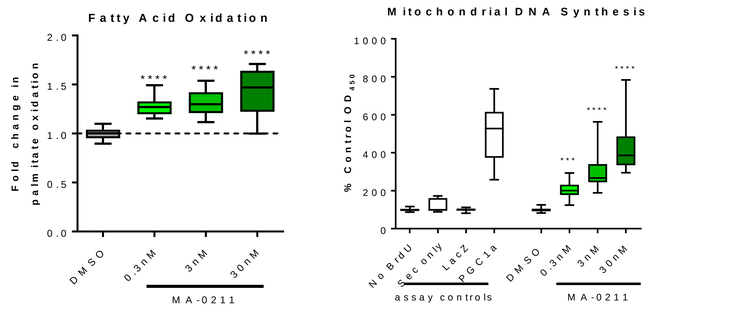
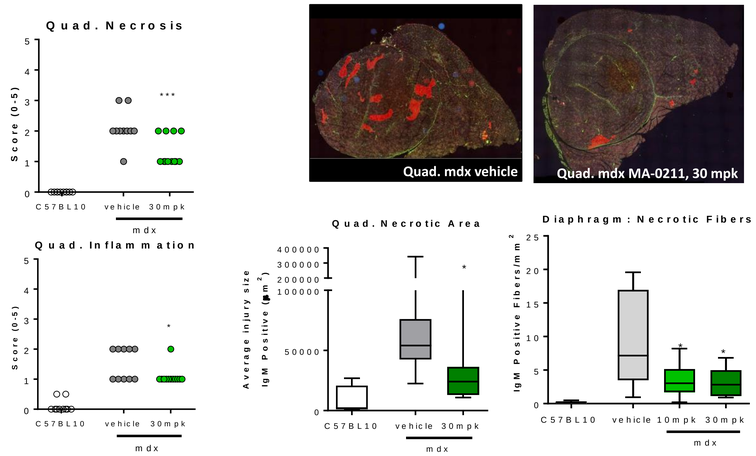


Figure 2: Fatty acid oxidation was increased after 24 hours of treatment in DMD myotubes from 17 month old donor; measured using Seahorse XF analyzer. Mitochondrial DNA synthesis measured by BrdU incorporation, a measure related to mitochondrial biogenesis, increases with 72 hour treatment. Similar results obtained from second donor.

Histological and functional Improvement in mdx skeletal muscle

Pathology



Histochemistry

Figure 3: mdx mice were treated orally, once daily for 5 weeks starting at about 5 weeks of age. Treated mice demonstrated a decrease in muscle necrosis and inflammation (scored in a double-blind fashion) and reduced necrosis (fewer necrotic fibers and smaller injury foci), measured by detecting IgM-positive muscle fibers. Data represents two independent studies.

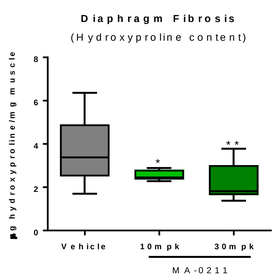


Figure 4: Reduced diaphragm fibrosis, measured by hydroxyproline content, was observed after 5 weeks of once daily treatment.

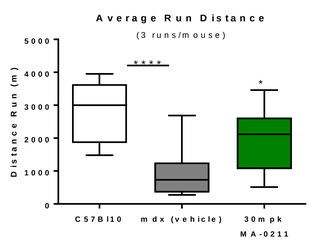


Figure 5: Treated mdx mice were trained and tested for endurance capacity using a treadmill. The average run distance across three runs per mouse was compared.

Prevention of cardiac functional decline in aged mdx mice

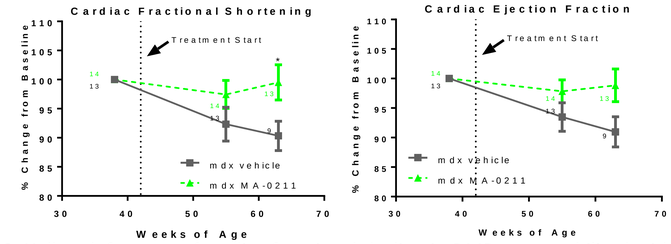


Figure 6: Hallmark features of cardiac functional decline in DMD were stabilized with MA-0211 treatment. Cardiac function was assed by echocardiography in aged mdx. After a baseline assessment, treatment began as a food admix at 42 weeks of age and continued for 5 months. Number on plot represents number of remaining subjects/group.

Biomarker improvement and beneficial shift in disease-associated metabolites in mdx mice

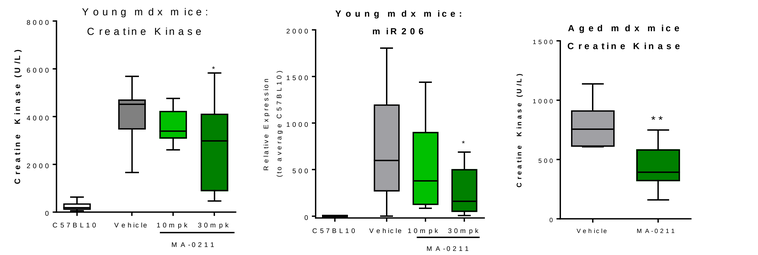
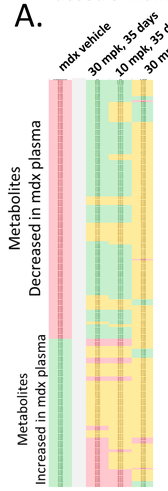


Figure 7: Plasma markers of muscle damage, creatine kinase and muscle-specific microRNA (miR206), are reduced with MA-0211 administration in young and older mdx mice. Young (5 weeks) mdx mice were dosed for 35 days, aged (42 weeks) mdx mice were dosed 5 months.

A.



B.

Metabolite	Mdx pheno.	Change with MA-0211	% change With MA-0211	Biology
Anserine/Homocarnosine	-44%	↑	+302%	Scavenges ROS
Arachidonic Acid (AA)	-27%	↑	+34%	Lipid metabolite, improves muscle performance/repair, anti-inflammatory
Palmitoleic Acid	-38%	↑	+26%	Fatty acid
Alanyl-Serine	+59%	↓	-28%	Incomplete breakdown product of protein catabolism
Isovalerylalanine	+35%	↓	-33%	Incomplete breakdown product of Leucine

Figure 8: (A) Mass Spec metabolomics plasma profiling was conducted in treated mdx mice identified 120 unique m/z ratio metabolites that were significantly differentially represented in mdx plasma compared to control. MA-0211 reversed the disease phenotype in a time and dose-dependent fashion. **(B)** Example metabolites and % change with treatment, representing potential biomarkers, are listed in the table.

Conclusions and Future Directions

✓ MA-0211 is an oral agent that has reproducibly demonstrated efficacy across multiple symptoms of DMD via improvement in mitochondrial function and metabolism.

✓ Mitochondrial/metabolic benefits are independent of dystrophin mutation and offer potential for combination with other treatments.

✓ IND has been submitted. Developing clinical plan in partnership with Astellas Pharma.

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