Edasalonexent (CAT-1004), an NF-κB inhibitor, enhances myotube formation *in vitro*, and increases exon-skipped sarcolemmal dystrophin in muscle of *mdx* mice

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Abstract

NF-κB is activated from infancy in boys with Duchenne muscular dystrophy and in *mdx* mice, and promotes muscle degeneration while inhibiting muscle regeneration. NF-κB driven micro-RNA also directly impair dystrophin protein translation, further destabilizing this protein and limiting the full potential of exon-skipping therapy in dystrophic muscles. Edasalonexent is an oral NF-κB inhibitor that is currently in the MoveDMD® trial in DMD boys aged 4-7, an age range where high burden of inflammation in the muscle is expected. Previously, edasalonexent has been shown to inhibit muscle inflammation and fibrosis, and to improve muscle function and exercise endurance in *mdx* mice and GRMD dogs. Here, we show that in primary human skeletal muscle myoblasts derived from multiple donors, treatment with edasalonexent enhances their differentiation into myotubes. In an *in vitro* pro-inflammatory context, simulated by the addition of IL-1β and TNFα, myotube formation was suppressed, and treatment with edasalonexent partially rescued myotube formation. In young *mdx* mice where muscle inflammation is prominent, edasalonexent treatment reduced inflammatory infiltration in the skeletal muscle while enhancing sarcolemmal integrity. In combination with an exon-skipping agent, edasalonexent treatment further enhanced the sarcolemmal dystrophin detected in the quadriceps of *mdx* mice beyond that produced by exon skipping alone. The increase in dystrophin levels with edasalonexent combination treatment extended to the heart, a tissue known to have low efficiency of dystrophin upregulation by these agents when used alone. These results demonstrate that inhibition of NF-κB by edasalonexent in a pro-inflammatory environment enhances myotube formation *in vitro*. Furthermore, edasalonexent treatment of dystrophic *mdx* mice enhances muscle fiber integrity, and in combination with an exon-skipping agent, enhances sarcolemmal dystrophin expression.

Background

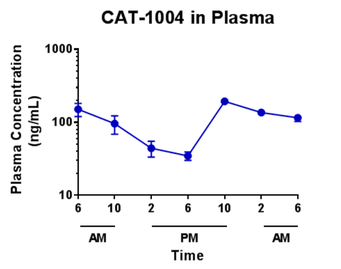
- **NF-κB driven micro-RNAs inhibit dystrophin protein translation**
- **Edasalonexent (CAT-1004) inhibits NF-κB**
- **These experiments explore the ability of edasalonexent to increase dystrophin expression in *mdx* mice in combination with the exon-skipping agent, AVI-4225**

In vivo Study Design and Experimental Procedures

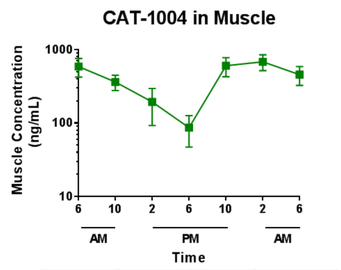
Strain	Treatment
C57BL/10 wild-type	Saline
C57BL/10 mdx	Saline
C57BL/10 mdx	AVI-4225 (40 mg/kg)
C57BL/10 mdx	CAT-1004 (1%)
C57BL/10 mdx	AVI-4225 (40 mg/kg) CAT-1004 (1%)

- *mdx* mice were dosed with CAT-1004 in diet for 7 days and plasma and muscle drug exposure was determined
- Wild-type or *mdx* mice were dosed with CAT-1004 in the diet beginning at 4 weeks of age until 10 weeks of age
- Mouse exon-23 skipping agent (PMO AVI-4225) was administered intravenously once per week
- At termination, separate pieces of muscle tissue were collected to extract mRNA, protein, and preserved for histology
- Skipping of mouse exon-23 was measured by RT-PCR
- Dystrophin protein was measured in muscle lysates by Western Blotting
- Muscle inflammation was measured in sections with H&E staining and fibrosis with Picrosirius Red staining
- Sarcolemmal dystrophin was detected in muscle sections by immunohistochemistry using a specific antibody

Pharmacokinetic and Pharmacodynamic Measurements



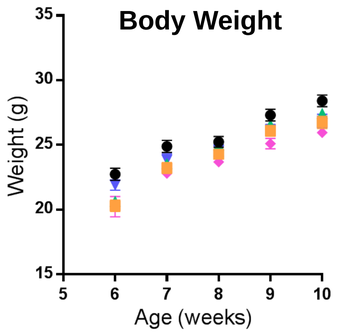
C mean (ng/mL)	C max (ng/mL)	C min (ng/mL)	AUC (hr*ng/mL)
110	194	35	2552

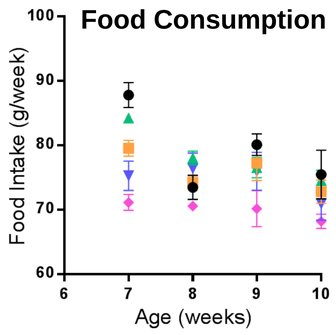


C mean (ng/mL)	C max (ng/mL)	C min (ng/mL)	AUC (hr*ng/mL)
409	684	97	9813

CAT-1004 diet administration in *mdx*

- Constant low exposure of CAT-1004 over time
- AUC comparable to clinical exposure achieved with 100 mg/kg dose
- C_{min} similar to clinical C_{min} at 100 mg/kg
- Muscle exposure ~4-fold higher than plasma exposure

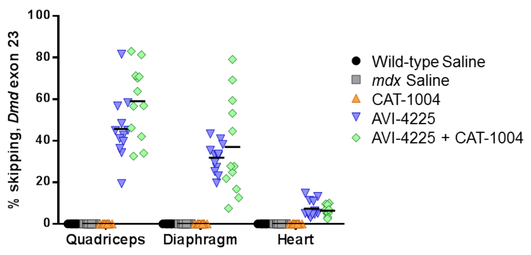


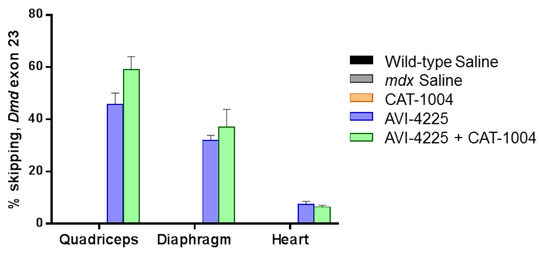


- Wild-type Saline
- *mdx* Saline
- *mdx* AVI-4225
- *mdx* CAT-1004
- *mdx* AVI-4225 + CAT-1004

No significant effect on body weight or food consumption

***DMD* Exon 23 Skipping**

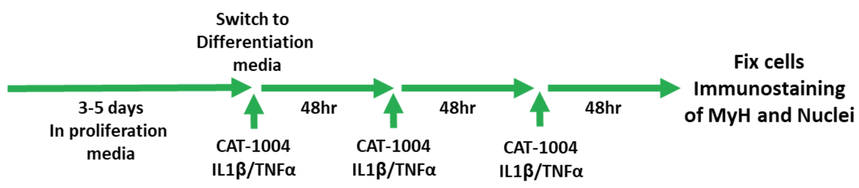


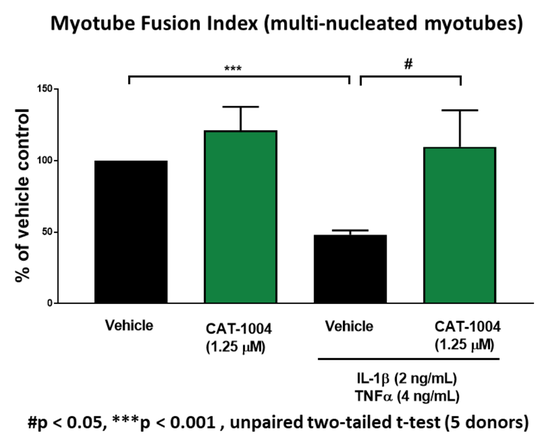


No significant effect of CAT-1004 on exon-skipped mRNA produced by AVI-4225

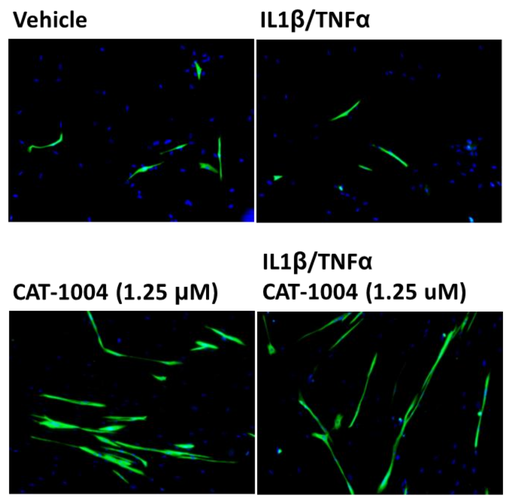
CAT-1004 Inhibits Inflammation To Promote *in vitro* Primary Human Myotube Formation

Primary human skeletal muscle myoblasts from 5 different donors were differentiated into mytobues as follows:

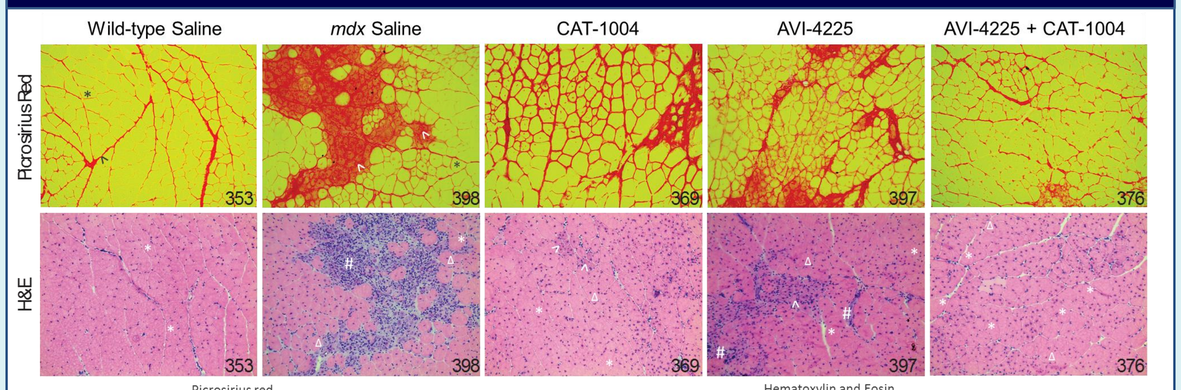




#p < 0.05, ***p < 0.001 , unpaired two-tailed t-test (5 donors)



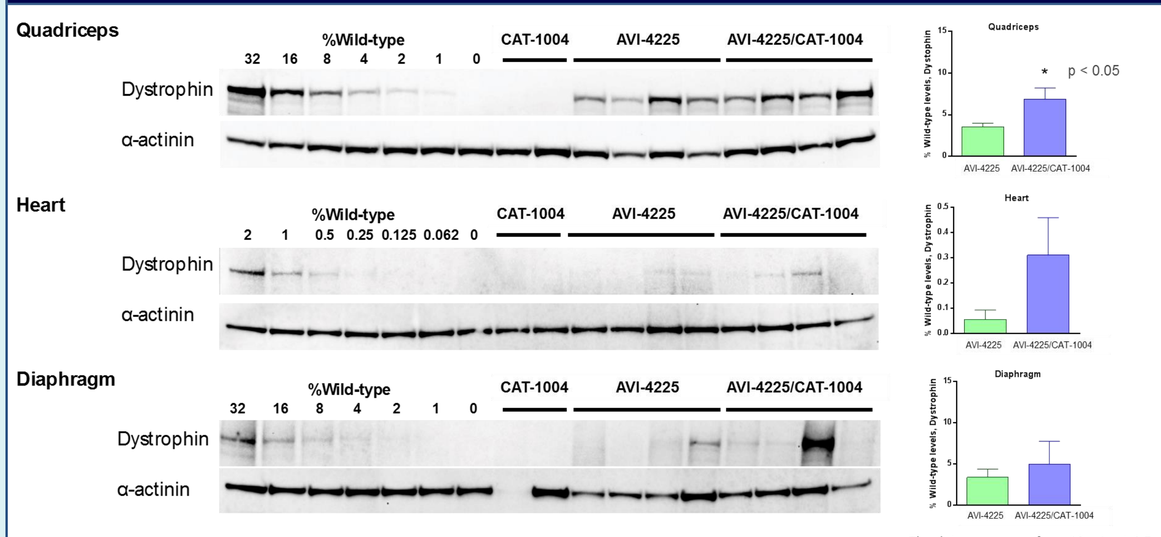
CAT-1004 and AVI-4225 Reduce Inflammation and Fibrosis in *mdx* Quadriceps



Picrosirius red
Sarcoplasm: Yellow *
Connective tissue and fibrosis(collagen): Red ^

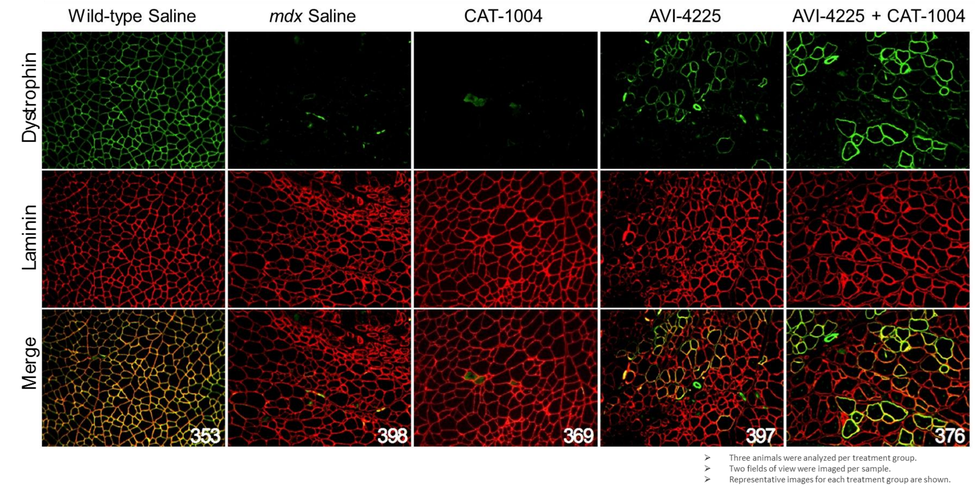
Hematoxylin and Eosin
Healthy muscle fibers: Pink. Nuclei at periphery. *
Nuclei: Blue
Infiltrating inflammatory cells: clusters of Blue #
Newly regenerated muscle fibers: Bluish pink ^
Regenerated muscle fiber: Centrally located nuclei Δ

CAT-1004 Enhances Dystrophin Protein Expression Produced by AVI-4225 in *mdx* Muscle



The data are means from 12 mice ± S.E.

Increased Dystrophin Localizes to the Sarcolemma in *mdx* Quadriceps



- Three animals were analyzed per treatment group.
- Two fields of view were imaged per sample.
- Representative images for each treatment group are shown.

Summary and Conclusions

- **Inhibition of NF-κB by edasalonexent in a pro-inflammatory context enhances myotube formation *in vitro*, and reduces inflammation and fibrosis *in vivo***
- **Inhibition of NF-κB by edasalonexent enhances protein translation and sarcolemmal expression of dystrophin produced by exon skipping with AVI-4225 in *mdx* mice**
- **These data suggest potential for combination treatment for DMD**

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