Myosin inhibitor EDG-4131 improves pathophysiology and molecular pathology in BMD model mice

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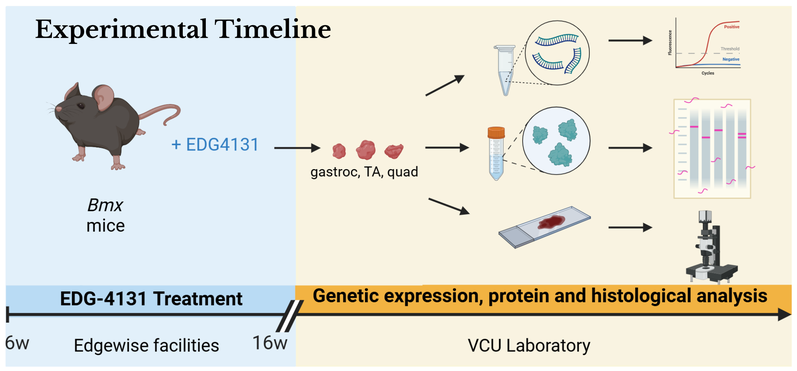
Introduction & Background

- Becker Muscular Dystrophy (BMD) is caused by in-frame dystrophin mutations, leading to a truncated, semi-functional protein, resulting in contraction-induced injury, and progressive muscle weakness.
- Our novel *bmx* mouse models BMD, mice show moderate weakness, inflammation, reduced dystrophin, and elevated dystrophin-targeting microRNAs (DTMs).
- Selective fast myosin inhibitors protect against contraction-induced injury and show promise for BMD. EDG-5506, a myosin inhibitor currently in clinical trials, shows efficacy, reducing muscle injury biomarkers.
- We previously demonstrated that vamorolone reduces inflammation and DTMs while increasing dystrophin in *bmx*. We reasoned that the myosin inhibitor EDG-4131 may act similarly to enhance dystrophin levels by reducing contraction-induced damage and DTMs.
- **Hypothesis: EDG-4131 will 1) improve *bmx* muscle pathophysiology & increase dystrophin protein levels by reducing DTMs.**

Methods



- male *bmx* mice harboring a deletion of murine *Dystrophin* exons 45-47 on a C57/BL6J background were orally treated with 40 mg/kg EDG-4131 for 6 weeks.
- Frozen muscle samples including the gastrocnemius and tibialis anterior (TA) were analyzed for downstream molecular and histopathological analysis.
- qPCR was used to measure DTMs and inflammatory transcripts
- Frozen muscles were sectioned for immunofluorescence and histological (H&E) analysis.
- Samples were lysed in a high SDS buffer for protein and capillary Western immunoassay (Jess, Protein Simple) was performed to quantify dystrophin protein levels.

Results

EDG-4131 reduces muscle pseudohypertrophy

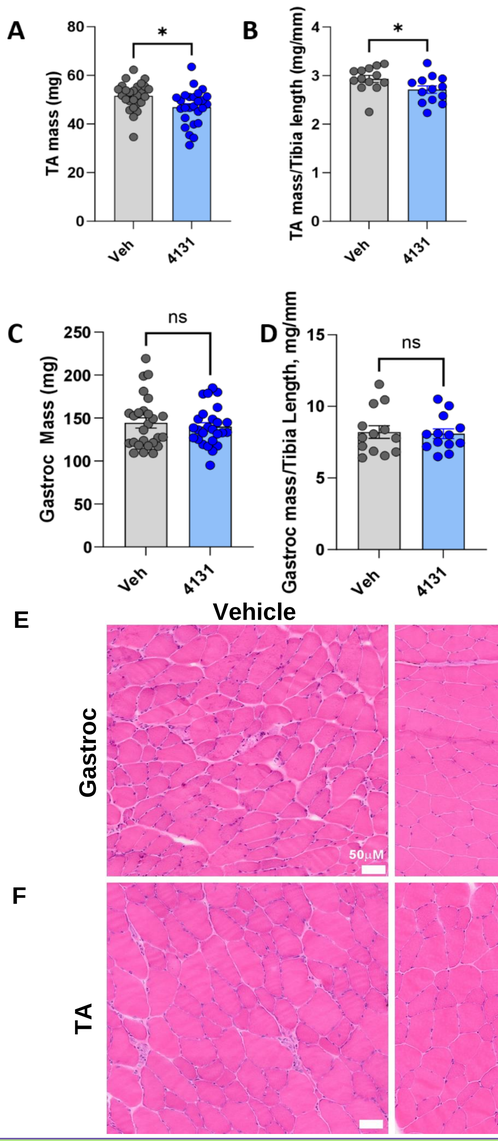


Figure 1. EDG-4131 reduces *bmx* muscle pseudohypertrophy and inflammation. (A,C) Absolute mass of muscle. (B,D) Mass normalized to tibia length. (E,F)Hematoxylin and eosin staining of (E) gastrocnemius and (F) tibialis anterior of vehicle (left) and EDG-4131 (right)-treated mice.

EDG-4131 reduces expression of some dystrophin-targeting microRNAs in *bmx* skeletal muscle

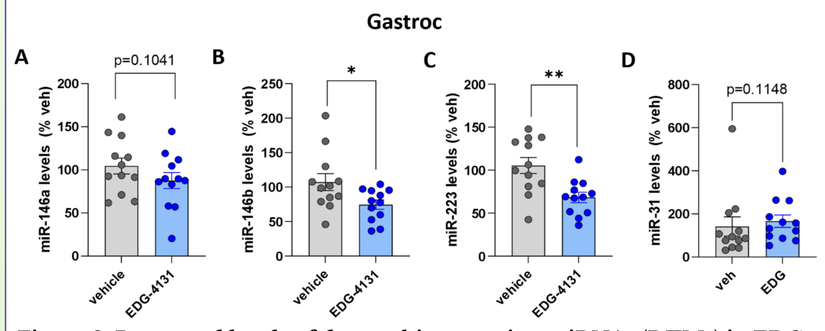


Figure 2. Decreased levels of dystrophin-targeting miRNAs (DTMs) in EDG-4131 treated mice. (A-D) qPCR indicated miRNA in vehicle or EDG-4131 treated gastrocnemius muscle. Tibilialis anterior data can be found in Supplemental Data in QR code.

EDG-4131 reduces inflammatory transcripts in *bmx* skeletal muscle

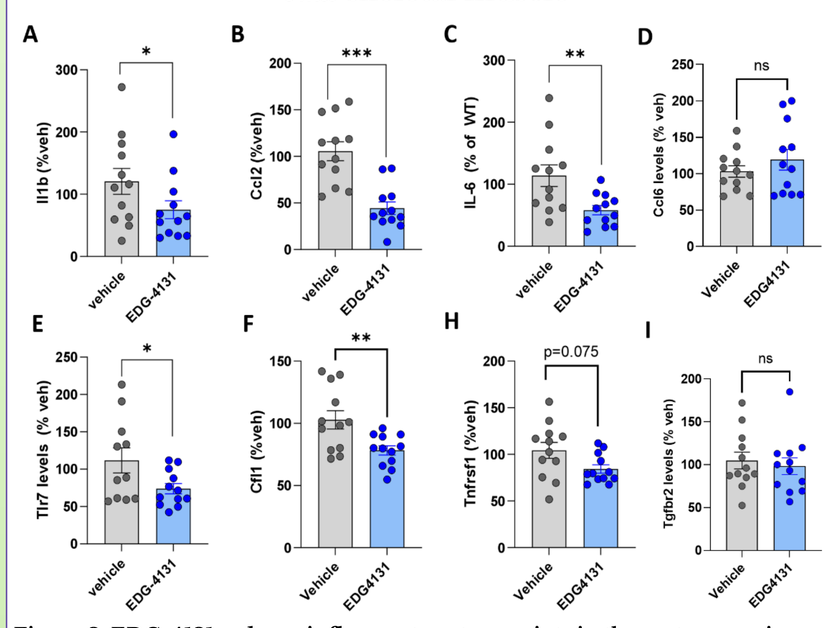


Figure 3. EDG-4131 reduces inflammatory transcripts in the gastrocnemius of *bmx* mice. (A-I) qPCR of indicated inflammatory transcripts in gastroc muscles show reductions with EDG-4131 treatment. Data for inflammatory transcripts in tibialis anterior can be found in Supplementary Data QR code.

EDG-4131 treatment may stabilize structural proteins in muscle

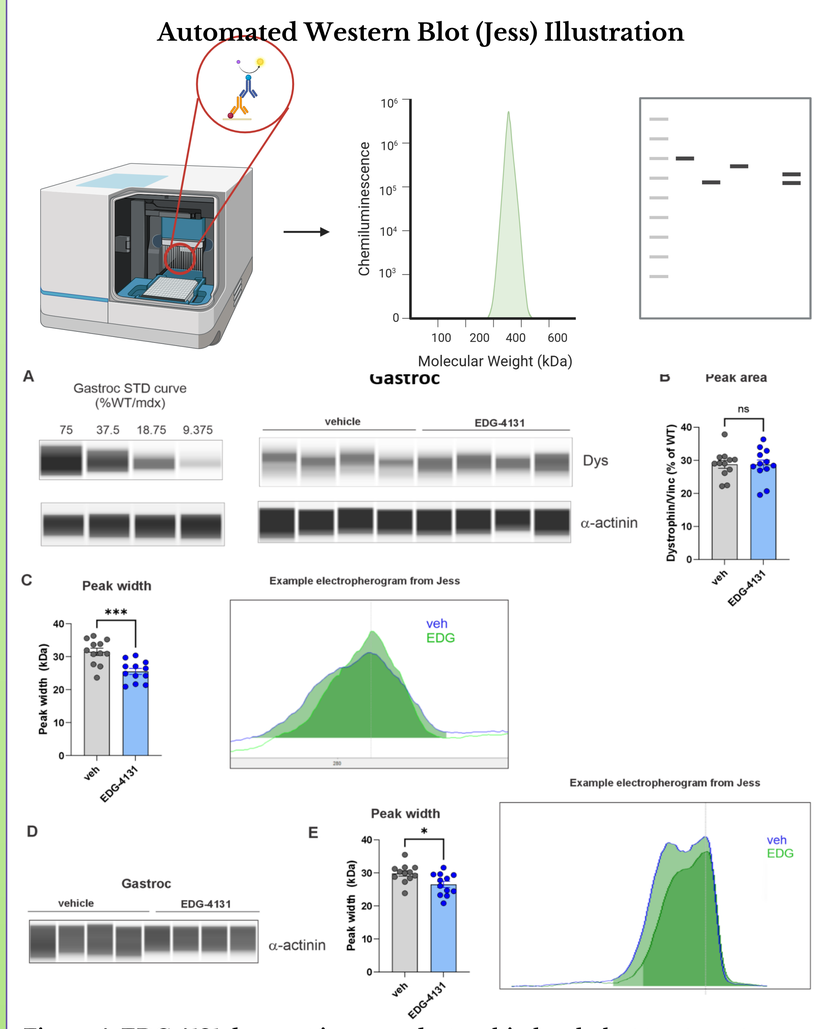


Figure 4. EDG 4131 does not increase dystrophin levels, but treatment shows a more concentrated band/peak in gastrocnemius. Dystrophin protein levels were assayed in the gastrocnemius via Jess capillary-based immunoassay (A) Gastrocnemius dystrophin levels in %WT/mdx standard curve and dystrophin protein levels in vehicle and EDG-4131 treated *bmx* mice. Virtual Jess blot shows 4 representative samples per group. (B) Quantification of dystrophin peak area normalized to α-actinin - %Dystrophin was calculated using the derived formula from standard curve. (C) Dystrophin peak width is significantly reduced with EDG-4131-treated muscles. Quantification (left) of peak width and representative electropherogram (right). (D) Representative α-actinin virtual blot samples illustrating that another structural protein, α-actinin shows an increased peak width and is reduced with EDG-4131. (E) Peak width quantification (left) and representative electropherogram (right). Tibilialis Anterior Data may be found in Supplemental Data QR code.

EDG-4131 treated muscles show visually brighter and more distinct dystrophin staining and reduced myofiber size variability

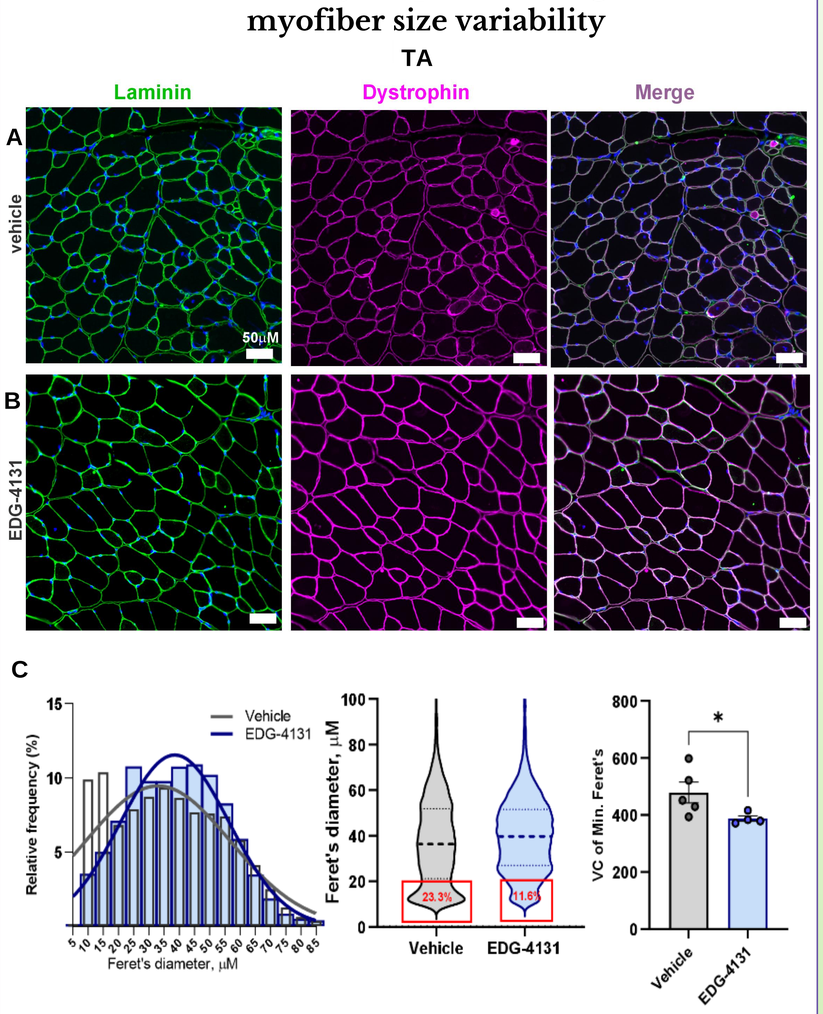


Figure 5. Dystrophin immunostaining is visually brighter in EDG-4131 treated tibialis anterior of *bmx* mice. (A,B) Dystrophin immunostaining (purple) was performed on vehicle & EDG-4131-treated TA sections. Scale bar = 50μm. (C) Left; Histogram of minimal Feret's diameter; Middle; Violin plot of minimal Feret's diameter; right; variance coefficient (VC) of minimal Feret's diameter. Additional myofiber analysis may be found in Supplemental Data QR Code.

Preliminary data: the ubiquitin ligase Trim32 is elevated in BMD muscle and reduced by EDG-4131

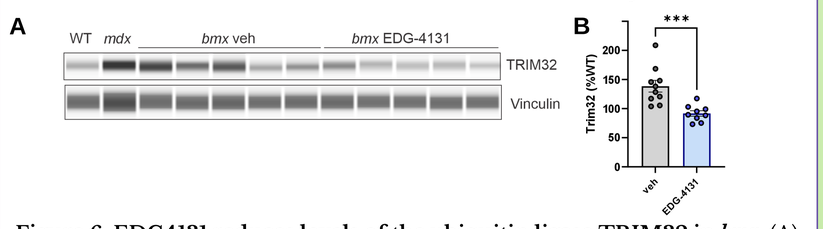


Figure 6. EDG4131 reduces levels of the ubiquitin ligase TRIM32 in *bmx*. (A) Virtual jess blot shows elevated TRIM32 in *mdx* and *bmx*; EDG4131 administration reduces levels toward WT (B) Chemiluminescence quantification (area under the curve) of Trim 32; results were normalized to WT and reported as %WT.

Conclusions

- EDG-4131 shows protective effects in *bmx* muscles resulting in improved muscle pathophysiology.
- Treatment reduces inflammatory gene expression, likely via reduction of contraction-induced damage.
- Overall dystrophin protein levels remained similar, however analysis of peak width and immunofluorescence suggest EDG-4131 may stabilize key sarcomeric and membrane proteins (dystrophin, α-actinin). We hypothesize this may be linked to differential ubiquitination and protein turnover.
- EDG-4131 reduces muscle turnover as demonstrated by reduction of small myofibers (Feret's diameter analysis)

Future Directions

- Histological analysis of gastroc muscles still in progress.
- Ongoing experiments will explore the mechanisms by which treatment stabilizes sarcomere and sarcolemma proteins and reduce proteosome and Trim32 activity.

Acknowledgements

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Additional information

QR Codes

- Supplemental Information:
- References:

Contact Information

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