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# An Investigation of Dasatinib and Quercetin as Enhancers of Myofiber Regeneration in DMD

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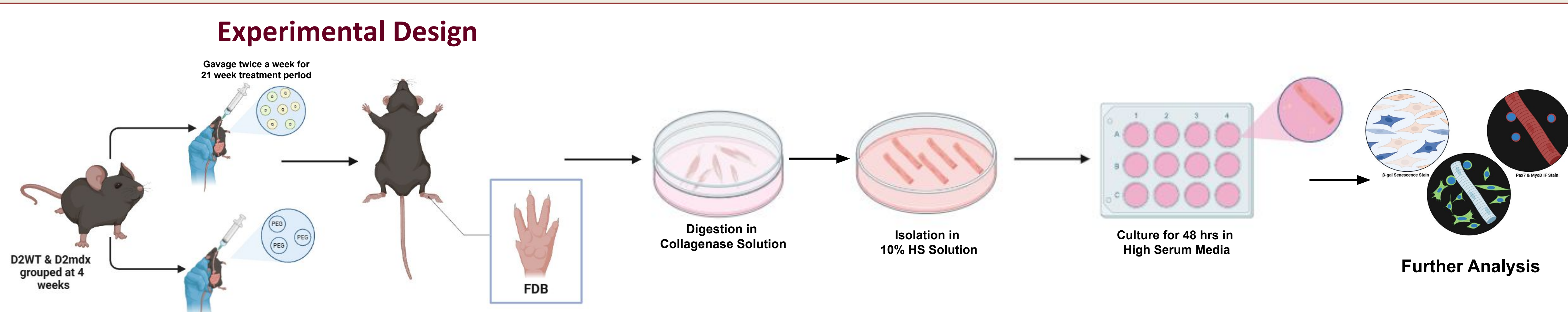
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## Introduction

- Duchenne Muscular Dystrophy (DMD) is a rare autosomal recessive disorder that results in the cell's inability to produce functional dystrophin. Muscle cells progressively degenerate due to stem cell exhaustion heavily impairing the regenerative capacity of muscle.
- As muscle cells are progressively lost and replaced with fat deposits and fibrosis, secondary mechanisms of the disease give rise to phenotypes associated with premature aging that become increasingly severe throughout the lifetime of a DMD patient.
- Damaged muscle cells become senescent. Senescent cells accumulate in muscle tissue causing inflammation, muscle atrophy, and reduced regeneration.
- While disease mechanisms are well documented, advancements in treatment options are in demand to mediate premature aging phenotypes and senescence for patients that do not qualify for gene therapies.
- Dasatinib and quercetin (D+Q) has been used synergistically to reduce senescence and inflammation in cancer therapy by initiating apoptosis in senescent cells.
- Observing muscle progenitor cell proliferation patterns may give insight into drug effects on myofiber regenerative capacity.

## Objectives

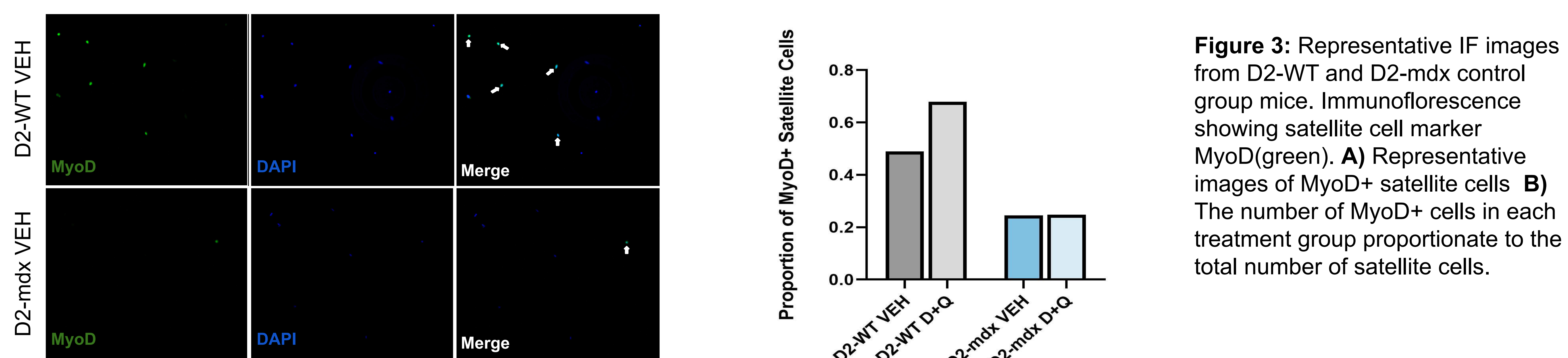
- This study aims to test the efficiency of D+Q in improving the regenerative capacity in DMD mouse (D2-mdx) myofibers.
- We hypothesized that the D+Q treatment would reduce inflammation in muscle tissue therefore enhancing satellite cell proliferation, a critical mechanism for effective muscle regeneration.



**Figure 1:** Experimental procedure of drug administration and myofiber isolation. Mice were administered Dasatinib (5 mg/kg) and Quercetin (50 mg/kg), dissolved in 10% polyethylene glycol 400, by gavage twice a week. Flexor digitorum brevis (FDB) muscles were harvested from 25 week old mice, then placed in 0.2% w/v collagenase solution to digest the ECM. Myofibers were then isolated by agitating FDB muscles in 10% HS. Myofibers were then cultured in a high serum environment to encourage satellite cell proliferation. Photos were taken at 24 and 48 hours of culturing. Cells were then fixed, then stored for further analysis.

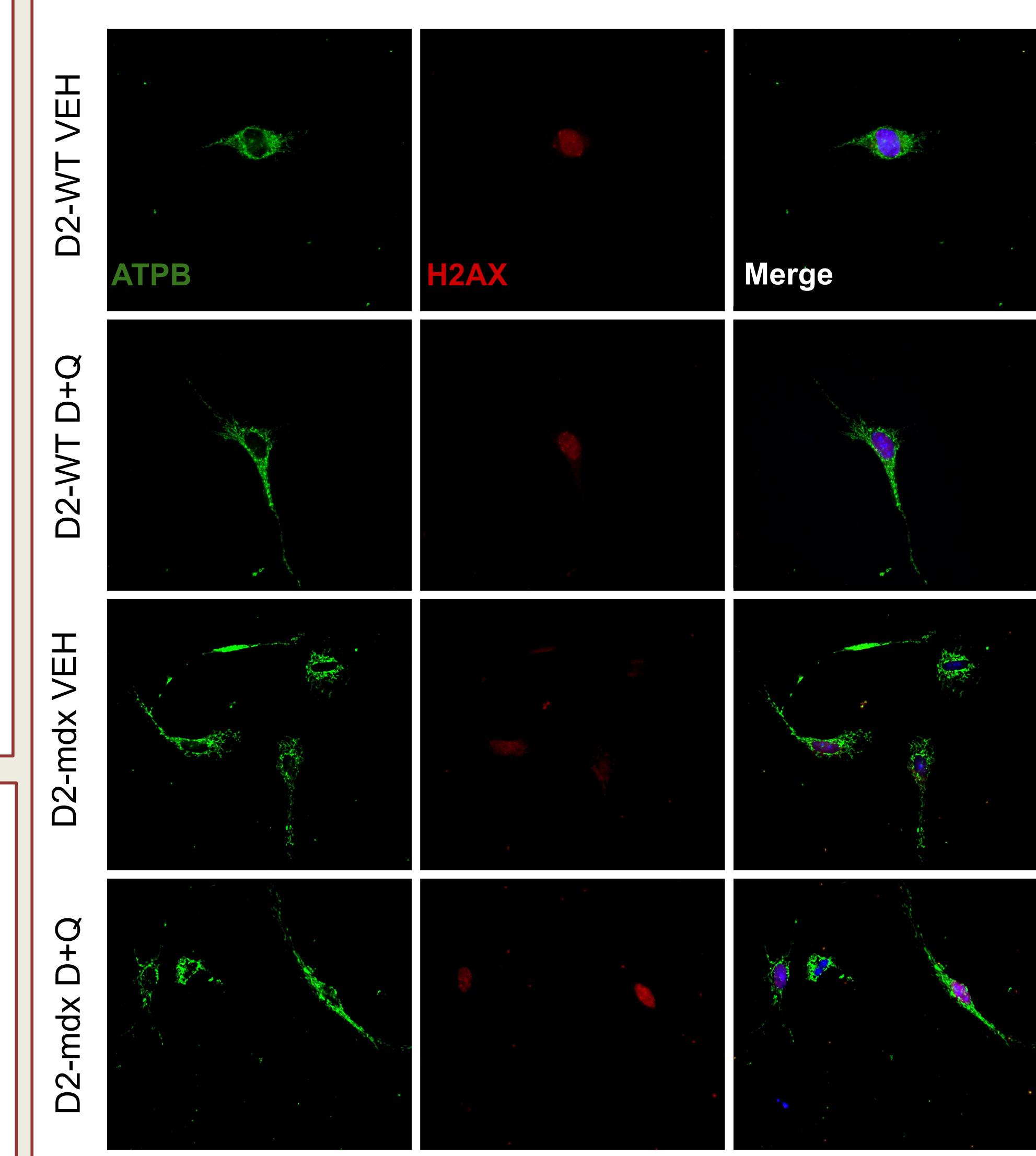


**Figure 2:** Myofiber images from each treatment group were captured at 24 and 48 hours. Satellite cells on the fibers were evaluated for proliferation patterns, and the number of visibly deattached cells was quantified. **A)** Representative images of cultured myofibers at 24 and 48 hours. **B)** No significant differences ( $p < 0.05$ ) were observed in the number of migrated satellite cells at 24 hours, however, both D+Q-treated groups exhibited increased cell proliferation. **C)** No significant differences ( $p < 0.05$ ) were observed in the number of migrated satellite cells at 48 hours between the control and D+Q treated D2-mdx groups. The number of migrated cells in the D2-WT control was significantly higher ( $p < 0.01$ ) than the D2-mdx D+Q treated group. (Mean  $\pm$  SEM; \* $p < 0.05$ , \*\* $p = 0.01$ , \*\*\* $p = 0.001$ , \*\*\*\* $p < 0.0001$ )



**Figure 3:** Representative IF images from D2-WT and D2-mdx control group mice. Immunofluorescence showing satellite cell marker MyoD (green). **A)** Representative images of MyoD+ satellite cells **B)** The number of MyoD+ cells in each treatment group proportionate to the total number of satellite cells.

## RESULTS



**Figure 4:** Satellite cells were stained for ATPB (green) and H2AX (red). All cells show signs of DNA damage, potentially due to mice age. Further fluorescence intensity analysis must be conducted to obtain conclusive results on.

## CONCLUSIONS & FUTURE DIRECTION

- Overall, fibers from D+Q treated mdx mice did not have a significant differences in the number of cells that migrated away from the fibers compared to their respective untreated mdx mice.
- This study is ongoing and the fibers are being further evaluated for differences in satellite cell content using immunofluorescence markers.
- These studies will guide selection of future pharmaceutical-based approaches targeting the senescence markers to be used independently or as a complement to other molecular therapies aiming to reduce the severity of DMD and improve quality of life for patients.