Development of an RNAi Therapeutic, ARO-DUX4, for the Treatment of FSHD

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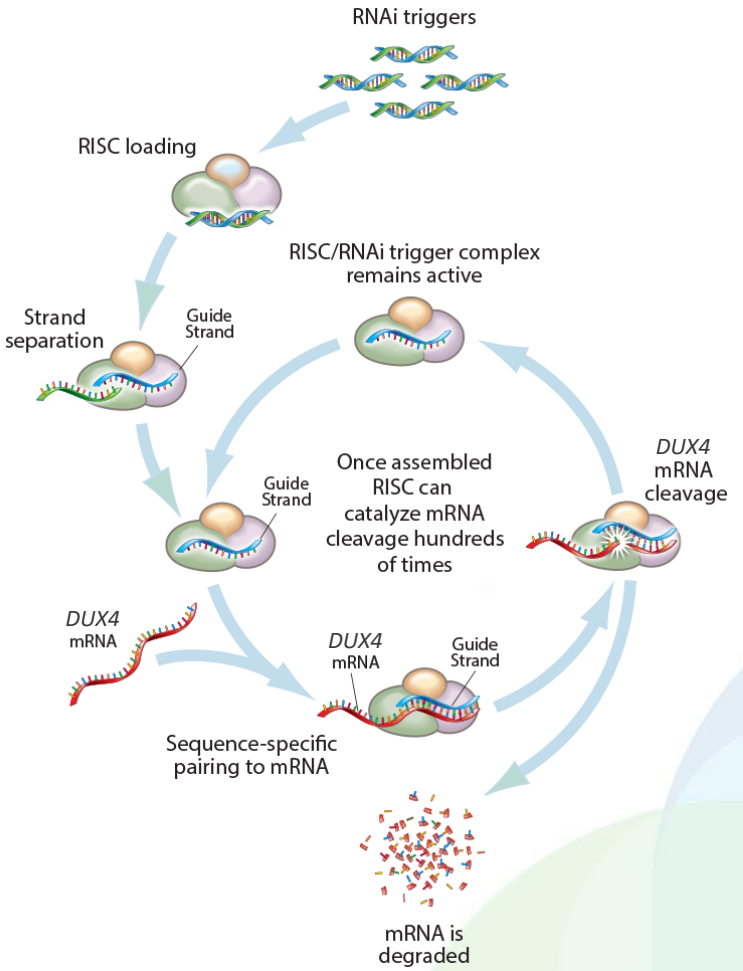
arrowhead pharmaceuticals

DUX4: A good target for RNAi

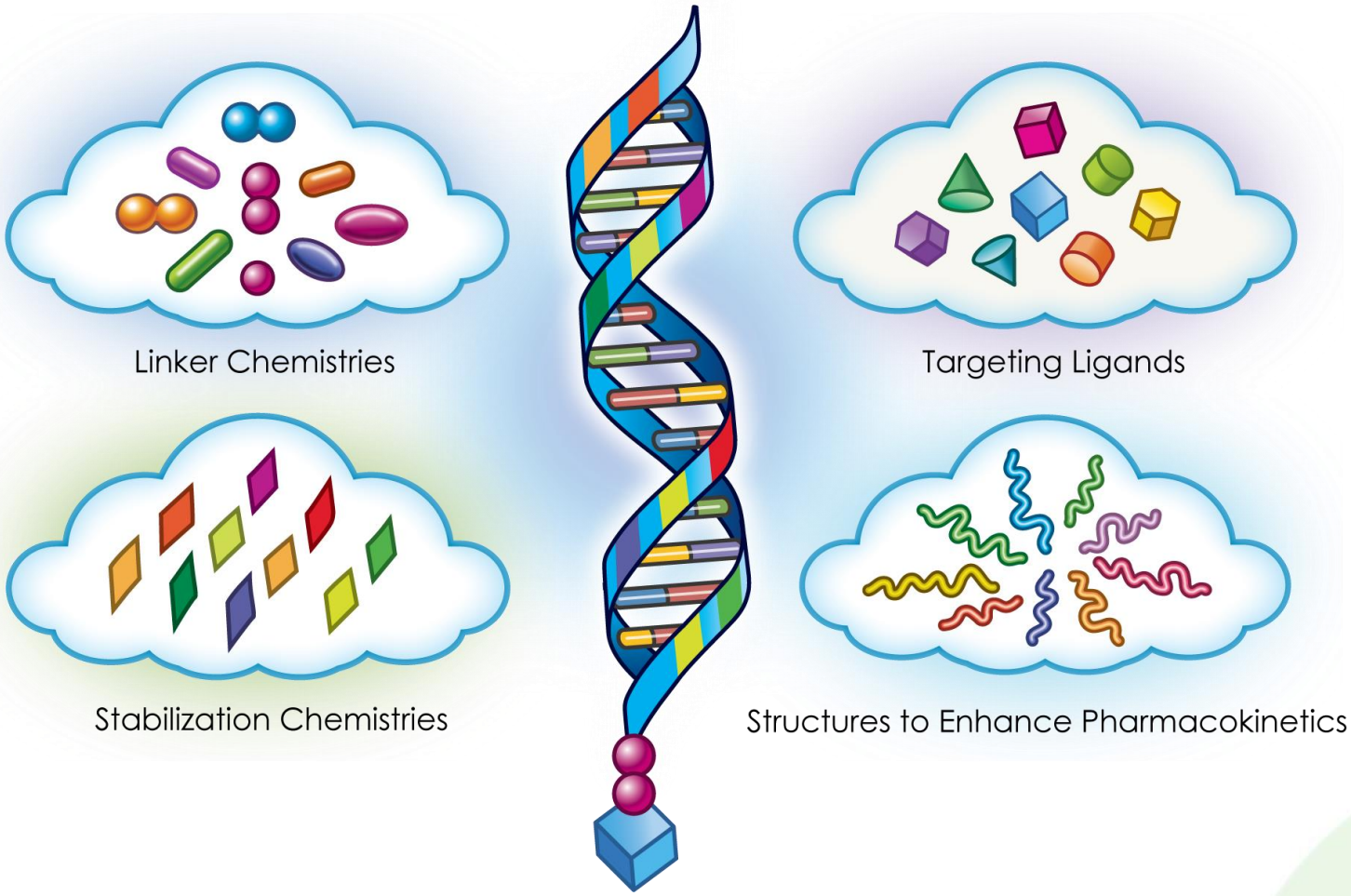
Facioscapulohumeral Muscular Dystrophy caused by misexpression of DUX4, a normally repressed transcription factor.

Misexpression results in alterations of DUX4 target gene expression and myotoxicity.

Direct RNAi knockdown of DUX4 is an effective and safe approach to treatment since DUX4 has no known physiological function in normal adult skeletal muscle.



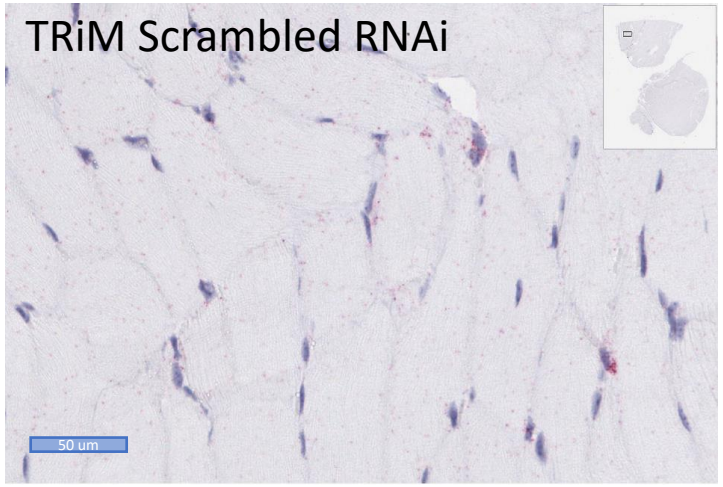
TRiM Platform targeting DUX4 in Skeletal Muscle

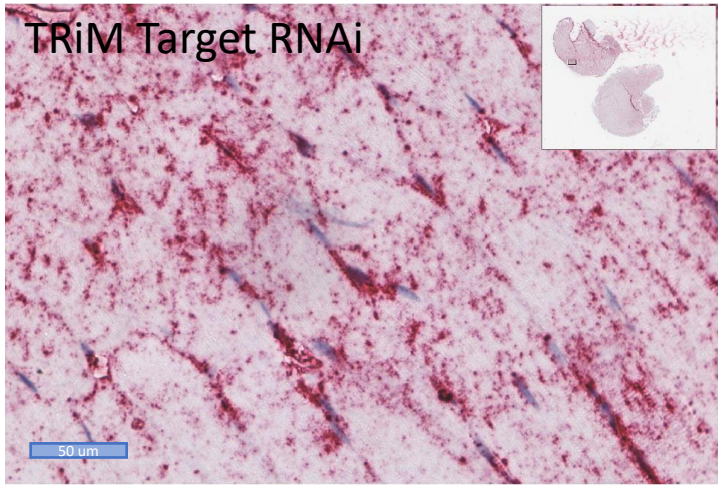


TRiM Platform Muscle Delivery

TRiM Muscle Platform Delivery
Mouse

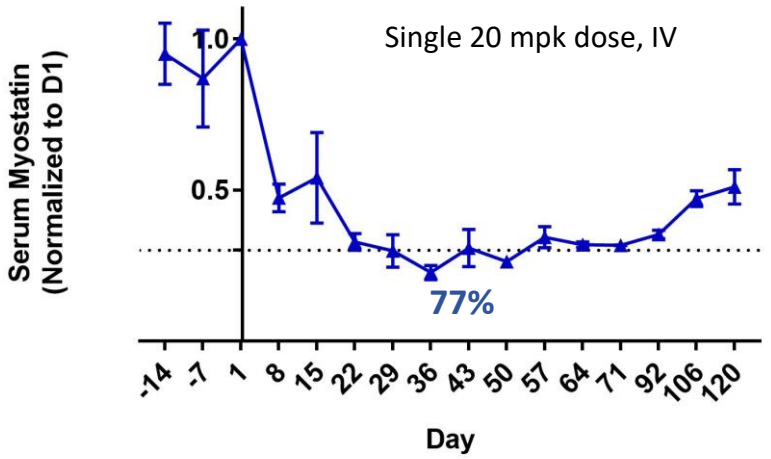
- 3 mpk, IV
- RNAscope detecting RNAi
- Gastrocnemius
- 76 – 99% myofibers contain TRiM RNAi

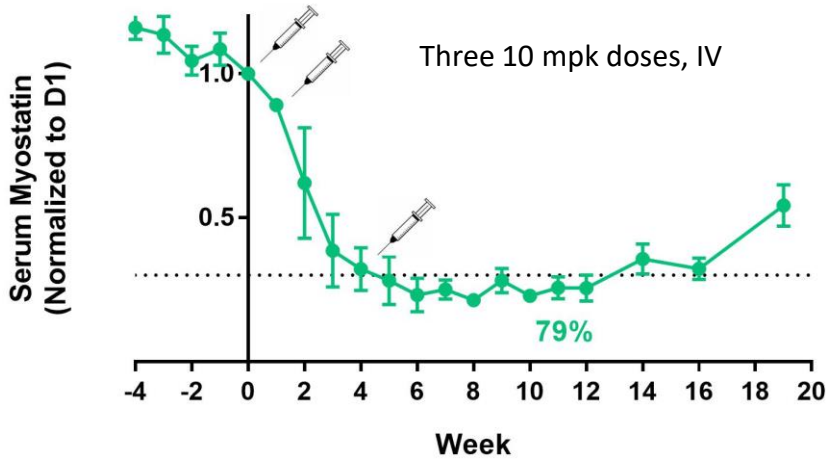




TRiM Muscle Platform Duration

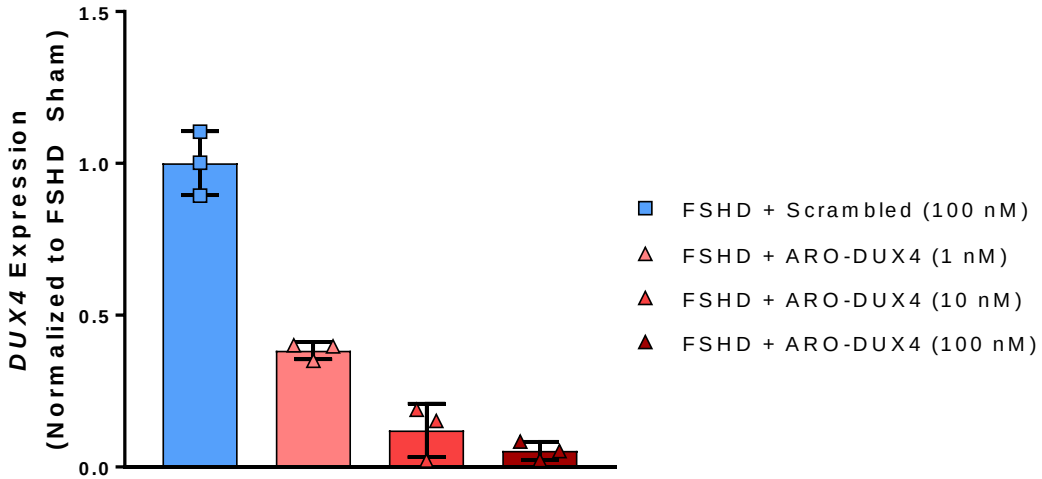
- NHP
- Surrogate RNAi Targeting Myostatin
- Single 20 mpk dose achieved 77% KD of serum myostatin
- Three 10 mpk doses (Days 1, 7 & 28) achieved >70% KD through Week 12



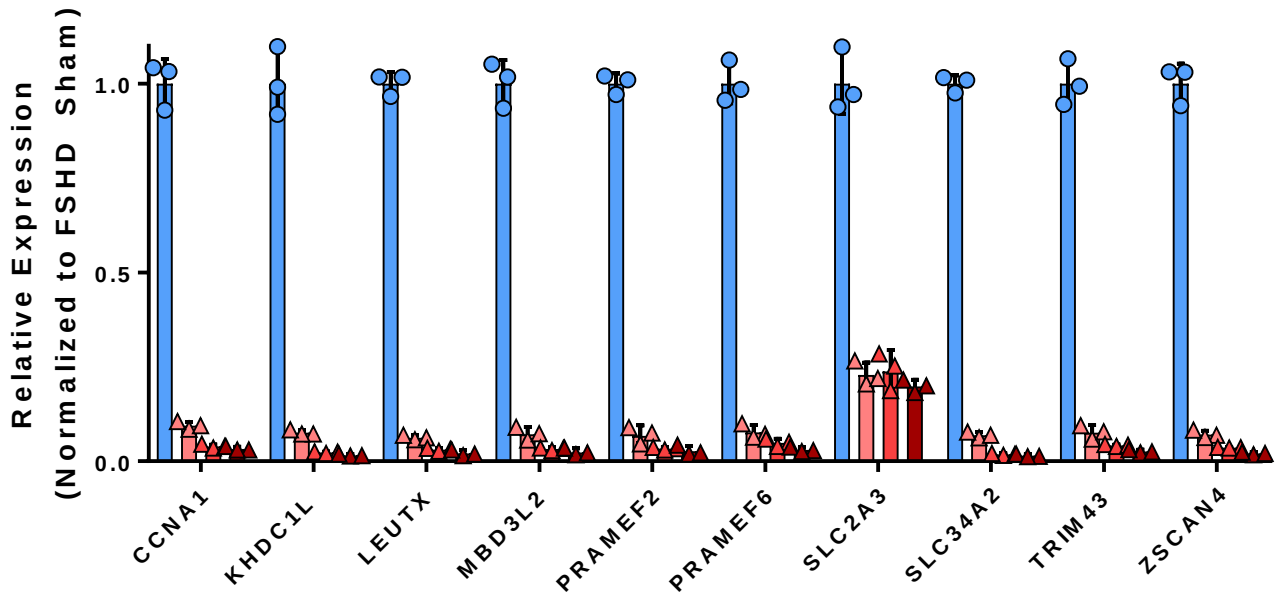


DUX4 Knockdown in Patient-derived Myotubes

DUX4



DUX4 Target Genes



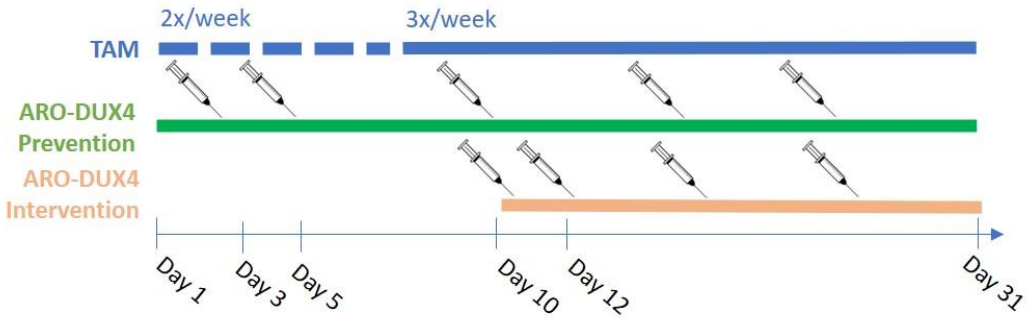
- ARO-DUX4 achieved dose-dependent knockdown of *DUX4* and deep reduction of DUX4 target gene expression in differentiated FSHD patient-derived myotubes.

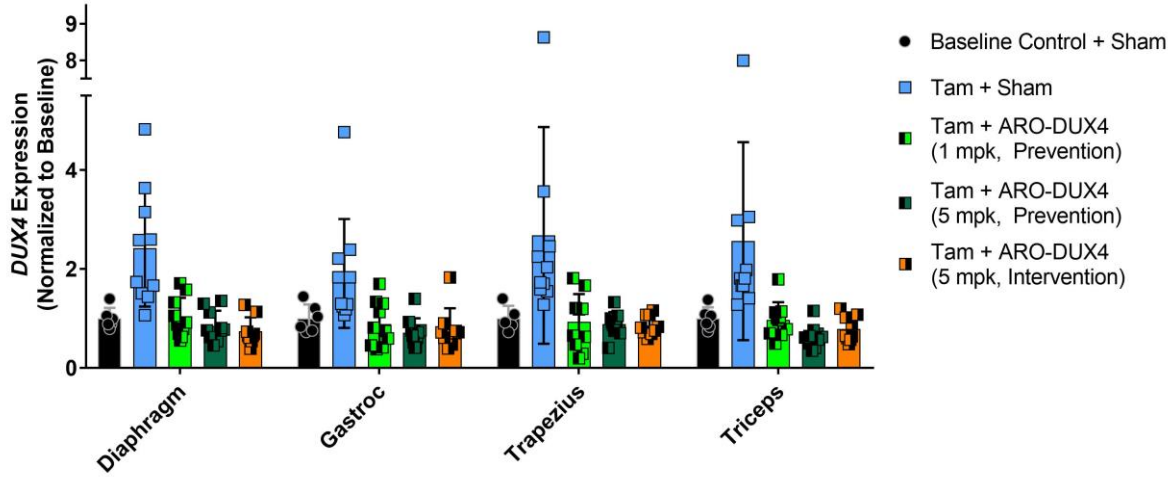
DUX4 Knockdown in Transgenic FSHD-like Mouse Model

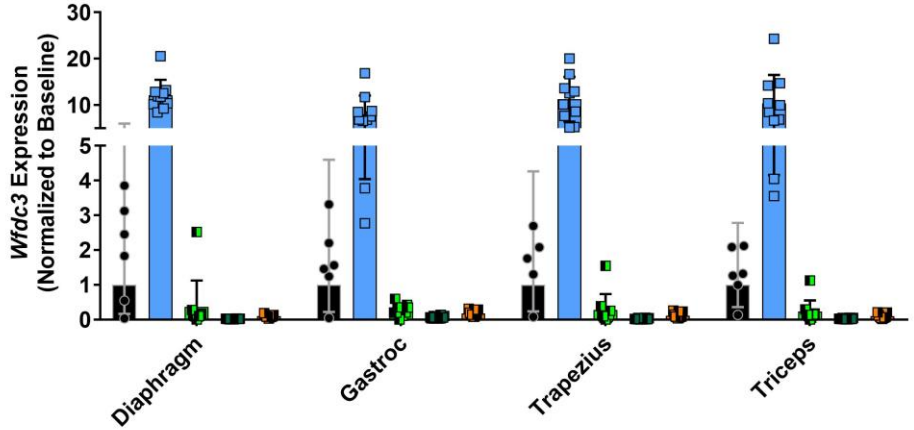
HSA-MCM/FLExDUX4 Mice

- Created by Peter and Takako Jones
- Tamoxifen-controlled, skeletal muscle-specific expression of human DUX4
- Increased expression of DUX target genes
- Develop FSHD-like muscle phenotype and functional loss
- Known "leaky" DUX4 expression in uninduced animals

ARO-DUX4 prevented and reversed tamoxifen-induced increase in *DUX4* and DUX4 target gene expression.



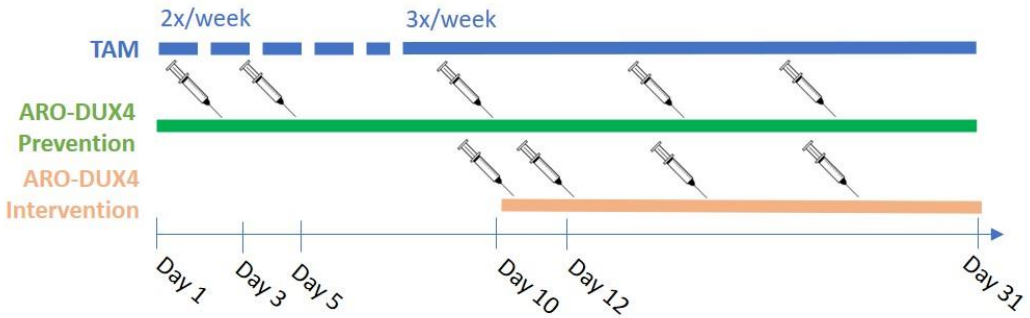


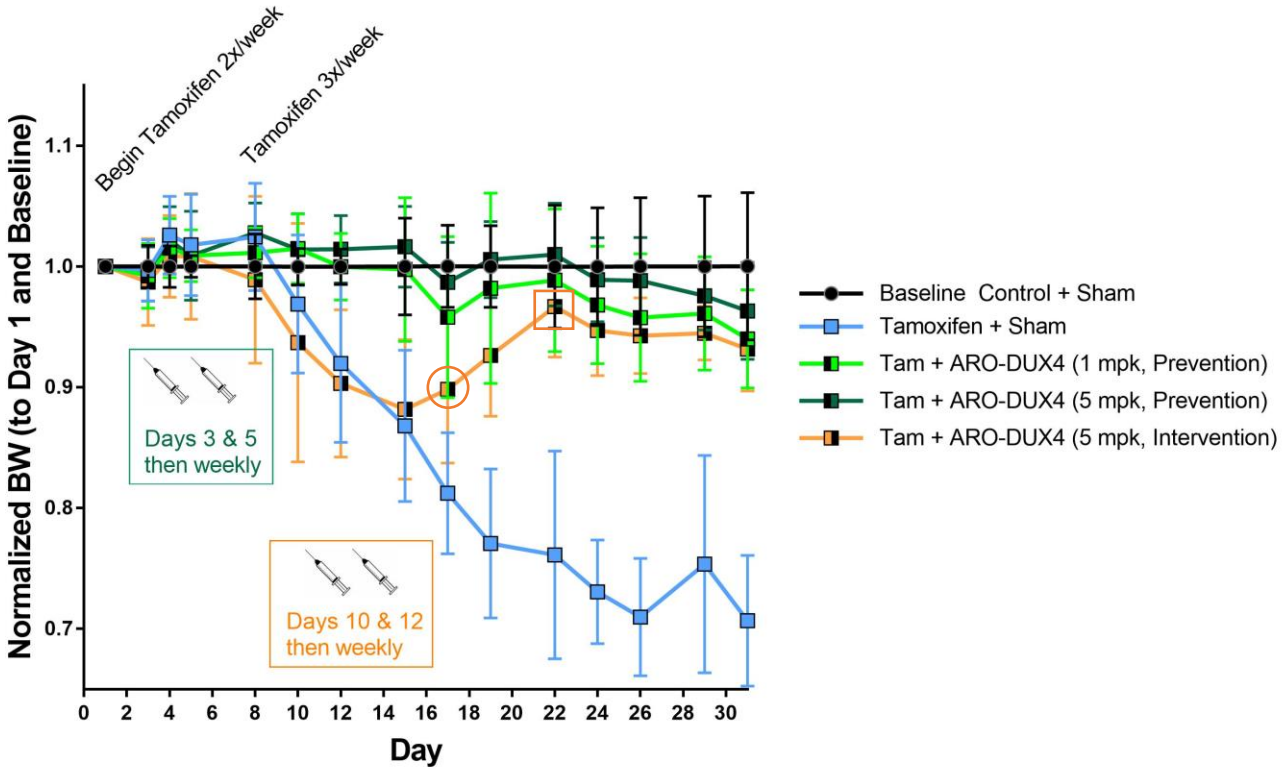


Prevention and Reversal of FSHD-like phenotype: Bodyweight

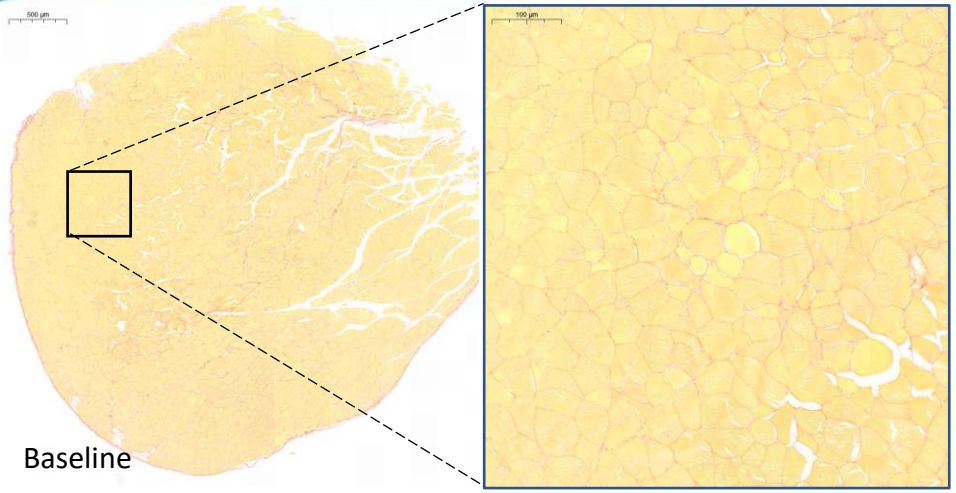
In HSA-MCM/FLExDUX4 mice, induced DUX4 expression resulted in significant BW loss apparent by Day 10

- ARO-DUX4 treatment:
  - Prevented DUX4-induced BW loss.
  - Reversed DUX4-induced BW loss by Day 17 allowing a return to baseline BW by Day 22.

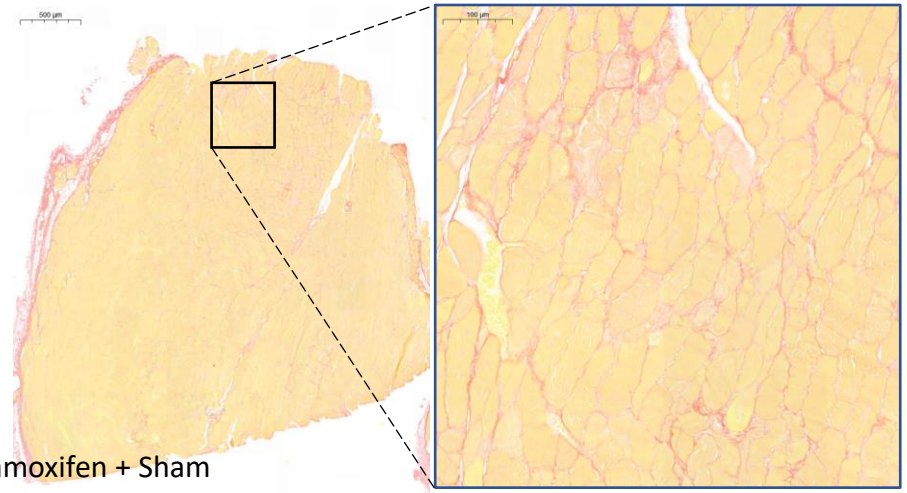




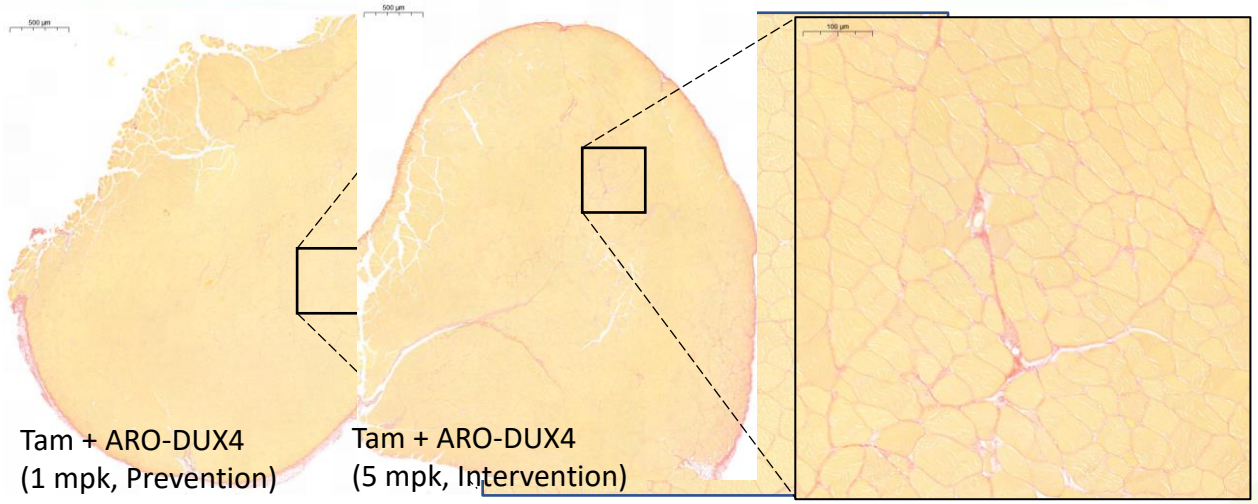
Prevention and Reversal of FSHD-like phenotype: Muscle fibrosis



Baseline

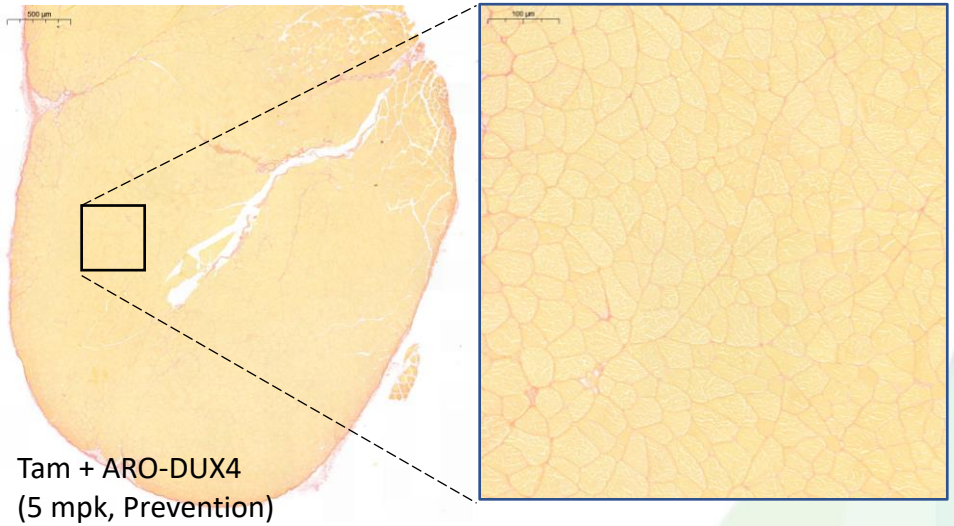


Tamoxifen + Sham



Tam + ARO-DUX4
(1 mpk, Prevention)

Tam + ARO-DUX4
(5 mpk, Intervention)

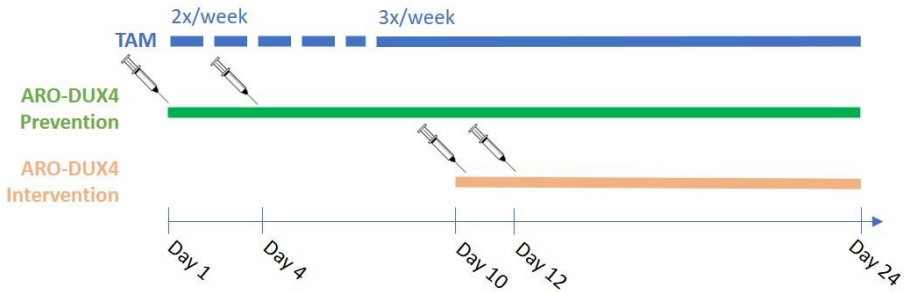


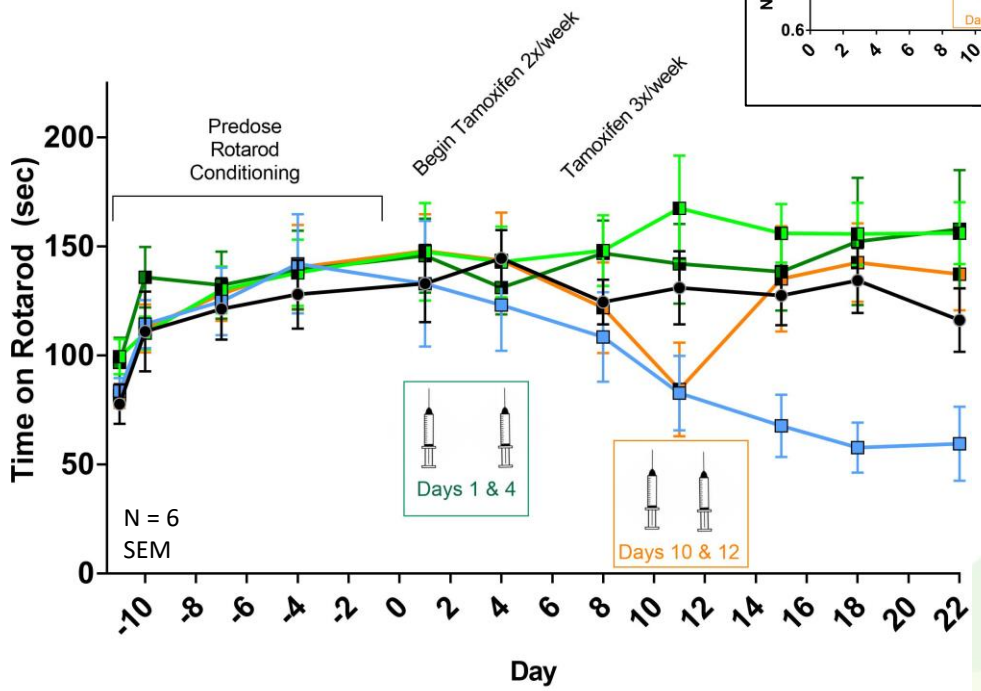
Tam + ARO-DUX4
(5 mpk, Prevention)

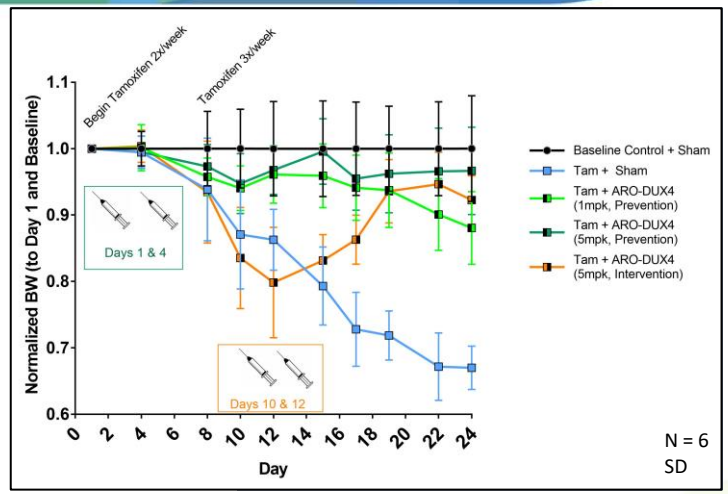
Day 31, Triceps

Prevention and Reversal of FSHD-like phenotype: Rotarod Functional Assay

- Tamoxifen induced DUX4 expression resulted in reduced Rotarod performance by Day 11
- ARO-DUX4 prevented Rotarod performance loss
- ARO-DUX4 reversed Rotarod performance loss by Day 15.







Summary

- TRiM platform delivers siRNA to myofibers with deep target knockdown lasting at least 3 months in NHP.
- ARO-DUX4 silences misexpressed *DUX4* and corrects the altered expression of DUX4 target genes in FSHD patient-derived myotubes.
- In HSA-MCM/FLExDUX4 mice, a transgenic FSHD-like mouse model, ARO-DUX4 knocks down *DUX4* and its target genes.
- ARO-DUX4 prevents and reverses the DUX4-induced
  - BW loss
  - muscle fibrosis,
  - Impaired rotarod performance
- Regulatory filings to enable clinical trials with ARO-DUX4 are expected to commence in Q3.

Acknowledgements

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