The FORCE™ Platform Achieves Robust and Durable DUX4 Suppression and Improves Muscle Function in a Facioscapulohumeral Muscular Dystrophy Mouse Model

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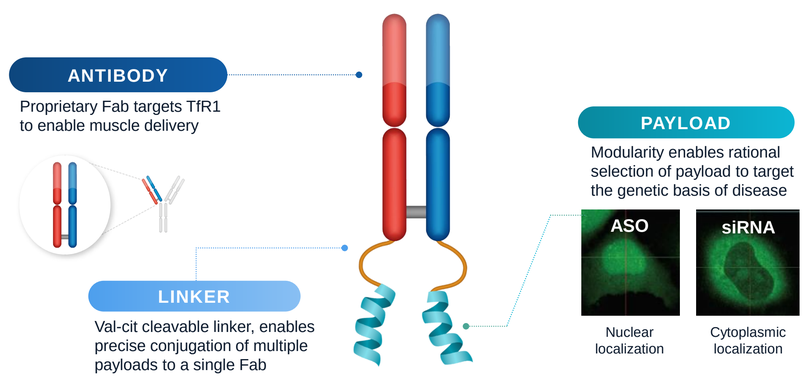
BACKGROUND

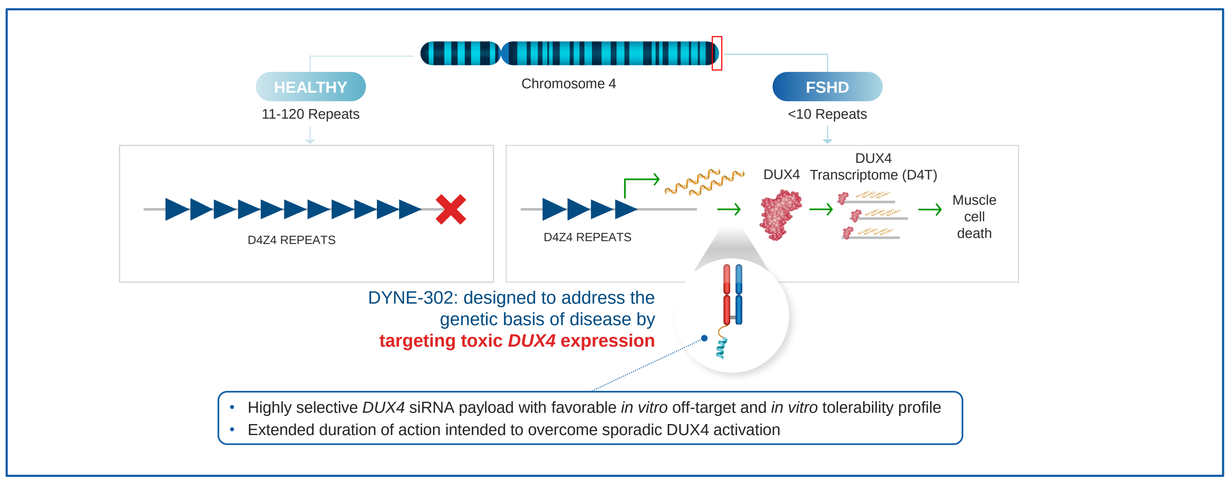
The FORCE platform was designed to allow effective delivery of therapeutics to muscle tissue through transferrin receptor type 1 (TfR1). The platform consists of 3 components: the Fab portion of an antibody directed against TfR1, a therapeutic payload, and a linker to connect them (Fig. 1). Facioscapulohumeral Dystrophy (FSHD) is a severe muscle disorder resulting from aberrant *DUX4* mRNA expression in skeletal muscle (Fig. 2). DUX4 expression activates a set of downstream genes collectively known as the DUX4 transcriptome (D4T) and leads to progressive myofiber loss and debilitating weakness. We leveraged the FORCE platform to develop DYNE-302 to deliver an siRNA designed against *DUX4* mRNA to skeletal muscle. The DYNE-302 siRNA payload is highly specific for *DUX4* mRNA and DYNE-302 demonstrated high *in vitro* potency in FSHD patient-derived myotubes. To establish the in vivo efficacy of DYNE-302, we crossed mice expressing human TfR1 (hTfR1) with mice expressing tamoxifen-inducible human DUX4 (iFLExD). The resulting hTfR1/iFLExD mice develop a slowly progressive myopathy due to sporadic DUX4 expression, or an acute myopathy with impaired muscle function upon tamoxifen induction of DUX4. A single intravenous dose of DYNE-302 resulted in robust D4T inhibition that lasted up to 3 months, with reduced myofiber pathology. Moreover, DYNE-302 demonstrated profound benefit on muscle function in the tamoxifen-induced acute model. Our data provide the preclinical foundation for therapeutic development of DYNE-302 for the treatment of FSHD.

Figure 1. Dyne FORCE Platform: Modern Therapeutics for Muscle Diseases



The FORCE platform consists of 3 components: antibody, linker, and payload. The platform allows delivery of therapeutics to muscle tissue through TfR1. DYNE-302 uses the FORCE platform to deliver an siRNA targeted to *DUX4* as the payload. Adapted from (1).

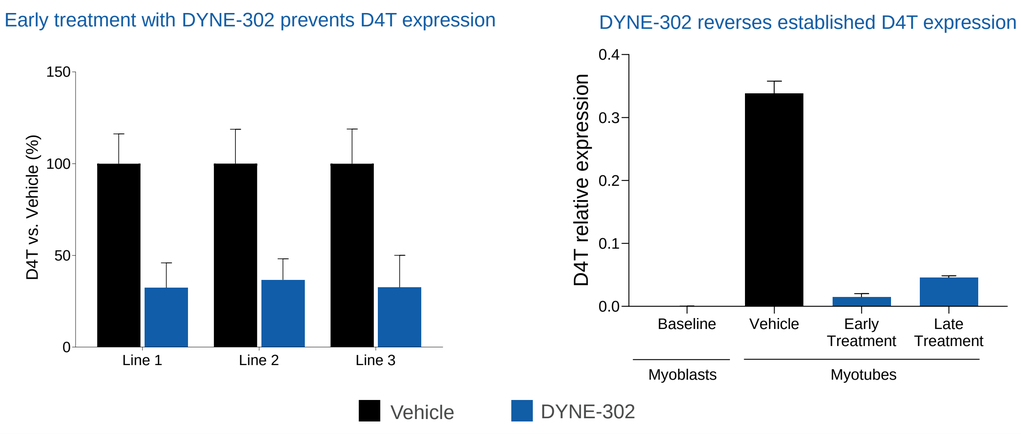
Figure 2. DYNE-302 Targets the Genetic Basis of FSHD



FSHD results from aberrant activation of the transcription factor DUX4 in skeletal muscle through either contraction of D4Z4 repeats (FSHD Type 1, 95% of cases) or through mutations in genes that silence the locus (FSHD Type 2, 5% of cases). Activation of DUX4 triggers expression of DUX4 target genes (D4T) and initiates a cascade that ultimately leads to skeletal muscle death. The siRNA component of DYNE-302 targets *DUX4* directly, blocking the most proximal cause of FSHD.

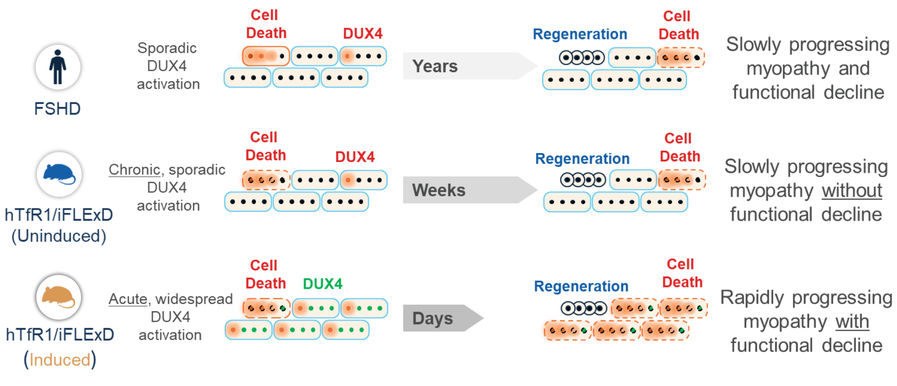
RESULTS

Figure 3. DYNE-302 Suppresses D4T Expression *in vitro* in FSHD Myotubes



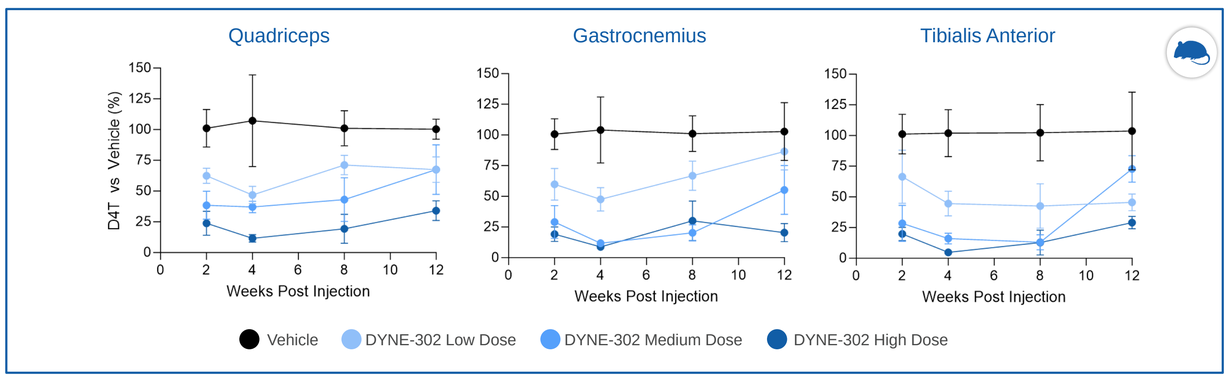
DYNE-302 added to patient-derived primary myoblasts at day 0 (early treatment, left and right graphs) or day 5 (late treatment, right graph) post-differentiation. Data are mean + SD; n = 3; D4T (DUX4 transcriptome) is the mean *of MBD3L2*, *TRIM43*, *ZSCAN4* mRNA expression. *RPL13A* was used as housekeeping gene for D4T expression.

Figure 4. The hTfR1/iFLExD Mouse Model Recapitulates Aspects of Human FSHD



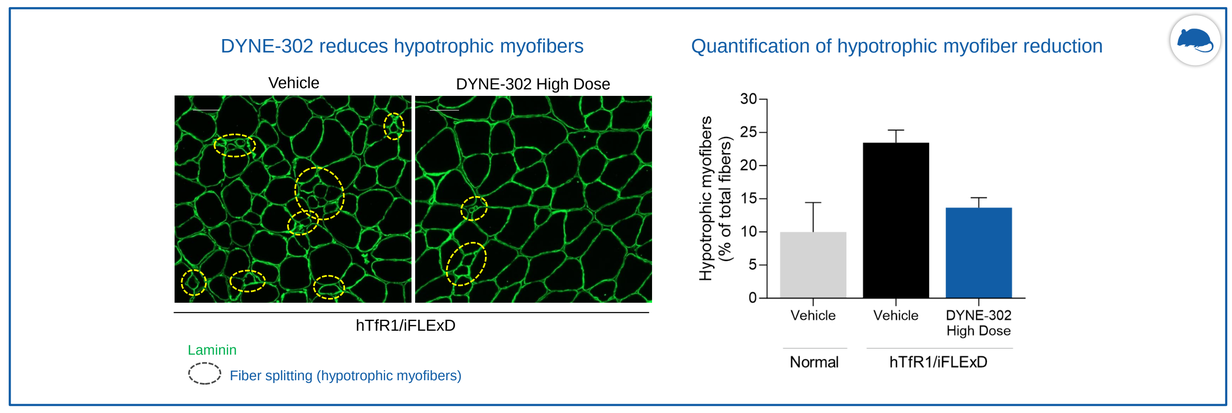
The iFLExD mouse model (2) was crossed to mice transgenic for human TfR1 under the control of the mouse TfR1 promoter. The induced and uninduced models differ based on the addition or omission of tamoxifen, which induces further DUX4 expression. Together, the two mouse models recapitulate different aspects of human FSHD.

Figure 5. Single Dose of DYNE-302 Achieves Robust, Durable, and Dose-Dependent D4T KD in Skeletal Muscle of hTfR1/iFLExD FSHD Mice



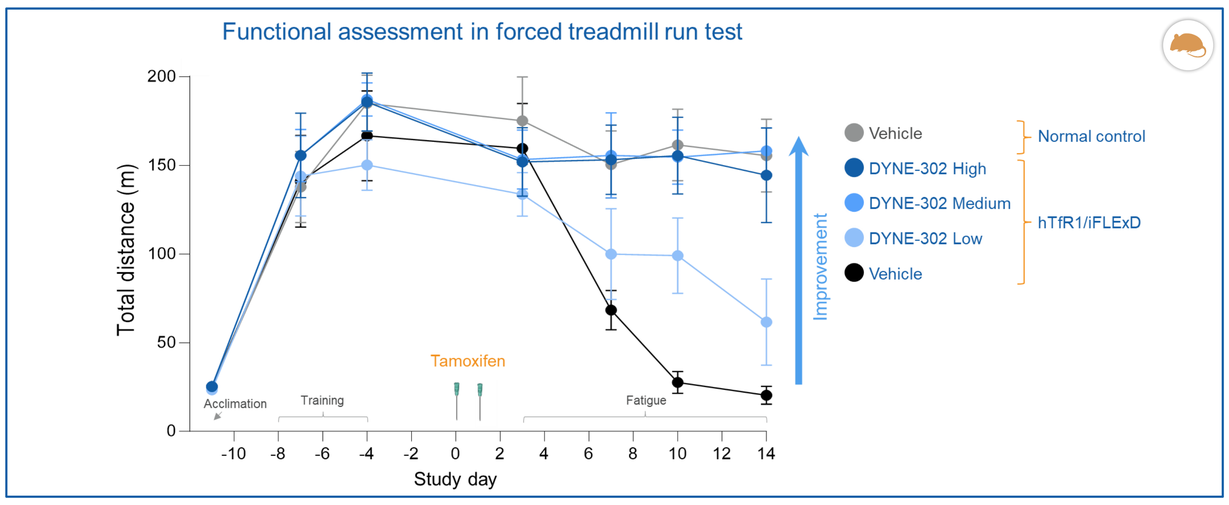
Uninduced hTfR1/iFLExD mice were dosed with vehicle or DYNE-302 on day 0, and D4T was analyzed at indicated time post-dose. Data are means ±SD; n = 4 – 12/group. D4T is an average of mouse *Wfdc3*, *Sord*, and *Serpinb6c* mRNA markers. *RPL13A* was used as housekeeping gene for D4T expression.

Figure 6. Single Dose of DYNE-302 Corrects Muscle Pathology in Quadriceps of the Uninduced hTfR1/iFLExD FSHD Model at 12 Weeks



Uninduced hTfR1/iFLExD mice were dosed with vehicle or DYNE-302 on day 0 and quadriceps collected for cryosectioning and anti-laminin immunostaining 12 weeks later. Immunostained sections were digitized using a slide scanner and myofiber ferret diameter was calculated algorithmically for each fiber, and percentage of hypotrophic myofibers (defined as myofibers with a diameter <20 microns) for each animal calculated. A similar myofiber pathology is observed in the muscle of FSHD patients (3). Data are means + SD; n = 4 – 9 animals per group.

Figure 7. Single Dose of DYNE-302 Demonstrates Functional Benefit in the Induced hTfR1/iFLExD FSHD Mouse Model



hTfR1/iFLExD mice were dosed with vehicle or various doses of DYNE-302 on day -14, trained to run in a forced treadmill run on days -7 and -4, and tamoxifen was administered on days 0 and 1 to all groups. Performance on the forced treadmill run (total distance run) was measured on days 3, 7, 10, and 14. Data are means ± SEM; n = 5 - 6.

METHODS

- DYNE-302 is a FORCE conjugate engineered to deliver an siRNA targeting human *DUX4* to muscle via human TfR1-mediated uptake.
- FSHD patient-derived myotubes were exposed to DYNE-302 and D4T expression assessed by qRT-PCR and RNASeq.
- Mice constitutively expressing human TfR1 (hTfR1) and sporadically expressing tamoxifen-inducible human DUX4 in skeletal muscle (hTfR1/iFLExD) were used as an FSHD model. hTfR1/iFLExD mice subjected to a single intravenous dose of DYNE-302 were analyzed for D4T by RT-PCR and for myofiber diameter by immunofluorescence. The effect of DYNE-302 on muscle function was measured by forced treadmill run after induction of DUX4 by tamoxifen. Mice subjected to vehicle injections served as controls. All procedures were reviewed and approved by the local Institutional Animal Care and Use Committee.

CONCLUSIONS

- DYNE-302 suppresses expression of D4T in myotubes from individuals with FSHD
- DYNE-302 demonstrates dose-dependent, durable D4T KD and normalizes muscle pathology in a chronic mouse model of FSHD
- DYNE-302 effectively preserves muscle function in an acute mouse model of FSHD
- Durability of pharmacodynamics in muscle suggests potential for quarterly dosing
- Effective delivery of siRNA to muscle confirms modularity of the FORCE platform

Data support the potential of DYNE-302 for the treatment of FSHD

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DISCLOSURE INFORMATION

All authors are employees or advisors of Dyne Therapeutics Inc. and may hold Dyne stock and/or stock options.

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