PROJECT

Supervisor:	Emilie VENEREAU
Title:	Preclinical Evaluation of a Designer HMGB1 as a Drug Candidate for Duchenne Muscular Dystrophy
Curriculum:	Cell and Molecular Biology
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/genetics-and-cell-biology/units/tissue-regeneration-and-homeostasis.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Current therapies for Duchenne muscular dystrophy (DMD) primarily aim to improve the muscle environment in order to slow disease progression and enhance regeneration. Glucocorticoids remain the standard of care; however, their long-term use is associated with significant side effects. The histone deacetylase inhibitor Givinostat (Duvyzat) is the first non-steroidal drug recently approved for all DMD genetic variants (1–2). It promotes muscle regeneration and reduces muscle wasting by enhancing the ability of fibro-adipogenic progenitors (FAPs) to support muscle stem cell differentiation (3–5). A promising therapeutic strategy may be to combine glucocorticoids or Givinostat with complementary approaches to increase efficacy while potentially reducing dosage and limiting side effects, thereby providing a more comprehensive treatment for DMD. Our previous work identified a variant of the High Mobility Group Box 1 (HMGB1) nuclear protein as a promising therapeutic candidate (6). HMGB1 is a DNA-binding protein that plays a key role in inflammation and tissue regeneration following injury (7–10). We have previously shown that the oxidized form of HMGB1 (dsHMGB1) exacerbates inflammation and represents a therapeutic target in muscular dystrophies (7). To harness its regenerative properties while minimizing pro-inflammatory effects, we engineered 3S, a non-oxidizable HMGB1 mutant. This variant has demonstrated strong potential in promoting tissue regeneration in acute injury models and in ameliorating the dystrophic phenotype in preclinical models (6). We are currently advancing the development of 3S in collaboration with Extend (National Technology Transfer Hub) and Evotec, while assessing its safety, optimal dosing, and potential synergy with glucocorticoids and exon-skipping therapies.

Rationale and hypothesis

We hypothesize that combining glucocorticoids or givinostat with 3S will yield synergistic effects by targeting complementary pathways and/or cellular compartments in skeletal muscle. This strategy may enhance therapeutic efficacy while enabling dose reduction and reducing side effects, offering a more effective approach for DMD treatment.

Objectives and specific aims

The objective of this project is to perform a preclinical study in a Duchenne muscular dystrophy (DMD) mouse model to evaluate the therapeutic effects of glucocorticoids or givinostat in combination with 3S treatment. By integrating functional assessments with histological and proteomic analyses, the project will determine the impact of the combined therapies and identify the molecular pathways affected. In parallel, ex vivo studies on dystrophic myoblasts will be conducted to investigate drug interactions at the cellular level. These experiments will be complemented by mass spectrometry–based proteomics to characterize the underlying mechanisms of action and pathway crosstalk between treatments.

Expected outcomes

This project will advance our understanding of the mechanisms of action of an innovative therapeutic approach for Duchenne muscular dystrophy (DMD) and may provide a foundation for developing a novel combination treatment strategy for the disease.

Skills that the student should acquire (max. 600 characters including spaces):

- Technical skills: cell culture (primary cells, cell lines, single myofibers), molecular biology (e.g. Q-PCR), imaging (e.g. confocal microscopy), flow cytometry, histology, mouse handling (colonies maintenance, DMD model, treatments with recombinant protein or FDa-approved drugs), production of recombinant protein, RNAseq, mass-spectrometry analyses.
- IT skills (e.g. Microsoft Office, GraphPad Prism software, ImageJ, Photoshop, Inkscape software).
- Communication skills: oral/poster presentations (lab meetings, internal seminars, national and international conferences), article writing (research articles and reviews).

References (max. 15)

1 Mozzetta C, et al. HDAC inhibitors as pharmacological treatment for Duchenne muscular dystrophy: a discovery journey from bench to patients. Trends in Molecular Medicine 2024;30:278–294.

2 Lamb YN. Givinostat: First Approval. Drugs 2024;84:849–856.

3 Minetti GC, et al. Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. Nat Med 2006;12:1147–1150.

4 Mozzetta C, et al. Fibroadipogenic progenitors mediate the ability of HDAC inhibitors to promote regeneration in dystrophic muscles of young, but not old Mdx mice. EMBO Mol Med 2013;5:626–639.

5 Consalvi S, et al. Preclinical Studies in the mdx Mouse Model of Duchenne Muscular Dystrophy with the Histone Deacetylase Inhibitor Givinostat. Mol Med 2013;19:79–87.

6 Careccia G, et al. Rebalancing expression of HMGB1 redox isoforms to counteract muscular dystrophy. Sci Transl Med 2021;13:eaay8416.

7 Celona B, et al. Substantial Histone Reduction Modulates Genomewide Nucleosomal Occupancy and Global Transcriptional Output. PLoS Biol 2011;9:e1001086.

8 Venereau E, et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. J Exp Med. 2012;209(9):1519-28.

9 Tirone M, et al. High mobility group box 1 orchestrates tissue regeneration via CXCR4. J Exp Med. 2018;215:303–318. Key Players of the Immunosuppressive Tumor Microenvironment and Emerging Therapeutic Strategies. Park K, Veena MS, Shin DS. Front Cell Dev Biol. 2022 Mar 8;10:830208. doi: 10.3389/fcell.2022.830208.