

 <p><b>UniSR</b> Università Vita-Salute San Raffaele</p>	<p><b>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</b></p>	<p><b>MO 20-5</b> ed. 01 del 21/02/2025 PO 20 Page 4 of 10</p>
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**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) focus on improving muscle environment to slow disease progression and boost regeneration. The histone deacetylase inhibitor Givinostat (Duvyzat) is the first nonsteroidal drug recently approved for all DMD genetic variants (1-2). It improves muscle regeneration and reduces muscle wasting by enhancing Fibro-Adipogenic progenitors (FAPs) capacity to support muscle stem cells differentiation (3-5). A promising therapeutic approach might be combining Givinostat with complementary therapies to enhance efficacy, and possibly reduce the dose to limit side effects, offering a more comprehensive treatment for DMD. Our previous studies identified a variant of High Mobility Group Box 1 (HMGB1) nuclear protein as a promising drug candidate (6). HMGB1 is a DNA chaperone contributing to inflammation and tissue regeneration after injury (7-10). We previously identified oxidized HMGB1 (dsHMGB1) as a therapeutic target in muscular dystrophies due to its role in worsening the inflammation. (7). To harness its regenerative potential while avoiding inflammation, we engineered 3S, a non-oxidizable mutant, which has shown promise in promoting tissue regeneration in acute injury models and mitigating dystrophic phenotype in preclinical models (6). We are advancing 3S therapy with Extend (National Technology Transfer Hub) and Evotec, while evaluating its toxicity, dosage, and synergy with glucocorticoids and exon-skipping technologies.

**Rationale and hypothesis**

We strongly believe that combining Givinostat and 3S may have synergistic effects by targeting different cellular compartments in muscle. Indeed, Givinostat primarily targets FAPs (4), while



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3S-HMGB1 directly acts on satellite cells and muscle fibers, accumulating in their nuclei (6, 9 and Ruggieri et al. To review). By combining these therapies, we aim to enhance therapeutic efficacy and reduce dose-dependent side effects, potentially providing a more effective strategy for DMD treatment.

**Objectives and specific aims**

The objective of the project is to conduct a preclinical study in a DMD mouse model, to assess Givinostat's therapeutic effects combined with 3S treatment. By integrating functional tests with histological and proteomic analyses, the student will evaluate the impact of the combined therapy and identify molecular pathways modulated by the treatments. The student will investigate how these drugs interact through *ex vivo* experiments on dystrophic myoblasts and fibroadipogenic progenitors (FAPs). Additionally, the student will exploit live imaging to study nuclear dynamics and features in wild-type or DMD myoblasts treated with 3S protein and/or Givinostat. These experiments will be complemented by proteomic analyses using mass spectrometry to investigate the molecular pathways involved in the interaction between these two drugs.

**Expected outcomes**

This project will deepen our understanding of the mechanisms of action of two innovative drugs for Duchenne Muscular Dystrophy (DMD), potentially laying the groundwork for a new combined therapeutic strategy to treat 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 Givinostat), production of recombinant protein.
- 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.



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- 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.