PROJECT

Supervisor:	STEFANIA CRIPPA
Title:	Modelling skeletal defects in MPSIVA to enhance HSPC-GT therapeutic efficacy
Curriculum:	Molecular Medicine Gene and Cell Therapy
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy.html

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

Background/gap of knowledge

Mucopolysaccharidosis type IVA (MPSIVA) is a genetic disorder caused by defects in GALNS activity, a lysosomal enzyme responsible for keratan sulphate and chondroitin sulphate degradation. This leads to accumulation of undegraded substrates, triggering cell damage in several tissues, including skeletal systems (1,2). Patients manifest severe skeletal dysplasia. Recent studies by our institute demonstrated the superior safety and efficacy of Hematopoietic Stem/Progenitor Cells (HSPC)-gene therapy (GT) to treat skeletal manifestations in LSDs (3-5). Based on these results, our laboratory is developing an innovative HSPC-GT approach to treat MPSIVA in the context of a novel platform approach. However, the pathophysiological mechanisms and cellular components responsible for MPSIVA are largely unknown, due to the absence of animal models fully recapitulating the human skeletal pathology (6,7). This limits our understanding of MPSIVA skeletal pathology and the potential to enhance and monitor the therapeutic efficacy of new therapies.

Rationale and hypothesis

To overcome these limitations, we hypothesize that the development of humanized skeletal tissue models using patient-derived cells will be essential for comprehensively investigating disease mechanisms and cellular contributors, thereby enhancing the therapeutic efficacy of HSPC-GT as a potential curative option for MPSIVA patients. Furthermore, these models will be employed to study the cross-correction mechanisms mediated by HSPC-GT approaches, or eventually to test novel therapeutic approaches to treat skeletal defects.

Objectives and specific aims

Aim1. Establishment of a biobank of patient-derived cells

Mesenchymal stromal cell (MSCs) lines, reproducing the enzyme deficit, will be generated through advanced gene editing tools. Primary MSCs will be also isolated from patients when available. iPSCs will be also generated by reprogramming of patients' cells or eventually iPSCs from healthy-donor will be gene-edited to reproduce patients' mutations.

Aim2. Establishment of MSCs- and iPSCs-based humanized skeletal models

The PhD student will be involved in the differentiation of MSCs to generate hypertrophic cartilage and model endochondral ossification in vivo (8). iPSCs will be employed to generate three-dimensional bone-like structures (9,10).

Aim3. Dissect molecular and cellular abnormalities underlying skeletal disease in MPSIVA patients

To reach this aim, gene profiling approaches, including single cells analysis and spatial transcriptomics, will be employed together with advanced microscopy to analyse the skeletal models at steady state and in HSPC-GT therapeutic settings, enabling the study of underlying mechanisms responsible for metabolic and functional disease and correction.

Expected outcomes

Overall, this project is expected to characterize the complex pathogenic cascade underlying tissue and cellular skeletal alterations in MPSIVA and elucidate the mechanisms of metabolic and functional correction, with the ultimate goal of improving HSPC-GT to treat MPSIVA.

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

The PhD student will acquire cellular skills, including cell culture isolation and characterization, cell reprogramming, tissue modelling, gene manipulation, lentiviral vector production, and molecular and biochemical techniques. He/she will also develop skills to perform in vivo study. The student will acquire the required scientific background and expertise to independently conduct his/her experiments, critically discuss the results and their biological significance, and

propose project advancements. The student will discuss the project during internal, institutional, and international meetings.

References (max. 15)

1. Tomatsu, S., et al. (2011). Mucopolysaccharidosis type IVA (Morquio A disease): clinical review and current treatment. Curr. Pharm. Biotechnol. 12, 931-945. 10.2174/138920111795542615
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4. Gentner, B., et al. (2021). Hematopoietic stem- and progenitor-cell gene therapy for Hurler syndrome. N. Engl. J. Med. 385, 1929-1940. 10.1056/NEJMoa2106596
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6. Tomatsu, S., et al. (2003). Mouse model of N-acetylgalactosamine-6-sulfate sulfatase deficiency (Galns-/-) produced by targeted disruption of the gene defective in Morquio A disease. Hum. Mol. Genet. 12, 3349-3358. 10.1093/hmg/ddg366
7. Berti M., at al. (2026). Development and characterization of a model of mucopolysaccharidosis type IVA for evaluating therapies targeting bone disease. Dis Model Mech. 2026 Feb 1;19(2):dmm052540. doi: 10.1242/dmm.052540.
8. Scotti C., et al. (2013) Engineering of a functional bone organ through endochondral ossification. Proc Natl Acad Sci U S A. 2013 Mar 5;110(10):3997-4002. doi: 10.1073/pnas.1220108110.

9. Craft AM., et al. (2015) Generation of articular chondrocytes from human pluripotent stem cells. Nat Biotechnol. 2015 Jun;33(6):638-45. doi: 10.1038/nbt.3210.
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