PROJECT 1

DoS: Alessandro Aiuti

Title: **Characterization of Mucopolysaccharidosis Type IV murine models to develop pre-clinical gene therapy strategies**

Curriculum: Gene and Cell Therapy

Link to OSR/UniSR personal page:
https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/pathogenesis-and-therapy-of-primary-immunodeficiencies/alessandro-aiuti.html
https://www.unisr.it/docenti/a/aiuti-alessandro

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Morquio syndrome is a multi-system inherited Lysosomal Storage Disorder (LSD) with prevalent skeletal involvement. Morquio type A, or mucopolysaccharidosis IVA (MPSIVA), is due to deficiency of N-acetylgalactosamine 6-sulfatase (GALNS) (1); Morquio type B, or mucopolysaccharidosis IVB (MPSIVB), is due to beta-galactosidase (b-GAL) deficiency (2). Both disorders lead to progressive accumulation of glycosaminoglycans (GAGs; e.g. keratan sulfate, KS) resulting in progressive cellular and organ damage. Severe dysplasia, dysostosis multiplex, and joint hyper-mobility impair patient quality of life in addition to cause several secondary defects leading to premature death. Enzyme replacement therapy (ERT), which is available for MPSIVA patients to slow down the progression of the disease, fails to correct bone defects. A similar treatment is not available for MPSIVB. Allogenic hematopoietic stem cell transplantation (HSCT) has been performed in a limited number of MPSIVA patients, however it has been shown to be inefficient to deliver sufficient enzymes to skeletal tissues. Pre-clinical studies (3) and data from the SR-TIGET phase I/II MPSI Hurler clinical trial have shown that hematopoietic stem and progenitor cells-gene therapy (HSPC-GT), based on the transplantation of ex vivo gene-corrected autologous HSPCs, restores at a supra-physiological level the enzyme activity, and importantly reduces GAG accumulation in several tissues and organs. Treated patients showed stability in motor performances, a normal growth according

to peers and improved joint mobility. Based on these results, we propose to develop a preclinical protocol of HSPC-GT for the treatment of Morquio syndrome. Pre-clinical development requires testing the toxicity and efficacy of this novel drug product into animal models which recapitulate the human disease pathophysiology. In our laboratory. a mouse model for MPSIVA (Galns-/-) and a mouse model for MPSIVB (Glb1-/-) are available. This project aims at characterizing the pathological phenotype of Galns-/- and Glb1-/- mice, with the ultimate goal to prove the superior efficiency of HSPC-GT to cure the skeletal and neurological defects of Morquio syndrome exploiting gene corrected HSPCs transduced in vitro with lentiviral vectors encoding for GALNS and GLB1. We will also study the cross-correction mechanisms through which transduced cells of hematopoietic origin (e.g. osteoclasts in the bone; macroglia in the brain) are capable to provide sufficient enzyme to restore the function of non-hematopoietic tissue-resident cells. In vitro cross-correction experiments will be performed using mesenchymal stromal cells (MSCs), MSC-derived osteoblasts, and fibroblasts from patients affected by Morquio syndrome. We will also investigate in detail the elastin production in fibroblasts from MPSIVB patients compared to healthy-donors with the aim of elucidating the role of elastin binding protein, a spliced product of GLB1 gene.

Overall this project will provide fundamental information for the clinical translation of HSPC-GT strategies in the treatment of patients affected by Morquio syndrome.

Skills to be acquired by the student:

Mice handling, including HSCT and HSPC-GT

Embedding and immunohistochemistry techniques

Biochemical assay (enzymatic activity, GAG determination)

Cell culture (HSPCs, MSCs, osteoclasts, fibroblasts)

Virus production and transduction protocols

References (max. 3)

1. Peracha H, Sawamoto K, Averill L, Kecskemethy H, Theroux M, Thacker M, Nagao K, Pizarro C, Mackenzie W, Kobayashi H, Yamaguchi S, Suzuki Y, Orii K, Orii T, Fukao T, Tomatsu S. Molecular genetics and metabolism, special edition: Diagnosis, diagnosis and prognosis of Mucopolysaccharidosis IVA. Mol Genet Metab. 2018.

2. Caciotti A, Garman SC, Rivera-Colón Y, Procopio E, Catarzi S, Ferri L, Guido C, Martelli P, Parini R, Antuzzi D, Battini R, Sibilio M, Simonati A, Fontana E, Salviati A, Akinci G, Cereda C, Dionisi-Vici C, Deodato F, d'Amico A, d'Azzo A, Bertini E, Filocamo M, Scarpa M, di Rocco M, Tifft CJ, Ciani F, Gasperini S, Pasquini E, Guerrini R, Donati MA, Morrone A. GM1 gangliosidosis and Morquio B disease: an update on genetic alterations and clinical findings. Biochim Biophys Acta. 2011.

3. Visigalli I, Delai S, Politi LS, Di Domenico C, Cerri F, Mrak E, D'Isa R, Ungaro D, Stok M, Sanvito F, Mariani E, Staszewsky L, Godi C, Russo I, Cecere F, Del Carro U, Rubinacci A, Brambilla R, Quattrini A, Di Natale P, Ponder K, Naldini L, Biffi A. Gene therapy augments the efficacy of hematopoietic cell transplantation and fully corrects mucopolysaccharidosis type I phenotype in the mouse model. Blood. 2010