PROJECT 1

DoS: LORENZO PIEMONTI

Title: **Pre-clinical approaches of cell therapy for type 1 diabetes with induced pluripotent stem cell (iPSC) – derived β cells.**
This project is supported by *SOStegno 70 Insieme ai ragazzi diabetici Associazione Onlus* (project "Beta is better") and the fundraising campaign "*Un brutto t1po*".

Curriculum: GENE AND CELL THERAPY

Link to OSR/UniSR personal page: http://research.hsr.it/en/institutes/diabetes-research-institute/beta-cell-biology.html and Beta cell differentiation Unit

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

Background. Pancreatic β cell replacement is a potential cure for patients with type 1 diabetes. Transplantation of islets from organ donors is a feasible and effective approach, but it is limited by the scarcity of pancreas donor and the need for lifelong immunosuppressive treatment. A new infinite source of β cells is then strongly needed and it has been consistently shown by our group and others (Pellegrini, Sordi, 2021a, 2021b) that human induced pluripotent stem cell (iPSC) can differentiate in vitro into functional insulin-producing cells. The overall objective of our studies is to treat type 1 diabetes by using insulin-producing cells obtained from iPSC.
On the road map towards the clinical application, selection of iPSC clones, high efficiency differentiation protocols and in vivo function of iPSC-derived β cells after transplantation remain major challenges.
Aim. The aim of this study is to dissect iPSC-derived β cell survival and function in a diabetic mouse model, using the most efficient clone of a newly generated GMP iPSC line, differentiated in vitro with highly efficient 3D protocol.
Experimental plan. At first, we will analyze gene expression that marks the most efficient iPSC clone from a donor and build a signature for early screening of iPSC clones. To this aim, we will sequence RNA of several iPSC clones that we recently reprogrammed from a healthy donor, differentiate them into β cell and combine RNAseq with differentiation efficiency data, in order to find differentially expressed genes. We will then apply and validate the obtained gene signature to the clones of a GMP iPSC line, generated in collaboration with Policlinico of Milan Cell Factory, and the in vitro differentiation protocol into β cells will be scaled up (in 3D culture) and made GMP-compatible.
Finally, we will transplant iPSC-derived β cells in murine models of diabetes, with and without iPSC-derived feeder cells (mesenchymal and endothelial cells). Cell engraftment, maturation, and interconnection with MSC and EC will be analyzed by integrated single cell and spatial transcriptomics.
Expected outcome. The outcomes of this project are the selection, based on an identified gene signature, of the best iPSC clones prone to differentiation into β cells, the setup of a GMP-compatible and 3D differentiation protocol and the mapping of transplanted β cell architecture and function.

Skills to be acquired by the student: The student will become expert of iPSC and β cell differentiation and acquire the following technical skills: iPSC characterization and differentiation into β cells (cell culture, flow cytometry, immunofluorescence, Luminex proteic assay, Taqman, static and dynamic perifusion of β cells, animal handling and cell transplantation, analysis of data). The student will collaborate with the the Center of Omics Sciences for RNAseq, single cell and spatial transcriptomics experiments.

References (max. 3)

1) Pellegrini Cytotherapy. 2021 Apr;23(4):311-319. doi: 10.1016/j.jcyt.2020.10.004
2) Pellegrini J Clin Endocrinol Metab. 2021 Jan 8:dgaa986. doi: 10.1210/clinem/dgaa986.
3) Augsornworawat Cell Rep. 2020 Aug 25;32(8):108067. doi: 10.1016/j.celrep.2020.108067.