PROJECT 1

DoS: Vasco Meneghini

Title: Development of gene editing strategies targeting glial cells for the treatment of rare neurodegenerative disorders

Curriculum: Gene and Cell Therapy

Link to OSR/UniSR personal page: https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/gene-and-neural-stem-cell-therapy-for-lysosomal-storage-diseases/vasco-meneghini.html

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

Alexander disease (AxD) is an autosomal dominant neurodegenerative disorder caused by missense mutations in the gene encoding the glial fibrillary acidic protein (GFAP), the major intermediate filament protein in astrocytes. Accumulation of GFAP aggregates in Rosenthal fibres impairs proteasomal activity and hyperactivates the stress response, thus compromising astrocyte functions and altering the homeostasis of the central nervous system (CNS). Currently, this orphan disease lacks a cure.

CRISPR/Cas9-based technologies enabling precise editing of disease-causing genes are a fascinating therapeutic option to treat several rare diseases [1], including neurodegenerative disorders [2]. Cas9 nucleases enable the non-homologous end-joining (NHEJ)-mediated disruption of mutated alleles or the targeted insertion for gene correction strategies through the homology-mediated end-joining (HMEJ) pathway. Base editors are an alternative to Cas9 nucleases to induce knock-down of mutated genes or correct point mutations. Due to the absence of DNA double strand breaks, base editors are expected to have a safer profile. Still, their efficiency and safety in targeting astroglial cell populations in vivo remains to be proven.

This project will provide in vivo proof-of-concept of efficacy and safety of novel CRISPR/Cas9-based approaches targeting GFAP mutational hotspots to recover pathological phenotypes in AxD murine models. We will apply novel gene- and base-editing technologies to specifically down-regulate the expression of mutated GFAP allele or to correct disease-causing mutations. We will compare their efficiency and safety in AxD in vitro models engineered for a rapid and precise screening of CRISPR/Cas9 systems. Taking advantage of our expertise in in vivo gene therapy (GT) for the treatment of leukodystrophies [3], we will optimize the intracerebral delivery of editing tools based on lipid nanoparticles or viral vectors in AxD mouse models, evaluating the editing efficiency, recovery of disease phenotypes, and safety of the proposed approaches. In this study we will integrate cutting-edge gene- and base-editing platforms, state-of-the-art nanoparticle and viral technologies, imaging and biochemical assays, and advanced NGS and bioinformatic analysis.

We expect to develop/optimize novel editing platforms for widespread in vivo targeting of CNS astrocytes. While the primary focus is on AxD, these platforms could be prospectively used for the study and treatment of other CNS disorders by targeting pathological pathways involved in primary astrocyte degeneration or dysfunctional/maladaptive astrogliosis.

Skills to be acquired by the student:

murine cell cultures, gene and base editing technologies, molecular biology, immunocytochemistry, biochemistry, live imaging, FACS and cell sorting analyses, viral vector and lipid nanoparticle tools, animal handling, small surgery procedures.

References (max. 3)

[1] Antoniani C, Meneghini V, et al. Blood. 2018 Apr 26;131(17):1960-1973. doi: 10.1182/blood-2017-10-811505.

[2] Meneghini V, et al. Front. Genome Ed., 03 March 2021. doi.org/10.3389/fgeed.2021.644319

[3] Meneghini V, et al. EMBO Mol Med. 2016 May 2;8(5):489-510. doi: 10.15252/emmm.201505850.