PROJECT 1

DoS: **Raffaella Di Micco**

Title: **Gaining insights on hematopoietic stem cell response to endogenous DNA damage to further advance gene and cell therapies**

Curriculum: **Gene and Cell Therapy**

Link to OSR/UniSR personal page:
https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/senescence-in-stem-cell-aging-differentiation-and-cancer/raffaella-di-micco.html

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

Hematopoietic Stem and Progenitor Cell (HSPC) gene therapy has emerged as a revolutionary intervention for the treatment of a wide-range of previously incurable inherited disorders. The success of these applications critically depends on the capacity to genetically engineer HSPC without compromising their biological functions. Emerging evidence, including our own, indicate that HSPC exposure to currently available gene transfer and gene editing technologies –which require prolonged *ex-vivo* culture, high viral vector doses and nuclease-induced DNA double strand breaks (DSB)– unexpectedly converge on the DNA damage response (DDR), a signaling cascade leading to cell cycle arrest. Protracted DDR hampers the hematopoietic reconstitution of gene-modified cells upon transplantation; instead, its transient inhibition significantly improves their functionality. An emerging set of data from our laboratory suggests that culture conditions employed for gene therapy applications may incidentally lead to endogenous DNA damage that in addition to the DDR triggered by exposure to gene therapy tools makes HSPCs to exceed a threshold of tolerable DDR and permanently exiting from the cell cycle. However the source, the type and the functional consequences of the observed DNA damage in human HSPCs are unknown. Taking advantage of an imaging-based approach the candidate will define kinetics of DSB and single strand break accumulation, as well as the dynamics and timing of HSPC cell cycle exit in clinically relevant HSPC sources. He/she will next combine this evaluation of DNA lesions with a whole genome analysis of genomic sites that are preferentially damaged in cultured HSPCs by a versatile, sensitive and quantitative method for DSB labeling in situ and sequencing (BLISS). Because we have previously shown that proliferation-induced DNA damage in fibroblasts does not occur indiscriminately throughout the genome, instead it is preferentially observed at sites of slow/altered DNA replication speed such as telomeres, the candidate will analyze telomeric DNA damage and study altered DNA replication patterns by DNA combing and electron microscopy. One intriguing possibility is that DNA damage accumulation observed in cultured HSPCs may derive from the forced removal of HSPCs out of their low-oxygenic bone marrow niche and be exacerbated by growth on plastic. To test this hypothesis he/she will undertake a mechanobiology approach and test several advanced bio-functionalized culture substrates mimicking physical cues and geometric constraints to be used as synthetic niche for HSPCs during ex-vivo gene-manipulation. This set of investigations will shed light on DNA damage related mechanisms potentially contributing to the functional impairment of HSPCs upon extended culture and genetic engineering and also provide unprecedented molecular details on how human HSPCs cope with endogenous stress that my arise during *ex-vivo* activation.

Skills to be acquired by the student:

The student will acquire a unique molecular expertise in the field of DNA damage, DNA replication stress and mechanobiology and combine it with available gene-therapy platforms and state-of the art protocols for genetic engineering of human hematopoiesis. He/she will become proficient with cellular assays to test human HSPC functionality *in vitro* and *in vivo* by xenotransplants. The student will be trained to become an independent thinker and will have the unique opportunity to interact with internal and international collaborators leaders in the field.

References (max. 3)

Precise Gene Editing Preserves Hematopoietic Stem Cell Function following Transient p53-Mediated DNA Damage Response. *Schiroli, Conti et al. Cell Stem Cell 2019*

Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. *Walter et al.. Nature 2015*

BLISS is a versatile and quantitative method for genome-wide profiling of DNA double-strand breaks. *Yan et al. Nature Communications 2017*