PROJECT 2

DoS: **Raffaella Di Micco**

Title: **Identification and targeting of senescence programs during hematopoietic stem cell aging**

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**):
Human Hematopoietic Stem and Progenitor Cells (HSPC) serve as a lifelong reservoir for mature blood cells. Accumulation of DNA damage in HSPC is a contributing factor to aging and bone marrow failure syndromes of inherited diseases characterized by telomeric shortening. We recently discovered that repeated activation of human aged bone-marrow derived HSPC out of their quiescence state elicits the DNA damage response (DDR) as a consequence of DNA replication stress. Yet, we have just scratched the surface of a plethora of still unexplored molecular mechanisms governing the biology of HSPC during aging. The candidate will assess whether DNA replication stress triggers HSPC senescence *in vivo* and affects the reconstitution upon transplantation of aged HSPC. He/She will undertake an imaging-based quantitative evaluation of several senescence markers (including SA-β-Gal, DDR, heterochromatin foci, micronuclei) in progenitors and primitive HSPC subsets by ImageStreamX, a technology that combines high content imaging with the quantitative power of flow cytometry. The candidate will then measure a panel of known pro-inflammatory cytokines associated with senescence *ex-vivo* and during the early and late phases of hematopoietic reconstitution. Mechanistically, the candidate will assess the contribution of telomeric DNA damage to age-related dysfunctions of HSPCs by immune- fluorescence in situ-hybridization technologies (immune-FISH) and define by single cell RNA sequencing and chromatin accessibility analysis the molecular determinants of young and aged HSPCs. Lastly, he/she will attempt to mitigate senescence barriers by specifically dampening DDR at telomeres and/or by targeting identified downstream pathways with the final goal to improve hematopoietic reconstitution of aged HSPCs. This set of investigations will provide a comprehensive assessment of the impact of senescence on HSPC reconstitution and identify innovative strategies to counteract senescence detrimental effects on the human graft. Moreover, concepts developed in this project will shed light on mechanisms driving bone marrow failure in patients affected by genetic disorders associated to short telomeres.

Skills to be acquired by the student:

The student will acquire a unique molecular expertise in the field of stem cell aging, senescence and telomere biology and combine it with state-of-the-art transcriptomic and chromatin accessibility analyses. 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 national and international collaborators, leaders in the field.

References (max. 3)

Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Di Micco et al. Nature Reviews Molecular and Cellular Biology, 2021*

Replication stress is a potent driver of functional decline in ageing haematopoietic stem cell. *Flach et al. Nature 2014*

Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Chang et al. Nature Medicine 2016*