PROJECT 2 (optional)

DoS: Prof. Guido Poli

Title: **Immuno-Virological Dynamics Triggered by Anti-HIV Therapy Suspension**

Link to OSR/UniSR personal page: https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/viral-pathogens-and-biosafety.html

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

A recently funded 3-year grant (PRIN2017; PI: Guido Poli) will be dedicated to dissect-out the dynamics of viral rebound and/or of its immunologic control upon suspension for a short time of combination antiretroviral therapy (cART) in HIV-1 infected individuals (Monitored Antiretroviral Pausing, MAP). As part of this general program, this PhD project will investigate the perturbation of monocyte subsets induced by MAP with particular regard to pro-inflammatory "M1" cells or anti-inflammatory/tissue-remodeling "M2" MDM [1]. We will investigate whether MAP will affect the expression of the primary HIV entry receptor (CD4) and/or co-receptors (CCR5 and CXCR4) as well as other markers of monocyte/macrophage activation and polarization. In this regard, it is still debated whether circulating monocytes represent a relevant target of infection in individuals receiving cART (likely because their infection is hampered by the expression of several restriction factors [RF]). In particular, members of the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminases inducing G-to-A hypermutations, and, particularly, of APOBEC-3A [2] seem to play a dominant role in curtailing monocyte infection whereas their modulation and the consequence for the susceptibility of monocytes to HIV infection upon MAP are currently unknow. Furthermore, in healthy conditions, monocytes are subdivided into those expressing the bacterial lipopolysaccharide (LPS) receptor CD14, but not the Fcγ receptor CD16, accounting for up to 80–90% of circulating cells, whereas HIV-1 infection has been associated with an increase of circulating $CD14^{+}CD16$-negative (^{neg}) and $CD14^{neg}CD16^{+}$ monocytes that, in turn, have been also described as the most frequently infected monocyte subset. Based on these premises, the project will aim at investigating the monocyte population before and after MAP in terms of:

Aim 1. The potential infection of circulating monocyte subsets based on CD14/CD16 expression before and after MAP as determined by quantification of cell-associated HIV DNA and RNA.

Aim 2. Definition of the monocyte polarization state based on "M1/M2" phenotypic and gene expression markers in respect to the three monocyte subsets mentioned above.

Aim 3. Levels of expression in purified monocytes and monocyte subsets of well-described transcription factors favoring virus replication (NF-kB, NFAT1, Sp1, AP-1, among others) and of anti-HIV restriction factors including: TRIM5α, TRIM19, TRIM22, APOBEC3G/3F/3A, SAMHD1, p21 and Tetherin among others [3] and their correlation with HIV infection *in vivo* and *ex vivo*.

Skills to be acquired by the student:

- Working safely in a biosafety level 3 (BSL3) environment;
- Safe manipulation of infectious HIV strains;
- Purification of monocytes from peripheral blood leukocytes of HIV-infected and seronegative individuals;
- Multiparametric FACS analysis of cell surface and intracellular markers of macrophage differentiation and viral protein expression;
- Molecular biology techniques for quantification of HIV nucleic acids (RNA, DNA) and of host cell RF and transcription factors;
- Protein characterization by Western blotting and immunoprecipitation.

References (max. 3)

1. Alfano M, Graziano F, Genovese L & Poli G. Macrophage polarization at the crossroad between HIV-1 infection and cancer development. *Arterioscler Thromb Vasc Biol* 2013, **33**:1145-52.
2. Graziano F, Vicenzi E & Poli G. Plastic restriction of HIV-1 replication in human macrophages derived from M1/M2 polarized monocytes. *J Leukoc Biol* 2016, **100**:1147-53.
3. Vicenzi E & Poli G. The interferon-stimulated gene TRIM22: A double-edged sword in HIV-1 infection. *Cytokine & Growth Factor Reviews* 2018, **40**:40-47.