PROJECT 2 (optional)

DoS: Paolo Ghia

Title: **Unravelling genetic intratumor B lymphocyte heterogeneity of chronic lymphocytic leukemia**

Curriculum: BAIO

Link to OSR/UniSR personal page:

https://research.hsr.it/en/divisions/experimental-oncology/b-cell-neoplasia.html

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

Chronic lymphocytic leukemia (CLL) is the most frequent adult leukemia in the West. The clinical course and outcome of CLL is highly variable. While no unifying genetic lesions have been found in CLL, recent studies have revealed recurrent mutations in a large number of genes, with each patient carrying a distinct set of mutations. It is suggested that intra-tumor heterogeneity may be the main force driving both tumor development and treatment resistance, with different gene mutations being detected at different tissue location or at different times of the disease course. Thus, it is important to achieve a comprehensive appreciation of the genomic alterations and their functional consequences in space and time during the natural history of CLL in order to fully appreciate the specific role of each mutation in its onset and progression.
The aim of the project is to investigate intratumor heterogeneity at genetic and functional level, from different topographic locations and at different time points of the disease taking advantage of internal cohorts of patients with CLL enrolled in ongoing clinical trials. In particular, we are planning to employ next-generation sequencing to perform genomic and transcriptomic analysis including single-cell and targeted deep sequencing, to:
- investigate intra-tumor genetic heterogeneity in time (longitudinal analysis) in a cohort of 15 patients with sequential sampling from the peripheral blood (PB) at multiple time points of the disease course;
- investigate intra-tumor genetic heterogeneity in different topographic regions (PB, Bone Marrow and Lymph nodes - LN) in a cohort of 30 patients with CLL undergoing LN biopsy per protocol. Circulating cell-free DNA (ccfDNA) will be analysed in parallel to validate it as potential surrogate for tissue involvement (liquid biopsy).
Initial functional validation will be implemented for genetic variants of interest by loss- and/or gain-of-function studies that will be achieved by either transfection of lentiviral vectors expressing epitope-tagged cDNAs or shRNAs or the use of CRISPR technology to generate specific alterations in CLL cell lines (e.g. MEC1).
Genetic, biological and clinical data will be integrated through advanced mathematical, statistical and bioinformatics methods.
It is expected that our integrated approach will allow dissecting the intratumor heterogeneity of a paradigmatic age-related malignancy like CLL and understanding its role in shaping the disease evolution and the resistance to therapy. In addition, it will potentially provide evidence to the use of ccfDNA to monitor tissue involvement in these patients, likely avoiding the need of a biopsy.

Skills to be acquired by the student (*Number of characters*, *including spaces*: max 600):

At the completion of the PhD project, the student will acquire deep knowledge of the molecular mechanisms responsible for the development of B cell lymphoproliferative disorders, practical expertize in cellular biology including handling, purification and sorting of primary human normal and neoplastic cells, and in molecular biology, including library preparation, NGS (whole-exome sequencing, targeted deep sequencing, RNA-seq) and basic bioinformatic analysis with the support of the Center for Omics Sciences (COSR) at our institution.

References (max. 15)

1. Mansouri L, et al. J Exp Med. 2015 Jun 1;212(6):833-431.
2. Maity PC, et al. Proc. Natl. Acad. Sci. U S A. 2020 Feb 25;117(8):4320-4327
3. Agathangelidis A, et al. Haematologica. 2020 Oct 1;105(10):e515