PROJECT 1

DoS: Paolo Ghia

Title: **Mechanisms of genetic and functional resistance to BTK inhibitors in CLL**

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, presenting with a remarkable heterogeneity of clinical manifestations as well as response to therapy. Despite the introduction of novel **therapies**, including Bruton Tyrosine kinase (BTK) inhibitors like Ibrutinib, achieving high rates of response and prolonged survival (1,2), CLL remains an **incurable disease**, due to the occurrence of **resistance,** that represents un unmet clinical need that deserves to be addressed. Initial studies have linked resistance to BTKi with *BTK* mutations (3-5) with or without *PLCG2* mutations. Interestingly, in many instances, mutations are present at subclonal level, with an allelic burden <10% of variant allelic frequency (VAF), thus questioning their relevance as the sole mechanism of resistance (6). To help understand the mechanisms of resistance to BTKi, not only the existence of additional gene mutations should be further explored, but also the possibility of a so-called **"*functional resistance*"**, due to either decreased dependence on proximal B-cell receptor (BcR) signalling or to its bypass through the stimulation of other immune receptors besides the BcR. As an example of "functional resistance" we have recently shown a compensatory increase of MAPK signaling in patients under treatment with BTKi (7).
Based on our recent studies that associated particular gene mutation with resistance to ibrutinib (manuscript submitted for publication), the aim of the project is to further investigate the mechanisms of resistance to BTKi at functional level. In particular, on one side we are planning to dissect the contribution of mutated genes in the resistance to therapy by modelling with CRISPR/Cas9 technology such mutations in the CLL cell line Mec1 and evaluate cell proliferation, survival, BcR signalling and response to drugs. We will also evaluate transcriptional and/or epigenetic regulation depending on the gene of interest. On the other side, we are planning to follow up on the observation of a compensatory increase of MAPK signaling in patients under treatment with BTKi and we will explore the efficacy of MAPK pathway inhibition as an alternative therapeutic option, both on primary samples and in relevant mouse models.

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

The student will acquire deep knowledge of the molecular mechanisms responsible for the development of B cell lymphoproliferative disorders, practical expertise in cellular biology including handling, purification and sorting of primary human and murine cell, in molecular biology, including CRISP/CAS technology, as well as in mouse handling. The student will also become proficient in design and management of scientific projects and in data presentation and discussion.

References (max. 15)

1. Ahn IE, Brown JR. Targeting Bruton's Tyrosine Kinase in CLL. Front Immunol 2021; 12:687458. PMID: 34248972
2. Eyre TA, Hori S, Munir T. Treatment strategies for a rapidly evolving landscape in chronic lymphocytic leukemia management. Hematol Oncol 2021; Oct 28. PMID: 34713475
3. Woyach JA, Furman RR, Liu T-M, Gulcin Ozer H, Zapatka M, Ruppert AS, et al. Resistance Mechanisms for the Bruton's Tyrosine Kinase Inhibitor Ibrutinib. N Engl J Med 2014;24:2286–94. PMID: 24869598
4. Herman SEM, Montraveta A, Niemann CU, Mora-Jensen H, Gulrajani M, Krantz F, et al. The Bruton Tyrosine Kinase (BTK) Inhibitor Acalabrutinib Demonstrates Potent On-Target Effects and Efficacy in Two Mouse Models of Chronic Lymphocytic Leukemia. Clin Cancer Res 2017; 23:2831–41. PMID: 27903679
5. Ahn IE, Underbayev C, Albitar A, Herman SEM, Tian X, Maric I, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. Blood 2017; 129(11):1469-1479. PMID: 28049639
6. Scarfò L, Bonfiglio S, Sutton L-A, Ljungstrom V, Pandzic T, Cortese D, et al. Btk and Plcg2 mutations in patients with chronic lymphocytic leukemia relapsing on ibrutinib: a European Research Initiative on CLL (ERIC) study based on real-world evidence. EHA Library. Scarfò L. Jun 12 2020; 294981
7. Gounari M, Ntoufa S, Gerousi M, Vilia MG, Moysiadis T, Kotta K, et al. Dichotomous Toll-like receptor responses in chronic lymphocytic leukemia patients under ibrutinib treatment. Leukemia 2019;33:1030–51. PMID:30607020