POST-DOC POSITIONS

Supervisor: Prof. Marek Mraz, MSc., M.D., Ph.D.
www.ceitec.cz/mrazlab

Information about the supervisor: H-index 30 (citations > 3500, 50 publications with IF), currently principal investigator of 4 grants (AZV 3x, NPO, in the past **ERC Starting grant**). Dr. Mraz has currently 7 PhD students, with 3 finishing soon). international collaborations: University of Southampton, Univ.California- San Diego, Mayo Clinic, Dana-Farber Cancer Institute, EMBL, University of Turin, member of EHA Comittee, Associate Editor of HemaSphere, reviewer in scientific journals: Blood, Leukemia, Leukemia Research

Topic title: LONG NON-CODING RNAs (lncRNAs) IN MICROENVIRONMENTAL INTERACTIONS OF B CELL CHRONIC LYMPHOCYTIC LEUKEMIA

Annotation: Marek Mraz research group has a long-term interest in non-coding RNAs and microenvironmental interactions of malignant B cells, and this research has been supported by an ERC Starting grant (2019-2024). We have previously described novel regulators of microenvironmental interactions including short non-coding RNAs, microRNAs (Sharma et al…Mraz, Blood, 2021; Musilova et al…Mraz, Blood, 2018; Cerna et al…Mraz, Leukemia, 2019). MicroRNAs were shown to play a pivotal role in B cell functions; however, the functions of long non-coding RNAs (lncRNAs) remain unclear. We aim to decipher for the first time the role of lncRNAs in B cell receptor (BCR) signaling and B-T cell interactions. Human genome contains large numbers of lncRNAs that can regulate various physiological cellular processes or contribute to the onset or aggressiveness of cancer. We will study lncRNAs in the context of chronic lymphocytic leukemia (CLL), which is driven by aberrations in the BCR pathway and B-T interactions. Regulation of BCR pathway and B-T cell interactions by lncRNAs is likely of relevance for CLL, but is also transferable to the biology of other B cell malignancies, autoimmune diseases and normal B cells. We identified 3 candidate lncRNAs involved in microenvironmental interactions of CLL. We will decipher the molecular functions of these lncRNAs using biochemical and cellular approaches and via a novel lncRNA knock-out mouse model. We have engineered mice for genetic loss of one of these lncRNAs, and the student will analyse the phenotype of these mice and breed them with known CLL mouse models (Eu-TCL1). Detailed biochemical/molecular studies will complement these data and we will also analyze primary samples from patients with B cell malignancies. We will identify functions of lncRNAs using CRISPR interference, RNA pulldown experiments, mouse models, and molecular biology technics. Furthermore, we developed a novel co-culture model inducing robust primary CLL cell proliferation (~50%) in vitro (Hoferkova et al, Leukemia, 2024). We aim to utilize this game-changing tool to perform the first-ever CRISPR screening of lncRNAs/genes regulating primary CLL cell proliferation. This will help better understand the disease biology and possibly identify novel molecular targets for therapy.

Topic title: LYMPHOID MICROENVIRONMENT MODELS AND THEIR USE TO STUDY TARGETED THERAPY AND RESISTANCE IN B CELL MALIGNANCIES

Annotation: Chronic lymphocytic leukemia (CLL) cells and indolent lymphomas are known to be dependent on diverse microenvironmental stimuli providing them signals for survival, development, proliferation, and therapy resistance. It is known that CLL cells undergo apoptosis after cultivation *in vitro,* and therefore it is necessary to use models of CLL microenvironment to culture CLL cells long-term and/or to study their proliferation. Several in vitro and in vivo models meet some of the characteristics of the natural microenvironment based on coculture of malignant cells with T-lymphocytes or stromal cell lines as supportive cell, but they also have specific limitations.
The aim of this research is to develop and use models mimicking lymphoid microenvironment to study novel therapeutic options, e.g. drugs targeting CLL proliferation, development of resistance in long-term culture or combinatory approaches, which cannot be analysed in experiments based on conventional culture of CLL/lymphoma primary cells. This project will utilize models developed in the laboratory and will further optimize and modify them. We have recently developed a co-culture model that is allowing to induce robust proliferation of primary CLL cells, something that was virtually impossible for decades (Hoferkova et al, Leukemia, 2024). Using kinase inhibitors, the biology of CLL and responses to targeted treatment will be interrogated. The student will utilize various functional assays, RNA sequencing, genome editing, drug screening etc., with the use of primary patient's samples and cell lines. The research might bring new insights into the microenvironmental dependencies and development of resistance to targeted therapy.

Topic title: ROLE OF TRANSCRIPTION FACTORS IN ONSET AND PROGRESSION OF B-CELL MALIGNANCIES

Annotation: Transcription factors (TFs) are important regulators of cell growth, development, and hematopoietic cell differentiation. Disrupting the mechanisms that are responsible for the proper function of the transcription apparatus can lead to the onset of blood cell malignancies. The abnormal function of TFs due to dysregulation or genomic aberrations are often associated with the development of leukemias, including chronic lymphocytic leukemia (CLL) and other B-cell malignancies. Much evidence from the latest research shows that CLL cells have an extra deregulated chromatin structure and show an increased incidence of activated enhancer and promoter areas, allowing TFs to bind and subsequently aberrantly activate potential oncogenes. Moreover, specific post-translational modification of some TFs have been noted as a result of dysregulated signaling in the leukemia microenvironment and this also contributes to disease progression. However, it remains largely unknown which TFs and how they contribute to the development and aggressiveness of CLL and other B malignancies. This project aims to describe the role of candidate TFs in the development and progression of B-cell malignancies with emphasis on CLL while also testing targeted therapy options, e.g. using specific inhibitors of TFs or chromatin modification regulators that are currently available or in development.

We have identified several TFs that might act as novel regulators of the B cell survival, proliferation and crosstalk with other immune cells. The PhD student will further investigate this using techniques such as genome editing (CRISPR), RNA sequencing, use of primary samples, and functional studies with various *in vitro* and *in vivo* mouse models. The research is also relevant for understanding resistance mechanisms to targeted therapy.