Topic 1) Bio-omics
LONG NON-CODING RNAs (lncRNAs) IN LEUKEMIAS
Supervisor: **Assoc. Prof. Marek Mraz, MSc., M.D., Ph.D.**

PROJECT: We are looking for a motivated PhD student for a project funded by ERC Grant (Marek Mraz lab, www.ceitec.cz/mrazlab). The project goal is to understand the role of noncoding RNAs in the regulation of microenvironmental interactions in B cell malignancies. It has been shown that most of human genome is transcribed into RNA, but only a small percentage encodes proteins, while the rest has a variety of regulatory functions. The lab is deciphering novel mechanisms of BCR signaling regulation mediated by microenvironmental signals, such as BCR signaling and T cell interactions. We showed for the first time that non-coding RNAs, namely microRNAs (miRNAs), regulate the BCR signaling which opened an interesting field of research (Musilova et al. Blood, 2018; Mraz et al. Blood, 2014; Cerna et al. Leukemia, 2019; Sharma et al, EHA congress, 2020). Now we would like to reveal the role of lncRNAs in BCR signaling and other microenvironmental interactions of B cell leukemias and lymphomas. We aim to describe for the first time the functional relation of lncRNA with BCR signalling.

We have identified candidate long-noncoding RNAs (lncRNAs) that might act as novel regulators of the crosstalk of BCR signaling/T-cell interactions/adhesion in B cell malignancies. This will be further investigated by the PhD student using technics such as genome editing (CRISPRi), RNA sequencing, functional studies with various in vitro (primary cells and cell lines), and in vivo models. The research is also relevant for pre-clinical development of novel RNA-based therapeutics, and resistance mechanisms to BCR inhibitors.
HOW TO APPLY AND OTHER INFO: see last page of the pdf

Topic 2) Bio-omics
REGULATION OF CELL MIGRATION IN B CELL LEUKEMIAS AND LYMPHOMAS
Supervisor: **Assoc. Prof. Marek Mraz, MSc., M.D., Ph.D.**

PROJECT: We are looking for a motivated PhD student for a project funded by ERC Grant (Marek Mraz lab, www.ceitec.cz/mrazlab). The project goal is to understand the molecular machinery that regulates the migration of malignant B cells between different niches such as lymphoid and bone marrow niche and peripheral blood. This is of great interests a general mechanism of how migration is regulated in cancer cells, but also specally in chronic lymphocytic leukemia (CLL), which is a disease dependent on the B cell recirculation between different compartments (reviewed in Seda and Mraz, 2015). In CLL, but also in other lymphomas, the malignant B cells permanently re-circulate from peripheral blood to lymph nodes and back, and blocking this recirculation can be used therapeutically since malignant B cells depend on signals in the immune microenvironment. However, the factors that regulate this are mostly unclear. The lab established several models for in vitro and in vivo studies of microenvironmental interactions and their interplay (unpublished in vivo model; Pavlasova et al. Blood, 2016; Pavlasova et al. Leukemia, 2018; Musilova et al. Blood, 2018; Mraz et al. Blood, 2014; Cerna et al. Leukemia, 2019).

We have identified candidate molecules that might act as novel regulators of the B cell migration or the balance between homing and survival in peripheral blood. This will be further investigated by the PhD student using technics such as genome editing (CRISPR), RNA sequencing, use of primary samples, functional studies with various in vitro and in vivo models. The research is also relevant for understanding resistance mechanisms to BCR inhibitors, pre-clinical development of novel drugs and their combinations (several patents submitted by the lab).
HOW TO APPLY AND OTHER INFO: see last page of the pdf

Topic 3) Bio-omics
ROLE OF TRANSCRIPTION FACTORS IN ONSET AND PROGRESSION OF B-CELL MALIGNANCIES
Supervisor: **Assoc. Prof. Marek Mraz, MSc., M.D., Ph.D.,** co-supervisor: **Mgr. Josef Vecera, PhD** (post-doc in Mraz lab)

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. Abnormal function of TFs due to dysregulation or genomic aberrations are often associated with the development of leukaemias, including chronic lymphocytic leukaemia (CLL) and other B-cell lymphomas. 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 signalling in the leukaemia microenvironment and this also contributes to disease progression. This project should 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 candidate TFs that might act as novel regulators of the B cell survival, proliferation and crosstalk with other immune cells. This will be further investigated by the PhD student using technics such as genome editing (CRISPR), RNA sequencing, use of primary samples, functional studies with various in vitro and in vivo models. The research is also relevant for understanding resistance mechanisms to targeted therapy.
HOW TO APPLY AND OTHER INFO: see last page of the pdf

Topic 4) Bio-omics
REGULATION OF BCR SIGNALLING BY DNA DAMAGE RESPONSE AND P53 PROTEIN
Supervisor: **Assoc. Prof. Marek Mraz, MSc., M.D., Ph.D.,** co-supervisor: **Mgr. Mirek Boudny, PhD** (post-doc in Mraz lab)

PROJECT: We are looking for a motivated PhD student that would like to work on the following project funded by the ERC (European Research Council) Starting grant. The variable clinical course of several B cell malignancies largely depends on p53 functionality and B-cell receptor (BCR) signalling propensity; however, it is unclear if there is any crosstalk between these pathways. We showed for the first time that there is a connection between p53 pathway and regulation of BCR signaling (Cerna et al…Mraz, Leukemia, 2018). We described that DNA damage response (DDR) activation leads to down-modulating the transcriptional factor FOXP1, which functions as a positive BCR signalling. It seems that the low FOXP1 levels limit BCR signalling partially via allowing for upregulation of a CD22 cell-surface, whose intracellular part serves as a docking site for phosphatases that limit BCR activation on the cell membrane. The student will further explore the connection between DNA damage response and the BCR signalling regulation. Additionally, the p53 aberration could also affect the basal levels of CD22/phosphatases, and thus contribute to the "tonic" BCR signalling, and general aggressiveness of the B cells. In vitro studies using Crispr technology and inducible shRNAs for p53 will be conducted. Additionally, we have collected over 100 samples obtained during the administration of chemo-immuno therapy in B-cell chronic lymphocytic leukaemia (CLL) patients, and these can be used to validate the in vitro observations.
HOW TO APPLY AND OTHER INFO: see last page of the pdf

Topic 5) Molecular Medicine
LONG NON-CODING RNAs (lncRNAs) IN THE PATHOGENESIS OF B CELL LYMPHOMAS
Supervisor: **Assoc. Prof. Marek Mraz, MSc., M.D., Ph.D.**

PROJECT: We are looking for a motivated PhD student that would like to work on the following project funded by the ERC (European Research Council) Starting grant. Follicular lymphoma (FL) is a type of blood cancer that originates from B-lymphocytes, and it is the most common type of indolent non-Hodgkin lymphoma. The clinical course of FL patients can be surprisingly variable (survival from months to decades) and FL still remains incurable. The course of the disease is characterized by repeated relapses leading to the evolution of resistant disease or to the high-grade transformation to a more aggressive diffuse large B-cell lymphoma (DLBCL). This is associated with remarkably poor prognosis and a high risk of early death.

Number of studies showed that multiple genetic lesions are associated with FL transformation (tFL); however, precise molecular mechanisms underlying this process is largely unclear. Importantly, the role of long non-coding RNAs in this process is completely unknown. However, we have recently described the role of short-noncoding miRNAs roles in transformed FL (Musilova et al…Mraz, Blood, 2018). The aim of the project is to reveal the molecular mechanisms involving lncRNAs and/or miRNAs responsible for FL transformation. The primary samples collected before and after high-grade transformation will be analyzed on the level of protein-coding as well as non-coding genes (NGS with Illumina, preliminary data available). This will be followed by searching for function of tFL-associated lncRNAs. This will help to better understand the disease biology and possibly to identify novel molecular targets that could be used therapeutically.
HOW TO APPLY AND OTHER INFO: see last page of the pdf

Topic 6) Molecular Medicine
PRIORITIZING DRUG COMBINATIONS IN LEUKEMIA BASED ON ANALYSIS OF TARGETTED THERAPY IN VIVO
Supervisor: **Mgr. Mirek Boudny, PhD** (post-doc in Mraz lab)**,** co-supervisor: **Assoc. Prof. Marek Mraz, MSc., M.D., Ph.D.**

The inhibition of BCR-associated kinases by so-called B cell receptor signalling inhibitors in B cell leukemia and lymphomas is a potent therapeutic strategy, and several BTK and PI3K kinase inhibitors are available for clinical use (Ondrisova and Mraz, 2020). Arguably, the therapy with BTK/PI3K inhibitors is the most promising therapeutic approach in chronic lymphocytic leukemia (CLL). However, the typically good initial clinical effect in

great majority of cases is later followed by a relapse or drug intolerance. Notably, the “BCR inhibitors” lead to the accumulation of CLL cells in the peripheral blood, which undergo apoptosis at a surprisingly slow rate with a peripheral blood lymphocytosis lasting for several months (Ondrisova and Mraz, 2020). This is in contrast to studies of normal B cells in mouse models where ablation of BCR receptor (largely analogical to BTK inhibition) results in quick (days) disappearance of all mature B cells. The key questions are: Are there adaptation mechanisms that allow CLL cells to survive in the peripheral blood when BCR signalling and their re-circulation to immune niches is inhibited by BTK/PI3K inhibitors? The long-lasting lymphocytosis during therapy with ibrutinib or idelalisib as single agents suggests that malignant B cells adapt and compensate for „BCR inhibition”, and this might be later followed by occurrence of mutations that provide true resistance such as the selection of clones with BTK mutations, or other aberrations.

Based on our preliminary data we have identified two novel mechanisms that CLL cells utilize in vivo to survive BTK inhibition by ibrutinib, and we propose 3 novel targeted combinatorial therapeutic strategies with BCR inhibitors. The aim of the project is to reveal the molecular mechanisms that specifically allow adaptation to the BCR inhibitors or their combinations with other drugs such as anti-CD20 or venetoclax (BCL2 inhibitor). The primary samples collected before and during therapy will be analyzed on the level of protein and RNA expression (NGS with Illumina, preliminary data available). This will be followed by searching for function to better understand the disease biology and possibly to identify novel therapeutic targets. Project applies technics such as genome editing (CRISPR), RNA sequencing, use of primary samples, functional studies with various in vitro and in vivo models.

HOW TO APPLY AND OTHER INFO: see last page of the pdf

WHAT DO WE OFFER:

- **modern laboratories, project funded by the prestigious ERC grant = high risk and high gain, state-of-the-art instrument, stable funding, competitive scholarship**
- **You will work in a team of young investigators that challenge some long-standing problems in the field of hematology/immunology. We do basic science, but with the objective to help patients in the future (we have access to primary samples with hem. malignancies).**

WHAT WILL YOU LEARN/DO:

- **How to think and work independently as a scientist**
- **Writing of abstracts and papers (and course in grant writing and presentation of data)**
- **How to present data and will attend conferences to present your research**
- **You will spend 1-2 months visit(s) in collaborating labs in Europe or US**
- **Collaboration with experts in wet lab research and bioinformatics**
- **Novel methods such as Next Generation Sequencing (Illumina) and genome editing (Crispr).**
- **How to critically analyze scientific data (regular journal clubs)**
- **Classical methods of molecular biology (e.g. immunoblotting, flow cytometry, qRT-PCR, cell cultures, cloning), and you will use our in vitro models for microenvironmental interactions, and artificial activation/inhibition signalling pathways to decipher the gene regulatory loops.**
- **You can supervise bachelor and diploma students if interested**

WHO ARE WE LOOKING FOR:

- **Motivated smart people that have the "drive" to work independently, but also willing to learn from other people in the lab and collaborate.**
- **Candidates should have a master's degree in Molecular biology, Biochemistry, or similar field and have deep interest in molecular biology and cancer cell biology.**

HOW TO APPLY:

- **To apply please submit a CV by email to: marek.mraz@email.cz (Subject: PhD School).**
- **Information about the laboratory at: ceitec.cz/mrazlab; The PhD will start approx. Sept 2021 (negotiable)**

OTHER INFO: The research is funded by ERC grant, and will be conducted at CEITEC MASARYK UNIVERSITY (campus Bohunice). Our laboratory extensively collaborates with the University Hospital Brno in the same campus to obtain primary samples from patients. The campus provides a vibrant, multidisciplinary and highly collaborative scientific environment. The lab is located in Brno, the second-largest city in Czech Republic that has the biggest concentration of biomedical research in the region. Brno is one of the major cultural hubs, with a vibrant and lively atmosphere housing ~60.000 students. The city has a very good public transport and plenty of interesting places to visit within the reach of trains (within small distance of several major cities such as Prague, Vienna, Bratislava, Budapest) and close to international airports.