Leukaemia is a heterogeneous disease in which malfunctioning blood cell precursors multiply at a high rate and infiltrate from the bone marrow into the peripheral blood. Untreated leukaemia is a fatal disease. To better understand the process of oncogenesis and the response of leukemic precursors to pharmacological agents, **model studies** of the multicellular environment of bone marrow compartments, called niches, where leukemic precursor development takes place, are needed. The vascular niche is mainly composed of mesenchymal stromal cells and endothelial cells, which produce factors that can promote disease development and protect leukemic precursors from the adverse effects of drug therapy.

The challenge is therefore, to develop suitable models for studying the multicellular local bone marrow microenvironment and, on the other hand, to use appropriate research methods. Available methods for studying cellular interactions are essentially limited to fluorescence techniques. These offer tracking of single labelled molecules, but the broad emission profile prevents detection of multiple markers simultaneously, as well as their size may interfere with the system under study. An alternative is **confocal Raman imaging**, which provides insight into global molecular structure, morphology and metabolism. In particular, **labelled Raman imaging** has the potential to be studied at the subcellular level using Raman reporters that accumulate in a specific cellular structure according to affinity for factors such as pH, membrane potential, hydro/lipophilicity and exhibit characteristic narrow bands in the signal-free spectral region of most cellular components.

Given the current limitations in advancing knowledge of oncogenesis and vascular niches, the **research hypothesis** of this project is to demonstrate the utility of confocal Raman microscopy for these studies, including the assessment of the integral biochemical state of cells and their organelles. In combination with the use of Raman reporters, cellular responses to drugs can be identified and used to assess therapeutic efficacy in the microenvironment of the bone marrow vascular niche. This provides insights into the metabolomics of single cells, shedding light on active cellular processes through high-resolution and state labelling of cellular organelles. The **aim** of this project is to develop a novel analytical approach based on Raman microscopy, supported by statistical data analysis and machine learning, to study a wide range of interactions in the microenvironment of the vascular niche with acute promyelocytic leukaemia and chronic myeloid leukaemia cell lines. Studies of cell-cell interactions and their mutual effects on phenotype and function in the microenvironment of the bone marrow vascular niche *in vitro* will be performed, followed by evaluation of the impact of therapeutic agents used in leukaemia therapy. The additional use of machine learning methods in the analysis of spectroscopic data will provide insights into the kinetic profiles of the cellular processes under study and the efficacy of therapies.

The **novelty** of the project lies in the use of Raman microscopy to study the bone marrow microenvironment and leukaemia development. An innovative analytical approach to study the presented system will be developed, and spectroscopic markers of metabolic and biochemical features of leukemic precursors and cells of the vascular niche will be placed in a publicly available database. Prospectively, the project can be extended to include leukemic and mesenchymal cells from patients instead of commercial cell lines, as the developed methods will be universal.