# Establishing an ex vivo Model of Clonal Hematopoiesis Using Primary Human Hematopoietic Stem Cells

The research group led by Prof. Dr. Katharina Götze in the Department of Oncology/Hematology at the Technische Universität München is currently seeking for a master's student for an internship (March-April), followed by a master's thesis (May-November) in our laboratory.

**Background:**

Hematopoietic stem/progenitor cells (HSPC) reside in the bone marrow and are responsible for blood cell production through a process known as hematopoiesis. Throughout life, these cells accumulate mutations, most of which are passengers without any functional consequences. However, some mutations can trigger clonal expansion, which is a gradual process that develops over time leading to hematological diseases such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML).

We focus on a pre-malignant stage known as clonal hematopoiesis of indeterminate potential (CHIP). CHIP is characterized by the presence of somatic mutations in HSPCs with a variant allele frequency greater than 2% in individuals who show no signs of hematologic disease, but increased risk of progression to myeloid malignancy.

The pathophysiology of MDS and AML is influenced by both cell-intrinsic (HSPC) and extrinsic signals coming from the BM microenvironment, which provides a network of diverse cell types that support the expansion of mutated clones. Whether this is also the case in CHIP remains unknown.

**Scope of the thesis:**

In our lab, we use primary human bone marrow samples to investigate how the bone marrow microenvironment influences disease progression in CHIP and aim to identify targetable mechanisms that could help intercept this progression.

One of the main challenges in this context is the lack models to study CHIP *in vitro*. To address this, the goal of this project is to establish an *ex vivo* model of clonal hematopoiesis by modeling CHIP-related mutations into primary CD34+ human bone marrow cells using the CRISPR-Cas9 technology.

**Experimental plan:**

1) Develop a *DNMT3A* knock-in and *TET2* knock-out model using human bone marrow cells
2) Characterize the model through a comparative analysis of wildtype and mutated clones
3) Perform functional validation by establishing co-cultures of engineered cells with primary healthy mesenchymal stromal cells (MSC)

**Methods:**

- Processing of human femoral heads, bone marrow aspirates and stem cell transplant filters to extract mononuclear cells
- Cell culture: leukemia cell line culture, isolation and culture of primary HSPC and MSC
- Gene editing using CRISPR/Cas9 system (design sgRNA, electroporation, Sanger-Seq)
- Multicolor fluorescence associated cell sorting (FACS)
- Standard molecular biology techniques (RNA and DNA isolation, PCR, etc.)
- Colony forming unit assays

**Requirements and contact information:**

We are looking for a highly motivated student with a background in cell and molecular biology, biomedicine or biotechnology with an interest in translational cancer research. We will value the following qualities:

- Responsible, accurate, and team-oriented way of working
- Fluent in English
- Prior experience in cell culture

We offer in-depth training and close collaboration with the clinic and expect the candidate to work independently after receiving initial training during the internship period.

Interested candidates who meet the qualifications are encouraged to submit their CV by **February 28th**, 2025, to Anna Navarro (anna.navarro@tum.de).