Investigating the effect of JAK2^{V617F} gene dosage on MPN phenotype by forward and reverse CRISPR/Cas9 genome engineering.

JAK2^{V617F} mutations can be found in the majority of patients with PV and half of those with ET and MF. It is currently unknown how the very same JAK2^{V617F} mutation has the capacity to initiate three different MPN phenotypes (PV, ET and PMF) in human patients. Clinical observation and sequencing studies of MPN patients support the hypothesis that JAK2^{V617F} gene dosage influences the disease phenotype. However, exact downstream consequences of JAK2^{V617F} dosage on human cells and its influence on disease initiation, phenotypic presentation and progression remain largely unknown.

Using a retrospective approach to analyze the consequence of JAK2^{V617F} mutation in patient-derived samples is very confounding due to unknown contributing factors such as the cell of origin that harbours the mutation, the epigenetic status of the mutated cells at the time of mutation acquisition.

In stark contrast, CIRSPR/Cas9 genome-engineering technology allows for the first time to investigate the functional and phenotypic consequences of individual mutations in a prospective manner on purified defined cell populations within the hematopoietic hierarchy.

Therefore, in this project we aim to prospectively evaluate whether JAK2^{V617F} and the allelic burden will change behavior, function and phenotype in HSPCs in vitro, and influence phenotypic presentation and development of frank MPNs upon xenotransplantation in vivo

We will employ novel CRISPR/Cas9-mediated allele-specific genomic-engineering in purified healthy HSPCs or patient-derived MPN stem cells**to introduce (Aim 1) or remove (Aim 2) JAK2^{V617F} mutations**. This allows us for the first time to probe if JAK2^{V617F} evolution/reversion changes cellular behavior in vitro and/or in vivo.