Publishable Summary:

Chronic lymphocytic leukemia (CLL) is a common type of blood cancer which is characterized by uncontrolled growth of mature-like B lymphocytes in the peripheral blood and lymphoid tissues. The main aim of this project was to prove that CLL B cells are activated to induce cell-autonomous signaling by mutual interactions of the B cell antigen receptor (BCR) on the same cells. Crystallographic studies performed in collaboration with CLL researchers showed that CLL-derived BCRs have the unique capacity for direct interaction and that the intrinsic structures that mediate the BCR-BCR interactions are similar in distinct CLL subsets. Our data showed that the BCR isotype is important for mutual interaction and signal initiation. In fact, we showed that membrane levels of IgM-BCR, but not IgD- BCR, are linked with enhanced metabolic activity.

Moreover, we showed that subset #4 CLL requires an intrinsic structure within the gamma constant region, which explains subset 4# CLL always expresses an IgG BCR. Most importantly, we identified an acquired single point mutation in light chain IGLV3-21 of subset #2 CLL and showed that this point mutation is required for mutual BCR-BCR interaction in crystals and for autonomous signaling in our cellular system. This point mutation is referred to as R110. We generated monoclonal antibodies specific for the R110 mutation and showed that this mutation is specific for CLL but is not restricted to subset #2. Intriguingly, we found that allele *IGLV3-21*01* is associated with the R110 mutation and could show that the amino acid composition of this allele is perfectly equivalent to the consensus sequence identified in the crystals to mediate the BCR-BCR interaction in CLL subset #2. Analyzing four independent cohorts from different geographical origin, we found that IGLV3-21^{R110}-expressing cases represent a distinct CLL subtype with poor prognosis independent of IGHV mutational status and CLL stereotypes. Thus, R110 mutation may act as a standalone driver that induces cell-autonomous signaling thereby stimulating CLL development of *IGLV3-21*01* expressing cells. Therefore, we proposed that allele *IGLV3-21*01* is a genetic risk factor for poor prognosis CLL. Most importantly, our data were confirmed by results published by other CLL researchers. This shows that our work as a whole is pioneering not only in the field of BCR signaling in CLL but also in the field of identifying acquired mutations associated with poor prognosis.

Population screening for the risk allele combined with targeted elimination of any detectable R110-positive cells could prevent the development of a malignant form of CLL, which ultimately represents a key outcome of this ERC project.