Severe congenital neutropenia is a rare genetic disorder affecting 3–8 people per million, where the bone marrow fails to produce enough neutrophils, a type of white blood cell essential for fighting infections. Without these cells, patients are highly vulnerable to bacterial and fungal infections. While treatment with granulocyte-colony stimulating factor can boost neutrophil production, around 20% of patients do not respond, requiring more invasive interventions like bone marrow transplantation. Moreover, neutropenic patients face a significantly increased risk of developing blood cancers, such as myelodysplastic syndrome or acute myeloid leukemia.

Recent research has identified mutations in the *CLPB* gene as a novel cause of neutropenia, but the mechanisms by which these mutations impair neutrophil function and lead to blood disorders remain unclear. The *CLPB* protein acts as a molecular "chaperone," helping other proteins fold correctly and maintaining cellular health under stress. Mutations in this gene can disrupt these vital functions, leading to defective neutrophils and potentially creating an environment where abnormal blood cells can proliferate and transform into cancer.

Our research seeks to uncover how *CLPB* mutations contribute to neutrophil dysfunction and increase the risk of leukemia. We hypothesize that specific mutations in the *CLPB* gene affect different functional domains of the protein, leading to varying degrees of symptoms severity. Furthermore, we assume that these mutations may set the stage for clonal evolution—where faulty blood cells acquire additional mutations, accelerating the progression toward leukemia.

To test these hypotheses, we are using cutting-edge approaches. First, we will generate high-resolution 3D models of the mutated *CLPB* protein to understand how specific genetic changes impact its structure and function. These insights will lay the groundwork for understanding *CLPB* dysfunction and guiding experimental validation. Since the accurate assessment of functional properties requires the production of these variants in a real experimental form, followed by checking the basic chaperonic function such as ATP hydrolysis, refolding and oligomerisation.

Next, we will use patient-derived induced pluripotent stem cells (iPSCs) to recreate blood cell development in the lab. These stem cells can mimic the production of neutrophils, allowing us to observe how *CLPB* mutations interfere with this process at different stages. Key experiments will include functional tests to assess neutrophil activity, such as their ability to fight infections through processes like phagocytosis, oxidative burst, and the formation of neutrophil extracellular traps.

Finally, we will study how additional genetic changes—known as "second-hit mutations"—combine with *CLPB* defects to drive leukemia. By introducing these mutations into iPSCs using a precise genome-editing technique called prime editing, we can simulate the genetic changes observed in neutropenic patients who develop leukemia. We will then analyze these cells to identify the pathways that lead to clonal expansion and malignant transformation. This approach will help us pinpoint molecular markers for predicting leukemia risk in neutropenic patients and may even prevent this progression.

The knowledge gained from this research could transform the diagnosis and management of severe congenital neutropenia. By understanding how specific *CLPB* mutations influence disease outcomes, we can better predict which patients are at the highest risk of severe complications or leukemia. Additionally, the findings may pave the way for new treatments that target the molecular pathways disrupted in severe congenital neutropenia, offering hope for patients with this challenging and life-threatening condition.