Classical Hodgkin lymphoma (cHL) is diagnosed annually in approximately 20000 new patients, predominantly in young adults. Currently used therapies do not cure at least 20% of cHL patients and approximately 25% of primary responding patients experience relapse within 3 years from diagnosis, highlighting need for more effective therapies. CHL is characterized by presence of malignant Reed-Sternberg (RS) cells, surrounded by massive infiltrate. Although immune cells comprise up to 90% of tumor mass, they are unable to mount effective anti-tumor response. There is an extensive network of bilateral connections between RS cells and infiltrating cells. RS cells express chemokines and cytokines that facilitate recruitment of infiltrating cells to the tumor site and their re-education, and produce immunomodulatory proteins that dampen anti-tumor immune response. Functionally skewed infiltrating cells in turn provide growth and immunomodulatory factors that support RS cell survival and growth. Increased numbers of Hodgkin lymphoma tumor-associated macrophages (HL-TAMs) correlate with shortened survival of patients with cHL, suggesting their tumor-supportive role. Available data on HL-TAMs demonstrate also that these macrophages are major source of the immunomodulatory protein PD-L1 expression in the cHL tumor mass. However, precise role of HL-TAMs and their interactions with RS cells are not well defined. Identification of mechanisms that underlie pathogenetic functions of HL-TAMs may lead to development of new therapeutic approaches in cHL.

Kinases are class of proteins particularly involved in regulation of cellular processes. They phopshorylate diverse intracellular proteins leading to their activation or inactivation. PIM-1, -2 and -3 proteins are kinases that modulate activity of multiple proteins involved in key cellular processes, such as proliferation, programmed cell death (apoptosis) or survival. Increased presence (expression) of PIM kinases have been documented in diverse solid and hematological malignancies. In these cancers, PIM expression was usually associated with worse prognosis, and available data derived from pre-clinical studies show that their blockade may induce death of the malignant cells.

In our studies, we have shown that PIM kinases are abundantly expressed in the malignant, Reed-Sternberg (RS) cells of cHL. PIM kinases increased RS cell survival and regulated expression of multiple genes involved in communication with infiltrating cells, and immune surveillance of the malignant cells. PIM inhibition in RS cells was associated with decreased expression of immunomodulatory proteins, and cytokines/chemokines directly involved in macrophage recruitment and reeducation toward protumoral TAMs. In our analyses of primary cHL sections, we have shown that PIM expression characterizes both RS cells and TAMs. **Since PIM kinases modulate activity of proteins implicated in immunosuppressive functions of TAMs, we hypothesize that expression of PIM kinases in macrophages contributes to the pathogenetic phenotype of HL-TAMs**.

In the proposed study, we will perform detailed analysis of PIM kinase expression in primary HL-TAMs, using immunofluorescence microscopy. Using cytokine and phospho-kinase arrays, we will identify RS-dependent factors that underlie PIM expression in the *in vitro*-generated HL-TAMs. By performing functional assays in coculture systems of HL-TAMs with RS cells or T lymphocytes, we will characterize consequences of PIM kinase inhibition in HL-TAMs on their immunosuppressive and tumor growth promoting capabilities. In the mouse xenograft model generated using RS cells, we will assess impact of PIM kinase inhibition on macrophage recruitment to the tumor site.

Fulfilling the planned aims of the project would allow to understand better mechanisms underlying mutual interactions between malignant cells and macrophages in cHL. Since RS cell survival depends on their interplay with infiltrating cells, data obtained from the study might contribute to the development of more effective therapeutic approaches in the future. According to our key hypothesis, PIM kinases are likely supporting the pathogenetic phenotype of HL-TAMs. Hence, pharmacological PIM inhibition *in vivo* would likely exhibit pleiotropic and complementary effects, simultaneously decreasing RS cell survival, downregulating immunomodulatory proteins by RS cells and TAMs, and attenuating signalling that supports macrophage differentiation towards pro-tumoral TAMs.