Permanent NFκB activity is a common feature of several lymphoma entities, including diffuse large B-cell lymphoma (DLBCL) and classical Hodgkin Lymphoma (cHL). NFκB activation stimulates proliferation and protects from death DLBCL and cHL malignant cells but also support tumor cell survival in certain solid cancers. For this reason, NFκB-targeting strategies have garnered considerable attention and are intensively explored. However, as in case of other oncogenic transcription factors, direct approaches to target NFκB were unsuccessful and no clinically available direct NFκB inhibitor has been developed to date. Similar to many other cancers, the lymphoma tissue consists of both malignant and non-malignant, tumor microenvironment cells (TMEs). In cHL, TMEs outnumber the malignant Reed-Sternberg (RS) cells and exhibit profound role in supporting survival and immune privilege of the tumor cells. Increased number of macrophages (tumor-associated macrophages, TAMs) predict therapy failure in cHL, indicating that TAMs are major TME components involved in the disease pathogenesis. RS cells recruit TMEs (including macrophages) to the tumor site and re-educate them. Functionally skewed infiltrating cells in turn provide growth factors that support survival of RS cells and express immunomodulatory molecules that dampen anti-tumor immune response. In RS cells, NFκB activity orchestrates the expression of pro-survival factors, cytokines/chemokines and immunomodulators involved in RS cell cross-talk with TMEs. In our ongoing study, RS cells induced NFκB activation and pro-tumoral phenotype of macrophages. Thus, activity of the NFκB pathway appears to play the central role in the biology of cHL, both in the tumor and certain TMEs. These observations suggest that identification of a common NFκB pathway vulnerability in lymphoma and TMEs might represent novel, rational therapeutic strategy. The activity of NFκB factors is controlled by the kinase-mediated phosphorylation of certain NFκB proteins. Of these phosphorylations, p65 S276/S536 and cRel S267 exhibit the greatest effects on transcriptional activity of NFκB. However, role of these modifications in B cell lymphomas remains largely unexplored. Our preliminary data suggest that inhibition of p65-S276 phosphorylation downregulates NFκB-dependent transcription in lymphoma cells and in TAMs. We have identified PIM1 as a p65 kinase expressed in both B-cell lymphoma cells and TAMs and initially characterized consequences of PIM kinase inhibition that include decreased NFκB target gene expression in lymphoma cells and RS-educated macrophages. Despite these highly promising data, there are several pending issues and questions that remain to be further explored and answered. In particular: (i) is cRel phosphorylation important for NFκB-dependent transcription in lymphoma cells and TAMs; (ii) what are specific transcriptional and biological consequences of p65 phosphorylation blockade in lymphoma cells and TAMs; (iii) are there any other targetable kinases besides PIM1 mediating p65 phosphorylation in these cells; (iv) does p65 S276 phosphorylation influence immunosuppressive phenotype of TAMs.

The aim of the present project is to discover the function of p65/cRel phosphorylations and identify kinases responsible for these modifications in B-cell lymphomas and TAMs. So far this has not been investigated by any group.

To achieve our goals, we will approach the issues from various sides. To assess role of p65/cRel modifications in NFκB-mediated transcription in cancer cells and TAMs, we will generate genetic models of cells expressing p65 or cRel mutants to mimic constitutively site-dephosphorylated (inactive) factors. We will identify genes that are expressed differentially in cells expressing wild type and mutant factors by RNA sequencing (RNAseq) and assess mechanisms underlying differential gene expression in detail. For this purpose, we will identify co-factors associated with p65/cRel modifications in proteomic analyses and assess their effects on the assembly p65/cRel-co-factor complexes in genome-wide experiments (by performing chromatin immunoprecipitations coupled to next-generation sequencing). We anticipate that integrative analyses of transcriptomic and epigenomic data will explain us how p65/cRel phosphorylations lead to deregulated gene expression in the malignant cells and RS-educated macrophages. Although we identified PIM1 as a p65-S276 kinase in DLBCL, RS cells and TAMs, it remains unknown whether it is the only functional kinase that phosphorylate p65 and cRel kinases in these cells have not been reported to date. For this reason, in planned proteomic analyses, we will identify p65/cRel phosphorylation site-specific kinases. Following identification, we will assess effects of their genetic ablation on site-specific p65/cRel phosphorylations, NFκB target gene expression and viability of DLBCL and RS cells. In analyses comparing differentially expressed genes in kinase-depleted cells with transcriptional profiles of cells overexpressing p65/cRel mutants, we expect to identify subsets of genes consistently down-regulated and playing key role in DLBCL and RS cell survival and pro-tumoral features of RS-educated macrophages. Using chemical inhibitors of the identified kinases, we will investigate expression of the identified genes expressed differentially and assess effects of these compounds of on malignant cell's viability. For the most promising compounds, we will assess their therapeutic activity and potential synergy with available agents blocking upstream components and modulators of NFκB pathway in DLBCL and RS cell lines. Having identified successful candidates synergistically attenuating growth of lymphoma cells, we will test their interactions *in vivo*, in patient-derived DLBCL xenograft models.

Fulfilling the planned aims of the project would allow to understand functions of p65/cRel fosforylations in the malignant B cells and TAMs, and identify targetable kinases that are responsible for these modifications. Given our preliminary results, we anticipate that inhibition these kinases would downregulate NFκB target gene expression, decrease lymphoma cell survival and attenuate protumoral/immunosuppressive features of TAMs. Collectively, we believe that the planned research might result in identification of novel therapeutic strategies for simultaneous targeting of the malignant B cells and their immunosuppressive TME.