Immunotherapy has recently emerged as the fourth pillar of cancer treatment, joining surgery, radiation, and chemotherapy. While early immunotherapies focused on accelerating T-cell activity, current immune-checkpoint inhibitors take the brakes off the anti-tumor immune responses. Successful clinical trials with PD-1/PD-L1 monoclonal antibodies and other immune-checkpoint inhibitors have opened new avenues in cancer immunology. However, the failure of a large subset of cancer patients to respond to these new immunotherapies has led to intensified research on combination therapies and predictive biomarkers. There is thus a still a great deal of exploratory research needed to clarify the fundamental mechanism and predictive biomarkers for the efficacy and adverse effects of this therapeutic strategy. To advance the development of PD-1/PD-L1 signal inhibitors in cancer therapy, it is important to continue research approaches, including molecular and genomic studies, to elucidate the interactions between host and tumor cells.

The research objective of our project is to decipher the involvement of various direct and indirect immune modulators of the PD-1/PD-L1 signaling in cancer cells. To this end we will develop chemical probes for discriminating signaling events in the PD-1/PD-L1 pathway. The PD-1/PD-L1 signaling pathway is crucial in dampening immunosurveillance for tumors and belongs to the broader immune checkpoint signaling system in cells. Tumors can escape host immune surveillance by expressing PD-L1, which negatively regulates immune responses by interacting with PD-1 on T cells. The recovery of T-cell activity, by blocking PD-1 signals on T cells (immune checkpoint blockade, ICB), yielded impressive clinical benefits for several types of malignancies. Nevertheless, there is still a great deal of basic research needed to clarify fundamental mechanisms of the PD-1/PD-L1 signaling.

Anticancer immunotherapies that block PD-1/PD-L1 signaling are currently based on monoclonal antibodies (mAbs). However, usage of mAbs for studying molecular and cellular mechanisms inherently carries a number of disadvantages, such as the immunogenicity of human mAbs (following repeated administration), poor solid tumor tissue penetration and poor control of pharmacokinetics. In contrast, small-molecule probes can have affinity and specificity features rivaling that of antibodies.

Development of chemical inhibitors for any of the immune checkpoint proteins lags the antibody development (mostly because of insufficient structural information). Only one nonpeptidic chemical scaffold that targets the PD-1/PD-L1 pathway with modest pharmacokinetics has been disclosed until now. The aim of the proposed research is to discover small-molecule antagonists which would effectively antagonize the PD-1/PD-L1 interaction, mimicking the effect of the known mAbs and having significantly improved cellular properties. The research also comprises a blueprint for targeting future protein-protein interactions. Thus, we propose to use and to synthesize a large mixture library of small-molecule fragments and screen for binding against the immune checkpoint proteins PD-1 and PD-L1 using a NMR-based fragment screening campaign. By merging, linking, or growing fragments, high-affinity leads may be obtained. Medium-to-high affinity binding compounds will be identified and then synthesized; their affinity to the targets will be assayed using nuclear magnetic resonance (NMR) spectroscopy followed by a co-crystal X-ray structure analysis. We have recently published the first crystal structure of human PD-1/PD-L1 and have unlimited access to purified proteins for NMR, X-ray and biochemical experiments.

The small-molecule probes developed by us will be used to dissect the potential cellular and molecular mechanisms by which different oncogenic genes, proteins, and miRNAs participate in immune regulation and, in particular, in tumor immune evasion. Accumulating evidence suggests that tumor suppressor genes, such as p53 and pRb, may negatively regulate immune responses. p53 has a well-established function as the "guardian of genome". We have been working on the activation of the tumor suppressive capabilities of p53 for fighting cancer (by targeting the negative regulators of p53: the Mdm2 and Mdmx proteins) since some time. We plan is to use our PD-1/PD-L1 and Mdm2/p53 and inhibitors for studying the prospective interdependence of p53 and checkpoint proteins in immunotherapeutic aspects of tumorigenesis.

Our research proposal addresses timely and important issues of the mechanism of the involvement of the PD-1/PD-L1 axis in tumorigenesis. Cellular active selective small molecules that bind to specific proteins are of uttermost importance to delineate biochemical pathways of proteins. Our research should result in protein-specific, potent chemical probes for the proteins that are prominently involved in tumorigenesis, and by doing this, we hope to provide invaluable compounds to the cancer community that can be used to characterize the underlying biochemical pathways and events that drive tumor formation.