Exosomes represent a class of extracellular vesicles released by cells to their environment. Within the space bordered by their membrane these vesicles contain various classes of nucleic acids, proteins, lipids and metabolites. Although an exosome membrane is not identical with a cell membrane and during the biogenesis of exosomes a part of their constituents is packed selectively, exosomal proteins (both membrane and lumen ones) are characteristic for their parent cell. Therefore, these components can be specific markers reflecting features of a parent cell (e.g., cancer cells), thus serving as an equivalent of a "liquid biopsy" and enabling non-invasive diagnosing and monitoring of disease progression. Exosomes released from cancer cells and present in body fluids of patients are responsible for, inter alia, transport of signals modulating the immune system. Blocking of immunosuppressive functions of exosomes released by cancer cells should, therefore, lead to stronger anti-cancer immune response (utilization of this phenomenon would be a novel promising approach to anti-cancer therapy).

The research on practical utilization of exosomes is hampered by the necessity to move from *in vitro* models (cell culture) where exosomes make a homogeneous population, to *in vivo* models where exosomes make a heterogeneous population of vesicles released by different types of cells. In serum of cancer patients exosomes released by cancer cells are present alongside exosomes produced by blood cells (platelets, lymphocytes and other cells of the immune system) as well as epithelial cells of blood vessels. Exosomes of lymphatic origin stand for the largest group among all exosomes identified in serum or plasma, which is a result of a large number of lymphocytes circulating in the blood. It is estimated that exosomes released by blood cells stand for 60-80% of the exosome population present in serum/plasma, which is an obvious problem if the composition of tumor-derived exosomes (TEX) is to be addressed.

Patients suffering from head and neck cancers (HNCs) associated with the human papillomavirus (HPV) infection have a more favorable prognosis than patients with cancers independent of HPV infection. This phenomenon is most probably connected with a different way of modulation of the immune system by these two types of cancer. In the proposed project we would like to verify the hypothesis that exosomes released by cancer cells contribute to differences between HPV(+) and HPV(-) tumors observed with respect to their progression and response to the treatment, and that molecular composition of these exosomes can be a potential prognostic biomarker.

In our preliminary report based on the *in vitro* model (cell lines derived from HNCs) we were able to identify specific membrane proteins present on the surface of exosomes released by HPV(+) and HPV(-) cells. We also found that the presence of such receptors influenced the way these exosomes modulated functions of immune system cells *in vitro*. Here we propose to validate and extend these observations using the *in vivo* model, i.e. TEX present in the plasma of patients with HPV(+) andHPV(-) HNCs. Due to the above-mentioned diversity of exosomes present in the blood, the critical path of the project is the necessity for selective isolation of the TEX population. Hence, we propose that the isolation of subpopulations of exosomes from plasma of HNC patients with different HPV status will be performed using the immunoaffinity-based method of exosome TEX isolation that was developed in the laboratory of prof. Whiteside (University of Pittsburgh, PA, USA) who is a scientific partner of this proposal. This method employs antibodies specific for membrane receptors present on the TEX surface. Next, we plan to perform proteomics analysis of TEX derived from plasma of HNC patients with different HPV status aiming at, among others, confirmation and extension of the results previously obtained using the *in vitro* models. Moreover, we plan to perform functional analysis of interactions between plasma-derived TEX and immune cells (in the context of influence of selected exosomal proteins on the phenotype of immune cells).

The proposed project presents an original approach to the problem of search for molecular factors responsible for differential prognosis of head and neck cancer with different HPV status. The project will allow for characterization of exosome markers, which could potentially be utilized in diagnosing and prognosis of HPV-dependent cancers. Moreover, the project will provide new knowledge on the role of exosomes in communication between tumor cells and normal cells present in its microenvironment. The results of the project may also have potential applicability in development of a novel approach to the so-called liquid biopsy of cancer.