Melanoma is an aggressive skin cancer with increasing incidence worldwide. Currently, the treatment of melanoma patients includes surgery followed by the adjuvant treatment with immune checkpoint inhibitors or inhibitors of BRAF/MEK kinases, which are adjusted individually depending on the patient's clinical situation. Despite these novel therapies, this cancer slips out of immune control and develops a primary or acquired resistance to therapy which occurs in about half of melanoma patients, remaining the major therapeutic problem.

Exosomes are a class of the so-called extracellular vesicles, which are released by cells into their environment and participate in cell-to-cell communication. Components of exosomes are specific for the releasing cells and could be used as specific markers reflecting the characteristics of the parental cells. Exosomes released by melanoma cells, so-called MTEX, and present in the body fluids (plasma/serum) of melanoma patients may be the equivalent of tumor cells and serve as an equivalent of the so-called "liquid biopsy" allowing non-invasive diagnosis of cancer. Additionally, among functions attributed to tumor-derived exosomes (TEX) is the suppression of immune response by a developing tumor, e.g., initiating the transformation of the immune cells to cells that suppress the immune response. TEX can not only facilitate tumor escape from immune surveillance, but also promote tumor growth by transferring signaling molecules to modulate tumor microenvironment. Therefore, functional studies of the interactions between MTEX and immune cells and cancer cells will bring the understanding of the complex communication occurring in the tumor microenvironment.

The overall aim of this project is to investigate the role of programmed cell death protein-6 (PDCD6IP) present in plasma-derived MTEX in exosome-mediated immune regulation and promoting melanoma progression. Quantitative targeted proteomics by mass spectrometry techniques and functional analyzes of MTEX interaction with primary immune cells and cancer cells will be implemented. We expect the project to confirm the potential use of PDCD6IP protein as a prognostic marker of melanoma progression after oncological treatment.

Our pilot analysis of the proteome signature of MTEX isolated from plasma of patients with metastatic melanoma showed that PDCD6IP is the protein with the greatest power to discriminate between patients with melanoma progression (PD) after oncological treatment from patients with no evidence of disease development or stable disease (NED/SD). Expression of PDCD6IP protein was decreased or absent in MTEX of NED/SD patients, while in PD patients it was consistently and significantly increased.

To explain why an elevated level of MTEX PDCD6IP is associated with melanoma progression, we will perform functional analyses of MTEX produced by the PDCD6IP-positive and PDCD6IP-depleted melanoma cells (the latter variant will be obtained by CRISPR-based knockout). To evaluate the potential role of exosomal PDCD6IP in immune regulation, both types of MTEX will be incubated with primary human immune cells then immune cell survival, proliferation, cytokine production, and effector functions will be compared. Using the same cell/MTEX model, we will evaluate phenotypic and functional changes in the recipient melanoma cells to determine whether the presence of PDCD6IP affects their growth, survival, and resistance to therapy. We expect these in vitro data to confirm the involvement of PDCD6IP in exosome-mediated immune regulation or tumor growth, providing a biological rationale for the proteomic data.

Realization of the proposed project will bring new knowledge on the role of exosomes in communication between tumor cells and immune cells present in the tumor microenvironment. The results of the project, apart from considerable cognitive significance, will have potential application significance for the development of an innovative approach to the so-called liquid tumor biopsy.

The project will be realized in cooperation with prof. Whiteside and prof. Ferrone, world-class experts in the field of molecular immunology of cancer, providing an excellent opportunity to perform the proposed research in an optimal environment enabling effective and successful implementation of the planned research. Scientific cooperation between all partners has been documented by mutual publications.