Angiogenesis is an important element of the tumor microenvironment, directly influencing the growth and metastasis of neoplastic cells. The cells of the immune system, including T lymphocytes and neutrophils are one of the first lines of defense, inter alia against tumor development. Increase in peripheral blood neutrophil count (more than nine times relative to the correct level) is, however, associated with the development of an adverse Neutrophil-to-Lymphocyte Ratio (NLR), which correlates with poor prognosis in patients diagnosed with gastric cancer, myxofibrosarcoma and neoplasms of the head and neck region. This is probably related to existence of a pro-oncogenic neutrophil population that promotes tumor development through, inter alia stimulation of proliferation, migration and angiogenesis of endothelial cells. So far, the mechanism of this stimulation has not been clarified, which makes it impossible to plan a therapy aimed at its inhibition.

Small extracellular vesicles belong to the group of extracellular vesicles and are released by all types of cells. They participate in many processes, including intercellular communication or modulation of the behavior of cells of the immune system. Components of small extracellular vesicles are specific for the cells from which they are released, so they can be specific markers reflecting the characteristics of these cells, as well as carriers of signals for target cells. In the case of small extracellular vesicles released by neutrophils, they have been shown to modulate proliferation of airway smooth muscle cells. Therefore, it is possible to stimulate angiogenesis by small extracellular vesicles without direct contact between neutrophils and endothelial cells. Results of studies on the mechanism by which neutrophils modulate the angiogenesis process in the tumor microenvironment may allow the design of a therapy aimed at inhibiting the angiogenesis process and tumor growth.

In the proposed project, the applicant will validate the hypothesis that small extracellular vesicles released by neutrophils (NEX) play a critical role in neutrophil-dependent modulation of angiogenesis.

Current research indicates that pro-oncogenic neutrophils accumulate in the vicinity of the tumor and support its development by stimulating the angiogenesis process. Pro-oncogenic neutrophils secrete large amounts of pro-angiogenic factors such as VEGF and MMP9. They affect endothelial cells causing increased proliferation and stimulate formation of blood vessel-like structures. The above-mentioned properties were not found in the case of "anti-tumor" neutrophils, which was confirmed in in vivo studies in a mouse model of oral cancer.

Moreover, assuming the role of type 1 interferon receptor in regulation of the switch between anti-tumor and pro-oncogenic phenotype of neutrophils, the applicant will validate the role of type 1 interferon receptor in NEX-mediated communication between neutrophils and endothelial cells. The obtained results will allow to assess the role of small extracellular vesicles released by neutrophils in modulation of the tumor microenvironment by pro-oncogenic tumor-related neutrophils. Furthermore, the study will help identify molecular components of NEX that may become a potential therapeutic target.

In order to explain the mechanism by which neutrophils regulate angiogenesis, the applicant will perform a functional analysis of small extracellular vesicles released by neutrophils with "pro-oncogenic" and "anti-tumor" properties. Neutrophils will be obtained from mouse progenitor cells conditionally immortalized using a retroviral transfection method. Small extracellular vesicles obtained from each of the neutrophil functional variant will be co-incubated with the endothelial cell line. In addition, the applicant will evaluate the effect of vesicles on endothelial cells in terms of proliferation, migration and the ability to form blood vessel-like structures. The applicant also plans to investigate the role of two overrepresented proteins present in small extracellular vesicles released from pro-oncogenic neutrophils on the phenotype of endothelial cells in vivo model. The obtained results will help to understand the role of small extracellular vesicles in modulation of the angiogenesis process and verify their potential therapeutic effect.

Implementation of the proposed project will enable to investigate the mechanism by which neutrophils can modulate the angiogenesis process necessary for cancer development. In addition, implementation of the project, apart from the significant cognitive importance, will determine whether small extracellular vesicles released by neutrophils may have a potential therapeutic application.

The project will be implemented in cooperation with the team of prof. Jadwiga Jablonska, who is an expert in the field of molecular immunology and the function of neutrophils in the neoplastic process, which will be an excellent opportunity to perform the proposed research in the optimal scientific environment enabling effective and successful implementation of the planned tasks.