Lung cancer remains the leading cause of cancer-related deaths in both sexes worldwide. As no apparent symptoms are present at the beginning of cancer development, lung cancer is detected at the advanced, difficult-to-treat stage. Thus, it is crucial to search for novel, more effective therapies to improve the prognosis for lung cancer patients. Recent medicine developments provided novel therapeutic tools that arm patient's immune system to fight against tumor. This approach is called immunotherapy. A wide range of immunotherapeutic options is currently tested in preclinical and clinical studies. Nevertheless, similarly to other treatments, immunotherapies may also be hampered by resistance mechanisms.

Tumor mass may be regarded as a malfunctioning organ consisting of various cell types embedded in a certain microenvironment. Importantly, tumors contain a small set of cancer stem cells that contribute to tumor regeneration, for example, after therapies. Besides cancer cells, tumor mass harbors immune cells, fibroblasts, the cells building blood vessels, etc. Together with the proteins, such as collagen, they form tumor microenvironment (TME) that plays an essential role in cancer development and response to therapies. High levels of collagens make the tumor stiff. Thus, it is difficult for the immune cells or large therapeutic drugs to infiltrate tumor mass and promote its shrinkage. Cancer cells frequently emerge in the human body. However, their development is blocked by the immune system. However, one of the hallmarks of cancer is the escape from immune system control. Tumors release factors that inhibit anti-tumor immune response. In summary, the presence of cancer stem cells, stiff microenvironment (e.g., due to the high levels of collagen), and inhibited anti-tumor immune response are the causes of failed cancer immunotherapy.

Our goal is to generate and test novel immunotherapeutic tools against human lung cancer that overcome the resistance mechanisms. We plan to create a research model with the reduced production of collagen to lower the stiffness of microenvironment and promote tumor accessibility for immune cells. To do so, we will use a lung cancer cell line and perform genetic engineering to diminish the level of the ZNF714 gene. We chose this gene because, in our previous research, ZNF714 positively affected the levels of collagen and other proteins building tumor microenvironment. We will conduct experiments to check whether these cells produce less TME protein and, if so, whether this feature will affect their ability to form a looser 3D structure accessible to immune cells.

In parallel, we will create an anti-cancer vaccine from tumor cells reprogrammed in vitro to stem cells. This vaccine, dubbed LC-iPS, will serve as a cargo delivering a wide range of tumor-specific antigens (e.g., proteins) that will train the immune cells to recognize tumor cells residing in the body. In particular, the LC-iPS vaccine will direct the immune system against cancer stem cells. Finally, to overcome the inhibition of the anti-tumor immune response, the vaccines will be modified to contain a molecule stimulating the immune system. We will test three vaccines with different immunomodulatory molecules in vitro to assess their influence on immune cell killing abilities. These analyses will allow the selection of the vaccine option with the highest potential to activate the immune system.

To validate the in vitro studies, we will further use a mouse model to integrate both parts of the project: ZNF714 deficient lung cancer cell line with lowered collagen production and modified LC-iPS vaccine with increased potential to stimulate the immune system. The lung cancer cell lines will be transferred to mice, and we will investigate their ability to grow, form metastasis, and contribute to TME protein production. Moreover, we will test their response to a known immunotherapy option and the experimental vaccine selected in the previous part of the project. The expected impacts of the project are two-fold. First, the study will allow for the detailed characterization of ZNF714 involvement in tumor functioning, its microenvironment and sensitivity to immunotherapy. Secondly, we will generate and evaluate a novel anti-cancer vaccine that combines lung cancer, stem cells, and immune system modulator. Both aspects were not tested before, which renders the project highly innovative.