Lung cancer is the leading cause of cancer death worldwide. The most common malignant lung cancer is non-small cell lung cancer (NSCLC). Treatment of NSCLC is a huge challenge because it is poorly sensitive to standard chemotherapy. In recent years, personalized therapies have played an increasingly important role in treating NSCLC, including molecularly targeted tyrosine kinase inhibitors (TKIs), which, however, is associated with the enormous toxicity of the therapy. In addition, many NSCLC patients develop resistance to TKIs by cancer cells during treatment with personalized medications. This means that in approximately 60% of NSCLC patients treated with TKIs, the therapeutic efficacy decreases or disappears, and, as a consequence, a change in the treatment regimen is necessary, or patients are prevented from being successfully treated. Therefore, the development of a new approach to anti-cancer drugs seems to be an essential task to improve the effectiveness of NSCLC therapy.

Targeted drug delivery systems (TDDS) are being developed to reduce the side effects of therapy. They are based on targeting a therapeutic compound or its carrier to neoplastic cells with specific markers on their surface. The expression of these factors may change during tumor development, which poses a problem in proper drug delivery. Cancer is not only a local accumulation of uncontrolled cancer cells but also various types of stromal cells, cancer cells, and the extracellular matrix interacting with each other. The environment within the tumor is called the tumor microenvironment (TME). Among the cells that mainly make up the stroma, we distinguish mesenchymal stromal cells and the vascular and immune cells. Thus, an alternative target for TDDS may be TME-related cellular surface molecules, exemplified by VEGFR-1 and -2 molecules. These receptors bind vascular endothelial growth factor (VEGF), the activation of which is responsible for the angiogenesis process.

The main goal of the project is to selectively deliver nucleic acid-based therapeutics to TME in order to inhibit tumor growth. The project will use siRNA to suppress the HIF1alpha gene, the activation of which plays a crucial role in communication between cancer cells and TME cells at many levels. Therefore, inhibition of this signaling cascade is a promising approach to increase the efficacy of anti-cancer therapy and prevent acquired resistance in the treatment of NSCLC. RNA-based therapies have gained significant importance in recent times and are considered a milestone in treating and preventing various diseases. Also, in the treatment of cancer, the use of oligotherapeutic constructs has resulted in good results. However, due to toxicity and susceptibility to degradation in serum, their *in vivo* use is still limited. One way to increase the potential for *in vivo* use of oligotherapeutics is the application of an appropriate TDDS. In the project, the vehicles that transport toxic therapeutic substances and link the two strategies are spheres based on bioengineered spider silk proteins targeting tumor vascularization. Moreover, to improve the effectiveness of the applied treatment, we propose combining this approach with the targeted delivery of tyrosine kinase inhibitors.

In the project, the applicants intend to produce functionalized silk spheres and carry out a detailed characterization of the obtained structures. First of all, the potential of the spheres to selectively recognize target cells expressing VEGF receptors and the effect of delivered siRNA on signaling pathwa**ys** involved in angiogenesis and tumor development will be explored. We will also examine the effectiveness of the proposed strategy in combination with the targeted delivery of tyrosine kinase inhibitors. The biological activity will be assessed *in vitro* in endothelial cells and the NSCLC model. The most effective combination of therapeutics will be tested using two *in vivo* models: the chicken embryo chorioallantoic (CAM) and the laboratory mouse models.

The innovative approach of anti-cancer therapy proposed in the project, consisting of the development of one common carrier targeting the tumor microenvironment cells for double cargo transport, will contribute to increasing the effectiveness of NSCLC therapy. The obtained research results will enable the design of new, more effective methods of treatment based on the targeted delivery of biological drugs and active small molecules not only in the treatment of lung cancer but also other types of cancer. They will significantly influence the development of the field of drug delivery, as well.