Nowadays, a solid tumor is not considered as a local accumulation of clonal cancer cells but rather as well organized “organ” composed of a variety of interacting stromal cell types, cancer cells, and extracellular matrix. The environment around a tumor is named the tumor microenvironment (TME). Among the cells that make up stroma, the main ones are mesenchymal supporting cells, and cells of the vascular and immune systems. The tumor-stroma cells are in constant interactions, and more importantly, these interactions modify constantly the cancer cells and TME. Thus, therapeutic targeting of cancer cells alone is not enough to kill cancer. Therefore, the multitargeted approaches are intensively investigated in which several cell types of cancer stroma are simultaneously inhibited.

Immunotherapy is one of the most intensively investigated cancer therapeutic approaches. Immunotherapy is a treatment that uses the defense system (immunological system) of the body to help fight cancer. It can be distinguished two forms of immunotherapy: i) the adoptive cell therapies that rely on introducing additional immune cells and proteins to make it recognize and attack cancer cells, and ii) application of therapeutics that stimulate an existing immune response to help it work harder to attack cancer cells. Immune cells are accumulated within the tumor “organ,” however, they do not function properly. The application of proper therapeutics can modify them to restore anti-tumor response.

We want to modify the immune cells within tumors by applying the oligonucleotide therapeutics delivered by silk nanocarriers. Our oligonucleotide therapeutics (oligotherapeutics) are CpG-siSTAT3 and aptCTLA4-siSTAT3. They inhibit the expression of the signal transducer and activator of transcription 3 (STAT3) molecule. The STAT3 signaling in TME is a key regulator of the hallmark of cancers. The CpG-*STAT3*siRNA is a drug that silences the STAT3 expression with simultaneous inductions of the TLR9 pathway in myeloid cells, while the aptCTLA4-siSTAT3 silences the STAT3 in lymphocytes. The inhibition of the STAT3 molecule in myeloid and lymphocytes in TME restores the anti-tumor response. Although the oligonucleotide-based constructs have already given promising results in cancer therapy, the *in vivo* applications still are limited due to their sensitivity to serum nucleases, some toxicity, and potential off-target activity. One strategy for avoiding these obstacles is to apply a targeted drug delivery system (DDS). Therefore, as a carrier for nucleic acid-based therapeutics, we propose spheres made of bioengineered spider silk.

The silk-derived biomaterials are biocompatible, biodegradable, non-toxic, and are great candidates for various biomedical applications. We are interested in using silk as a drug delivery system (DDS). Previously we elaborated system based on silk spheres for selective delivery of a cytotoxic drug (Doxorubicin) to Her2-positive breast cancer cells. In the present project, we want to use silk spheres for safe, targeted, and effective delivery oligotherapeutics. Based on the preliminary data, silk spheres not only efficiently delivered *in vitro* oligotherapeutics to targeted cells but also modified (extended) the kinetics of processing and activity of the CpG-siSTAT3 comparing with the delivery of naked CpG-siSTAT3 therapeutics. We hypothesize that this modification will significantly improve the CpG-siSTAT3 activity *in vivo*.

We will develop and characterize the silk spheres that carry the oligotherapeutics, and the therapeutic potential of the molecules will be examined *in vitro* and *in vivo*. We will examine whether the resulting spheres will provide oligotherapeutics to TME immune cells in mouse models of breast cancer and whether it will be beneficial in terms of tumor destruction.

Based on the results, we will develop a new approach to anticancer therapy that will rely on the activation of immunological response in the tumor microenvironment. This technology is universal and can potentially be applied to treat any solid tumors.