The reasons for choosing the research topic:

Cancer is constantly a major challenge to modern medicine. In recent years, immunotherapy has emerged as a potent weapon against cancer. In particular, two strategies governed the attention of the researchers and the medical society. The first one relies on the use of immune checkpoint inhibitors (ICI) - the monoclonal antibodies that have the potency to block the inhibitory signals derived from cancer cells and unleash the full potential of cytotoxic effector cells. The second strategy- the so-called adoptive cell transfer - takes advantage of the patient's own genetically modified effector cells that express chimeric antigen receptors (CARs). CAR receptors enable specific recognition of proteins present on the surface of tumor cells. This recognition triggers their cytotoxic response, that consequently leads to cancer cell elimination. Although both of these approaches are promising in principle, they still struggle in the field of solid tumors. Recently, more attention is drawn to the basic biological processes responsible for that outcome. For instance, it was shown that the PD-L1 molecule, both on tumor cells as well as in tumor milieu, undergoes changes and modifications in a structure that eventually might disturb its recognizing by the blocking antibody. Moreover, the cells that reside in tumor milieu apart from presenting PD-L1 on the surface also secrete various factors that inhibit effector cell cytotoxic functions. In the case of CAR therapy, there are two main limitations: the definite number of tumor-specific targets and in case of using less specific, the risk of recognition targeted molecule on healthy cells instead of tumor cells. Currently, more light was shed on the activation of CAR receptors. It was shown feasible to modulate the specificity of those receptors and warrant them safe for healthy tissue. This modulation is made by tuning down the "strength" of the CAR-target binding.

Given the abundance of PD-L1 molecule on the tumor cells as well as on cells residing in tumor milieu in our initial results, we have developed PD-L1 CAR effector cells and tested their cytotoxic potential against tumor and tumor microenvironment cells. Depending on PD-L1 density on the surface of target cells, we observed not only the target- but also the time-dependent cytotoxicity of PD-L1 CAR-modified effector cells. Thus, in this proposal, we want to explore this phenomenon, discover the forms of PD-L1 that are present on tumor cells and cells of tumor milieu, and finally to make an attempt to build the PD-L1 CAR specific for tumor cells only.

The aim of the project:

The aim of the project is to elucidate the mechanisms regulating expression of PD-L1 and its different variants in breast cancer cells as well as cells that reside in tumor milieu to identify a novel approach to improving the safety of the PD-L1 CAR-based strategy.

Implementation of the project:

In the current project, we have planned the implementation of four research tasks. In Task 1, we will assess the PD-L1 variants that are present on the surface of normal and tumor cells as well as cells of tumor milieu. In Task 2, we will modulate the affinity of PD-L1 CAR towards the chosen PD-L1 variants. Next, in Task 3 we will evaluate the efficacy of the newly constructed CARs in directing cytotoxic cells to specifically eliminate cancer cells and cells of tumor milieu. Finally, in Task 4 we will get an insight into the mechanisms of the activity of newly designed PD-L1 CARs.

Expected results:

CAR-based therapy is still limited in terms of molecules that can be efficiently targeted. Taking into account abundant PD-L1 expression on cancer as well as on the tumor milieu components, and also the advances in CAR research, we expect, that the idea of PD-L1 CAR-based therapy has a significant potential to become quite an example of universal CAR for oncological applications, as long as it can be precisely tailored and rendered more specific for the tumor site.