Cutaneous melanoma arises from the malignant transformation of melanocytes. Melanocytes are skin cells capable of absorbing UV radiation and producing melanin, a natural pigment responsible for skin pigmentation. Factors that promote the malignant transformation of melanocytes include UV radiation, the number and shape of nevi, age, and genetic predispositions. Despite improvements in patient survival rates due to the development of new therapies, melanoma remains a serious clinical challenge. Current treatment approaches include radiotherapy, chemotherapy, surgical procedures, and novel drug therapies, including targeted therapy using selective $BRAF^{V600}$ inhibitors (dabrafenib, encorafenib, and vemurafenib) and MEK1/2 inhibitors (trametinib, binimetinib, and cobimetinib). Targeted therapy is used in patients with *BRAF* mutations, which are present in 50–70% of melanomas. Another widely used treatment is immunotherapy with immune checkpoint inhibitors (ICIs). Under physiological conditions, immune checkpoints protect healthy tissues from excessive inflammatory responses, but melanoma can exploit this mechanism to suppress immune responses. Currently used immunotherapies include antibodies targeting programmed cell death receptor 1 (PD-1) – nivolumab and pembrolizumab; its ligand (PD-L1) – atezolizumab; cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) – ipilimumab; and lymphocyte activation gene 3 (LAG-3) – relatlimab.

The response to immunotherapy depends on the tumor microenvironment, and one of the key factors is interferon-gamma (IFN-γ) secreted by immune cells. As a pro-inflammatory cytokine, IFN-γ strengthens the immune response by increasing antigen presentation by immune cells and recruiting effector immune cells to the tumor site. One of the major problems in pharmacological treatment is the development of resistance. Resistance to immunotherapy may result from prolonged IFN-γ exposure or the lack of response to IFN-γ. Melanoma cell capability to adapt to the microenvironment, including their response to IFN-γ, is linked to phenotype plasticity. A key regulator of melanoma plasticity is the microphthalmia-associated transcription factor (MITF). High MITF level characterizes a differentiated melanoma cell phenotype. MITF levels show an inverse correlation with the expression of the nerve growth factor receptor (NGFR). Phenotype alterations may increase melanoma cell susceptibility to non-apoptotic forms of cell death, although high NGFR levels may promote immune evasion. One of the newly described markers of both melanoma differentiation and response to ICIs immunotherapy is signal-regulatory protein alpha (SIRPα). High SIRPα expression correlates with a differentiated phenotype and response to immunotherapy.

The aim of this project is to assess the phenotypic changes induced by IFN-γ and determine whether these changes sensitize melanoma cells to disulfidptosis. The study will be conducted in drug-naïve and targeted therapy-resistant melanoma cells treated short-term with IFN-γ to mimic the early response to immunotherapy and long-term exposure as a model of resistance to immunotherapy with ICIs. Disulfidptosis is a recently described non-apoptotic form of cell death occurring in cells with high *SLC7A11* expression, a component of the xCT transporter, under glucose-starved conditions resulting from GLUT1 inhibition. Under these conditions, disulfide stress increases, leading to disruption and degradation of the β-actin cytoskeleton. In this proposal, we will use WZB117, an inhibitor of GLUT1/2/4 and GNE-140, a lactate dehydrogenase (LDH) inhibitor, which can induce a metabolic switch in melanoma cells by decreasing glucose uptake. We hypothesize that these compounds will induce disulfidptosis in cells exposed to IFN-γ and allow for detailed investigation of the mechanisms leading to cell death via disulfidptosis.

We expect that the data obtained in the project will gain insight into phenotypic changes in melanoma cells during initial response and acquired resistance to immunotherapy with ICIs. The chosen model will mimic clinically relevant situations and assess whether IFN-γ–induced changes can be potentially exploited in developing new therapeutic strategies with the use of potential disulfidptosis inducers, such as LDH or GLUT1 inhibitors.