Melanoma is an extremely malignant skin cancer. The use of selective inhibitors of $BRAF^{V600}$ kinase, the presence of which is characteristic of 50-70% of melanoma cases, and MEK1/2 has much improved the treatment of melanoma patients. Unfortunately, clinical response is limited due to resistance acquired during treatment. In turn, immunotherapy with immune checkpoint inhibitors may lead to improved long-term outcomes but results in late responses in a small fraction of patients. For patients with *BRAF* wild-type melanomas, including *NRAS*-mutated melanomas, the situation is critical due to limited therapeutic options if immunotherapy cannot be used. Therefore, it is justified to search for new or complementary therapies in order to increase the efficacy of the treatment of melanoma patients. In 2022, a novel type of cell death dependent on copper ions (cuproptosis) has been described. The vulnerability of cancer cells to cuproptosis relies on their metabolic phenotype, specifically oxidative phosphorylation. The following project will extensively assess the sensitivity of melanoma cells of distinct genetic subtypes (harboring mutation either in *BRAF* or *RAS*) to cuproptosis-inducing agents.

The aim of the project is to investigate the relationship between the metabolic phenotype of melanoma cells, naïve and modified by the therapy in the short-term and after the development of resistance, and the sensitivity of cells to agents inducing cuproptosis. As a melanoma research model, we will use patient-derived cell lines either with the $BRAF^{V600E}$ or RAS^{Q61R} variant that will be exposed to dabrafenib (inhibitor of $BRAF^{V600}$) in combination with trametinib (inhibitor of MEK1/2) or trametinib used alone. In the project, we will establish melanoma cell lines resistant to targeted drugs used in combination. These cell lines will be also exposed to interferon-γ to create a research model that mimics the immune microenvironment. This experimental setup represents to some extent the various clinically-relevant conditions for which sensitivity to cuproptosis-inducing agents will be determined. To better reflect the conditions accompanying tumor growth, an *in vitro* model with reduced oxygen concentration (6%, normoxia *in situ*) will be used. The obtained results will be additionally verified in a mouse model of melanoma (*in vivo* studies). Modern methods of molecular and cell biology will be used, including live cell microscopy, confocal microscopy, flow cytometry, metabolic tests, qRT-PCR, Western blotting, CRISPR/Cas9 gene editing and gene overexpression. The contribution of the ATOX1 protein, which is involved in intracellular trafficking of copper ions, to the regulation of the susceptibility of melanoma cells to the induction of cuproptosis and the relationship between autophagy and cuproptosis will be investigated.

The expected effect of the project is to delineate the mechanisms regulating the vulnerability of melanoma cells to a novel and uncharacterized type of cell death (cuproptosis) in an experimental setup that will represent specific clinically-relevant conditions. From a broader perspective, this knowledge may contribute to the use of compounds that selectively induce this type of cell death alone or in combination with available molecularly targeted drugs to improve the clinical response of melanoma patients.