Glioblastoma Multiforme (GBM), a grade IV brain tumor, is the most common malignant primary brain cancer in adults, associated with one of the most dismal prognoses of all cancers. The treatment of GBM remains extremely challenging due to a low chance for total surgical resection and the resistance of cancer cells to therapy. Intratumor heterogeneity is one of the leading determinants of therapeutic resistance. The dismal prognosis of GBM is, at least in part, caused by difficulty in eliminating glioblastoma stem cells (GSCs). Current standard treatments target proliferating cells, while GSCs are considered mostly quiescent and thus resistant to conventional therapies. GSCs are involved in tumor proliferation, progression, metastasis, and resistance to therapies; therefore, their eradication is definitively required to treat GBM patients successfully.

Cancer is a complex disease controlled by several intracellular pathways; therefore, single-drug therapy does not provide a satisfactory clinical outcome. Undoubtedly, drug combination may enhance anticancer effects due to different target pathways and prevent drug resistance. Moreover, drug synergy may reduce the effective doses required for the eradication of cancer cells. In this research, the compounds that inhibit signaling pathways and drugs whose combination aims to enhance the anticancer effect will be used. Noteworthy, in this project, we will use the drug repurposing strategy. Drug repurposing is based on finding for approved drugs new clinical applications other than those initially intended. One of the reasons for the growing interest in drug repositioning is that these drugs' pharmacokinetics and safety profiles are already known.

Our proposal will be executed using patient-derived cell lines. Cells will be divided into two populations to preserve the glioblastoma stem cells population (GSCs) and induce cell differentiation ("classical" cancer cells). These populations differ significantly in their sensitivity to treatment, so each of them should be taken into account. Experiments will be done using a physiologically relevant 3D cell culture model, which better than traditional 2D cell culture captures the complexity of tumor biology. Our proposal will use a mathematical modeling technique based on specialized computational methods, which will allow for the rapid identification of synergistic optimized combinations with a significant reduction of experimental costs and efforts. The mechanism of action of selected drugs and combinations will be tested using appropriate molecular biology methods. The most effective combination will be further tested using two *in vivo* models: the chorioallantoic membrane model of the chicken embryo (CAM) and mice xenograft model. Our innovative approach is expected to result in biological insights into how cancer cells respond to interferences of specific pathways; how the cross-talk between signaling pathways activate as a compensation response may impact the therapeutic response; and how to overcome these limitations. This knowledge may provide a base for translating mechanistic insights into rationally designed regimens and potentially personalized cancer treatment.