Tumors localized within central nervous system (CNS) represent an undisputable challenge for contemporary neurooncology. WHO classification distinguishes their four subtypes: I/II grade incl. pilocytic and diffuse astrocytoma; and III/IV grade, which corresponds to the „high grade" CNS tumors (i.e: anaplastic astrocytoma and **glioblastoma multiforme; GBM).** Notably, the efficiency of therapeutic strategies of the „high grade" tumors (especially of GBM) leave much to be desired. Therefore, classical GBM treatment (incl. the application of temozolomide, radiotherapy and/or surgical resection) are commonly followed by microevolution of chemo-resistant and invasive GBM and its recurrence. This results in a relatively high death rate of GBM patients. Recently, the application of **doxorubicin (DOX**; either in „monotherapy" or as and supportive drug) has been proposed as an alternative and complementation of regular GBM treatment strategies. **On the other hand, the development/microevolution of DOX-resistance have not been addressed in GBM systems.** These can include: activity of ABC (ATP-binding cassette) transporters; autophagy-dependent „removal" of dysfunctional (chemo-damaged) organelles; enzymatic (MGMT-related) repair of drug-induced DNA damage and the induction of (pseudo)senescence to counteract the action of cell cycle-specific drugs. All these processes **require a strong energy input from the metabolic apparatus of GBM cells.**

Our preliminary data suggest the induction of drug-resistance and a concomitant microevolution of the invasive GBM cell populations under DOX-induced stress. These processes are correlated with the mitochondrial rearrangements and the signs of metabolic reprogramming within GBM populations. **They prompted us to hypothesize that mitochondrial dynamics determine the ability of GBM cells to switch between various „metabolic modes" in the stress conditions.** In turn, this „metabolic flexibility" facilitates GBM cell adaptation and resistance to DOX-induced stress. Mitofusins (MFNs) act as the modulators of mitochondrial architecture under micro-environmental stress. Therefore, we also assumed that MFN-dependent mitochondrial fusion, followed by cellular hyperthrophy and intensified production of energy carriers (ATP; NAD(P)H) facilitates the selective expansion of DOX-resistant GBM cell lineages under DOX stress.

To address these notions, we will trace the time-course/symptoms of the metabolic reprogramming undergone by DOX-exposed GBM cells. We will correlate them with the MFN-dependent rearrangements of the mitochondrial architecture. Next, using an array of chemical inhibitors and genetic approaches (incl. ectopic MFN up- and downregulation) we will assess the role of MFNs as the determinants of interrelations between metabolic adaptation, drug-resistance of GBM cells. Finally, elucidation of the effects of the long-term DOX-exposition of GBM cells on their metabolic phenotype will enable us to unravel the consequences of MFNs and these interrelations for the microevolution of GBM drug resistance. To achieve these aims, we will employ a number of biochemical (i.e Seahorse XFp-assisted metabolic profiling and enzymatic assays), microscopic (fluorescence microscopy, TIRF and TEM microscopy) and cytometric (ImageStreamX) techniques. **These studies will not only describe the consequences of DOX application for the welfare of GBM cells, but also address a novel function of MFNs in determining the microevolution of „metabolic flexibility" and drug-resistance of GBM. We believe that the obtained results will contribute to the knowledge on the long-term consequences of DOX application for GBM progression and to the optimization of DOX-based GBM treatment strategies.**