Alzheimer's disease (AD) is a neurodegenerative disorder that accounts for more than half of all dementia cases in the world. The main causes of AD include alterations in the expression and metabolism of amyloid β precursor protein (APP), leading to the generation of amyloid β peptide (Aβ), which exists as soluble monomers, oligomers, and insoluble fibrils and senile plaques. Although the latter are a neuropathological hallmark in the brain cortex and hippocampus of AD patients, numerous studies indicate oligomers (Aβo) as the most pathogenic form of Aβ. One of the targets of toxic action of Aβ are mitochondria, the dysfunctions and impaired morphology of which are already observed at the early stage of the disease. Mitochondrial disturbances imply enhanced expression of pro-apoptotic proteins and reduced expression of anti-apoptotic proteins, suppressed PI3K/AKT/GSK3β pathway, and finally neuronal apoptosis. Additionally, Aβ triggers overactivation of microglia followed by enhanced release of pro-inflammatory cytokines, which exacerbates neurotoxicity of Aβ.

Above-mentioned alterations are accompanied by disturbed metabolism of prosurvival bioactive sphingolipid, sphingosine-1-phosphate (S1P), and S1P-dependent signalling via specific receptors ($S1PR_{1-5}$). Modulation of four of the five S1P receptors ($S1PR_{1,3,4,5}$) by fingolimod (FTY720), a drug approved for the treatment of multiple sclerosis, has been demonstrated to alleviate some changes. Another, more selective ($S1PR_{1,5}$) drug from this family, siponimod (BAF312), has not been tested in AD yet. Therefore, the main goal of this project is to compare the influence of both modulators, fingolimod and siponimod, on mitochondrial function, cellular death and survival, and inflammatory changes, in a cellular model of AD induced by Aβo. Secondly, we also would like to analyse the role of particular S1P receptors in the aforementioned processes, what will allow to explain possible differences in the neuroprotective effects of the studied modulators.

Mouse hippocampal neuronal cell line (HT22) and microglial cell line (BV2), treated with Aβo will be used in the current project as a cellular model of AD. Next, cells will be treated with: phosphorylated (pharmacologically active) form of fingolimod, siponimod, and with selective agonists of S1P receptors: ponesimod (for $S1PR_1$), CYM5541 (for $S1PR_3$), CYM50308 (for $S1PR_4$), and A-971432 (for $S1PR_5$). Firstly, the influence of Aβo on mRNA and protein levels of S1P receptors ($S1PR_{1,3,4,5}$) will be verified. Mitochondrial membrane potential, metabolic activity, and level of intracellular ROS will be measured to evaluate mitochondrial function. Cellular death and survival will be assessed by annexin V/propidium iodide (PI) staining and analysis of mRNA and protein levels of selected pro-apoptotic and anti-apoptotic proteins as well as protein and phosphorylation level of PI3K, AKT and GSK3β. Finally, analysis of mRNA and protein levels of selected proinflammatory cytokines together with microscopic observation will be carried out to examine inflammatory changes.

Results obtained from this project will provide knowledge about the efficacy of two S1P receptor modulators in mitigating certain toxic effects of Aβo. Moreover, the current project will contribute to a better understanding of the role of particular S1P receptors in Aβo-induced toxicity, what may be useful in the development of new drugs for the treatment of AD.