The main goal of this study is to determine the effects of resveratrol, 5-aminoimidazole-4-carboxamide riboside (AICAR) treatment, swim training, and a combination of these treatments options (hybrid therapy) on Amyotrophic Lateral Sclerosis (ALS) mice lifespan and motor function. In addition, the aim of this project is also to investigate the mechanism(s) responsible for these changes related to skeletal muscle and nerve tissue in the group of mice undergoing the therapy that induces the best results.

Amyotrophic Lateral Sclerosis (ALS) is an incurable, chronic neurodegenerative disease characterized by a selective motoneurons death in the motor cortex, brainstem, and spinal cord that control any action of the muscles. Hitherto, riluzole is the only available therapeutic option for ALS. However, its therapeutic effect is limited, extending survival by a few months without any improvement in muscle function.

There are plenty of data related to ALS pathogenesis. Among them, the most attention is paid to oxidative stress and mitochondrial abnormalities. Recently, in terms of neurodegeneration, special attention has been paid to the skeletal muscle cell structures formed by the mitochondria and the endoplasmic reticulum membranes, containing protein (e.g., Caveolin-1) and lipid (especially cholesterol) components, called MAMs. However, the role of muscle in ALS has been extensively studied and gave rise to highly inconsistent results. On the one hand, the data from the last decade shows that overexpression of $SOD1^{G93A}$ in skeletal muscle initiates motoneurons death and induces profound muscle atrophy. Nevertheless, on the other hand, no evidence targeting muscle to boost its volume/function provides compelling, lasting, or meaningful protective effects against motoneurons degeneration and clinical motor deficits in rodent models or even patients.

Our recent data demonstrated that the progression of ALS is accompanied by a modification of skeletal muscle MAMs components. During ALS development, a reduction in the Cav-1 protein level and accumulation of cholesterol in skeletal muscle crude mitochondria (containing mitochondria and MAMs) was observed. Of great interest is that swim training prolongs the lifespan of ALS mice. More importantly, this phenomenon is accompanied by changes in skeletal muscle. Swim training induces modification of MAMs components (increased Cav-1 and decreased cholesterol levels), associated with bioenergetics improvement and lowering oxidative stress. Moreover, improved energy metabolism in skeletal muscle of ALS mice was also observed after swim training.

The research included in this project will be conducted on transgenic mice B6SJL-TgN[SOD1-G93A]1Gur (ALS mice), B6SJL-Tg(SOD1)2Gur/J (control, littermates mice), and stable cell lines: mouse muscle myoblast C2C12 and human nerve SH-SY5Y expressing $SOD1^{G93A}$ (model of ALS) and SOD1 (control).

Despite the positive or potentially positive effects of these therapeutic options (swim training, resveratrol, and AICAR) on ALS, no data investigates the combination of these factors on ALS lifespan and motor functions. Indeed, these three factors act via similar mechanisms. Still, in our opinion, they should complement each other and, in this way, may become a more complete, large-spectrum treatment in this devastating disease.
The knowledge of the mechanism(s) related to oxidative stress, mitochondrial biogenesis, and energy metabolism, responsible for prolonging ALS mice lifespan, seems extremely important from the scientific point of view and practical issues. Furthermore, this study may also identify the mechanism(s) of mitoprotection induced by modifications of MAMs components, which may provide new possibilities to treat: neurodegenerative diseases and other disease-related to mitochondrial dysfunction.