The anti-inflammatory potential of extracellular vesicles of the parasitic nematode *Anisakis simplex* in human gastric and intestinal organoids developed from induced pluripotent stem cells

In industrialized countries, the prevalence of inflammatory disorders has reached epidemic proportions. According to the World Health Organization, chronic inflammatory disorders affecting any organ of the body constitute the greatest health problem and one of the leading causes of death in the world, with more than 50% of all deaths being attributable to inflammation-related disorders.

The negative correlation between helminths infections and inflammatory disorders has been widely debated. The *hygiene hypothesis* and the so-called *old friend hypothesis*, which are well known among both scientists and the general public, suggest that the recent increase in inflammatory disorders is related to a lack of exposure to microorganisms, especially gastrointestinal nematodes. The *hygiene hypothesis* especially, suggests that the lack of exposure to gastrointestinal nematodes during childhood stunts the immature immune system, resulting in the more frequent development of inflammatory disorders. The ability of helminths to manipulate the host immune system is well documented and there are many examples in the literature of the beneficial effects of helminths in diseases with an inflammatory component. There is evidence that helminths infections, especially with GI nematodes, may help regulate uncontrolled inflammatory responses associated with mentioned previously inflammatory disorders in humans. However, the use of live parasites carries known risks. In the last decade, the discovery that GI nematodes release extracellular vesicles (EVs) that can enter host cells was a breakthrough discovery in parasite research. Despite their small size (50–2000 nm), EVs are known to contain various molecules, including nucleic acids, proteins, lipids, or metabolites. Nucleic acids, especially miRNAs, and proteins are thought to play a key role in host-parasite communication. Given the ability of GI nematodes to compromise immunity, EVs and their cargo, may play a critical role in evading the host immune system. Therefore, the use of helminth EVs in the context of regulating inflammatory responses associated with inflammatory disorders seems increasingly likely.

The latest trend in the study of the molecular basis of the influence of parasites on the host organism is the use of organoid cultures. These are complex, three-dimensional (3D) structures composed of multiple cells that serve as representative models for specific organs. Organoids allow the study of aspects of human physiology and disease that cannot be replicated in animals due to significant differences between species.

Anisakis simplex, one of the most emerging parasitic nematodes in Europe, is characterized by a complex life cycle, where humans can become accidental hosts. In preliminary studies, we confirmed that *A. simplex* larvae produce EVs (Anis-EVs) that have an anti-inflammatory effect on the immortalized human colorectal adenocarcinoma cell line (CACO-2), based on the profile of cytokine secretion and gene expression analysis. We also characterized Anis-EVs cargo: miRNAs (Anis-miRNAs) and proteins (Anis-PROTs). **To our knowledge, there is no single report describing the effect of Anis-EVs and their specific cargo on molecular changes in human gastric and intestinal organoids with induced inflammation.**

The aim of our study is to investigate effect of Anis EVs directly and indirectly (selected miRNAs and proteins) on inflamed human gastric and intestinal organoids. To achieve this goal, a transcriptomic and phosphoproteomic approach using high-throughput methods (RNA sequencing and liquid chromatography coupled with tandem mass spectrometry) will be applied, and Anis-EVs and their specific cargo (Anis-miRNAs and Anis-PROTs) will be used with inflamed human gastric and intestinal organoids as research models. In addition, the role of Anis-EVs, Anis-miRNAs and Anis-PROTs in the activation of inflammatory signaling pathways in inflamed organoids will be determined. The impact of Anis-EVs, Anis-miRNAs, and Anis-PROTs on selected oxidative stress biomarkers and on secretion of selected inflammatory biomarkers in inflamed organoids will also be investigated.

The scientific goal of the project is closely related to recent trends in global research aimed at understanding the potential of helminth therapy in inflammatory disorders. **The use of complete EVs or only some of the molecules contained in them, in combination with currently available modern research methods (organoid culture, RNA-seq, nLC-MS/MS) could provide a variety of scientists and the general public with further data on the effect of parasitic nematodes products on inflammation.**