SUMMARY

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the central as well as peripheral nervous system, the etiology and pathophysiology of which are not been fully understood. Often, the onset of the disease is difficult to diagnose, as symptoms can be mistaken for temporary weakness in the body. Progressively, the symptoms become more characteristic and include: progressive central motor neuron damage (including muscle weakness and increased muscle tone), slurred speech and difficulty swallowing, and in the final stage, respiratory distress and death usually 5 years after diagnosis. Amyotrophic lateral sclerosis occurs in all parts of the world with an annual incidence of 3-5 cases per 100,000 population. The European Reference Network for Rare Neuromuscular Diseases' guidelines for a treatment schedule in the diagnosis of ALS are very limited. The main recommendations include the introduction of the basal drug Riluzole, which is taken from the time of diagnosis until the end of life. The temporary recommendation is the use of medicinal products such as Edarawon and AMX0035, and by the end of 2023, the guidelines for the drug Tofersen should be announced, as a first-line treatment for patients with the genetic form of ALS patients with a mutation in the superoxide dismutase 1 gene (*SOD1*). Recent epidemiological studies have shown that the beneficial effect with Riluzole persists only during the first 6 months of treatment and extends life by only 10-20%. The above-mentioned facts highlight the need to develop new therapies that will provide patients with a longer and better quality of life.

Currently, research is underway into the use of cell therapy to delay the progression of ALS. Research work in recent years has focused on understanding the causes of the onset and progression of the disease and points to as one potential factor - immune system dysfunction. On the other hand, there are many reports that mesenchymal stromal cells (MSCs) can modulate the immune response, as well as modify the expression of pro- and anti-inflammatory proteins and trophic factors for neurons. The secretion of neurotrophic factors by MSCs can positively support the maintenance of motor neuron and glial cell function necessary for proper neuronal activity. It has been observed tha MSCs have a positive effect on the protection against apoptosis for: motor neurons, astrocytes and microglia. The therapeutic use of autologous MSCs in patients has significant limitations, as ALS is a disease that occurs in middle and older age. MSCs sourced from ALS patients present many dysfunctions, and this can adversely affect the effectiveness of therapy. In addition, MSCs from ALS patients were found to have reduced proliferative potential. In view of the negative

aspects that occur, MSC therapy based on an allogeneic cell preparation seems to be the optimal solution. One of the attractive options is mesenchymal stromal cells, isolated from Wharton's jelly (WJ-MSC). The advantage of the allogeneic approach is the possibility of using an allogeneic "of-the-shelf" cell product, without the invasive interference that occurs when producing an autologous preparation of cells isolated from patients' marrow or adipose tissue. Another advantage is their ability to secrete immunomodulatory and neurotrophic factors. Wharton's jelly of the umbilical cord is a rich and safe source of MSC cells due to the ease of the procedure for obtaining them. Cells derived from the fetal tissues are different from MSCs obtained from adult tissues (bone marrow, adipose tissue). The obtained WJ-MSCs are more homogeneous and have higher proliferative potential.

The aims of the implementation doctorate were to develop an advanced therapy medicinal product (ATMP) based on Wharton's jelly mesenchymal stromal cells (WJ-MSC) for the treatment of amyotrophic lateral sclerosis. WJ-MSCs products were manufactured under current GMP conditions in accordance with applicable procedures and using qualified and certified materials and equipment. Production takes place in Class A (laminar flow) in a Class B environment. The manufacturing steps of the ATMP included qualifying the umbilical cord material based on medical history, maternal blood results for the presence of infectious agents, and preliminary visual inspection of the tissue. From the qualified tissues, primary cultures of WJ-MSC cells were established using the Wharton's jelly explants method in a culture system free of animal-derived components. The achieved cells were subjected to qualitative and quantitative verification for the release of the final product, confirming their compliance with the criteria recommended by the International Society for Cellular Therapy (ISCT), including checking characteristic surface markers (negative Lin-: CD34, CD45, CD19, CD14, HLA-DR; positive: CD73, CD90, CD105), as well as assessing their karyotype, the presence of endotoxins and mycoplasma, and microbiological sterility. Afterwards, extended characterization was carried out on cellular products released for marketing in accordance with the ATMP.

The innovative approach consisted of precise, extended characterization of WJ-MSC cells in terms of their surface markers, secretory profile (secretome) and immunomodulatory properties. Immunomodulatory properties were evaluated using an *in vitro* co-culture model consisting of peripheral blood mononuclear cells (MNCs) from healthy donors with allogeneic WJ-MSC cells. The co-culture was evaluated for proliferation and assessment of MNC cell subpopulations, such as T cells, B cells, monocytes, NK cells, Th cells, Tc cells, Th1 cells, Th2 cells, Th17 cells, regulatory T cells, as well as their activation profile based on

the characteristic surface markers CD69, CD25 and HLA-DR. The development of a WJ-MSC cell bank and its clustering, made it possible to assign products with similar biological activity. Based on the results, a patent application was developed: "Method of classifying MSC cells for therapeutic properties". The object of the invention is a method of clustering cell products based on MSC cells for their therapeutic properties and selection of the cell product by matching donor to recipient. In the future, clustering, may help with the implementation of personalized medicine bringing with it an enhanced and matched therapeutic effect of cellular products.

Another goal was to administer an investigational mesenchymal cell-based medicinal product from Wharton's Jelly (WJ-MSC) in a clinical trial with the acronym ALSTEM. The clinical trial assessed the effects of Wharton's jelly cells (WJ-MSCs) on the immune system of patients diagnosed with ALS. Due to the availability of unique biological material such as samples of peripheral blood, urine and cerebrospinal fluid of people diagnosed with ALS, among other things, the immunological status of patients who received WJ-MSC cell products in the ALSTEM clinical trial was investigated. Patients' therapeutic response was determined by the ALS Functional Rating Scale-Revised (ALSFRS-R) scale trend factor. The ALSFRS-R scale is considered the gold standard used to measure ALS disease progression, as well as response to new drugs and therapies. The trend factor of the ALSFRS-R scale distinguished three groups of patients, which were described as:

- RESPONDER value of ALSFRS-R coefficient > 0, indicates positive effect of treatment
- NULL value of ALSFRS-R coefficient = 0, indicates neutral effect of treatment
- NON-RESPONDER value of ALSFRS-R coefficient < 0, indicates no response to treatment

The evaluation was based on the immunophenotype of peripheral blood subpopulations and the profile of selected cytokines, chemokines and growth factors in plasma and cerebrospinal fluid. The concentration of selected immunoreactive analytes was determined on a MAGPIX instrument (Luminex platform). Analyzing the data obtained from biochemical and cytometric analyses of blood, urine and cerebrospinal fluid, as well as assessments of vital functions, a preliminary dataset for predicting patients' response to WJ-MSC cell therapy was created.

In summary, the goal of the project and dissertation was to develop a therapeutic product of advanced therapy based on Wharton's jelly mesenchymal cells (WJ-MSCs) for the treatment of amyotrophic lateral sclerosis (ALS). A major key element of the implementation activity was the full exploration of the potential of WJ-MSC cells, their precise

characterization, studied at the immunological and genetic levels for their clustering, which is a clear novelty in the field. Another of the goals is to characterize and group ALS patients participating in the clinical trial according to their therapeutic response to the WJ-MSC cell product. ALS patients in the clinical trial underwent a neurological examination, as well as evaluation with several tools assessing disease progression (confirmed by the ALSFRS-R scale decline rate). Analyses of patients' biological material, among other things, at the immunological level, have yielded predictive parameters that predict a positive patient response to treatment with WJ-MSCs.