S T A T E M E N T

by **Assoc. Prof. Dimitrinka Yordanova Atanasova-Dimitrova, PhD**,
Member of the scientific jury, approved by Order No. P-109-469/09.11.2023
of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

of the PhD thesis
of **Martin Nikolaev Ivanov, MD**
on topic
"PROLIFERATION AND DIFFERENTIATION OF PROGENITOR CELLS IN THE SUBVENTRICULAR ZONE FROM THE TELENCEPHALON OF ADULT PRIMATES"

For awarding the educational and scientific degree "Doctor of Philosophy"
In the scientific specialty "Anatomy, Histology and Cytology"
In professional direction 7.1 Medicine,
An area of higher education 7. Health care and sports
Scientific supervisor of the PhD student: Assoc. Prof. Stoyan Pavlov, MD, PhD

DEAR MEMBERS OF THE SCIENTIFIC JURY,

This statement was prepared for public defense of the PhD thesis presented by the PhD student Martin Nikolaev Ivanov, MD, for the acquisition of the educational and scientific degree "Doctor of Philosophy" in the scientific specialty "Anatomy, Histology and Cytology".

The presented documents and materials correspond to the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its Implementation and the Rules for the Development of the Academic Staff at the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna. The procedure has been correctly followed.

Martin Nikolaev Ivanov was born on February 24, 1994. In 2018, he graduated from the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna (MU-Varna) and acquired the professional qualification of a master's Doctor of Medicine. Immediately after completing his higher education, he began working as a part-time Assistant Professor in the Department of Anatomy and Cell Biology of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna. After one year, he was appointed as an Assistant Professor to the same Department, leading anatomy and cell biology exercises for students of Medicine and Dentistry, first and second year. Since 01.02.2019, he has been enrolled as a full-time doctoral student at the Department of Anatomy and Cell Biology with Order No. P-109-81 of the Rector of MU-Varna. For three months in 2019 (01 July 2019 to 01 October 2019) and 16 days in August 2020, he was a visiting researcher

at the Max-Planck Institute for Biophysical Chemistry in Göttingen, Germany, in the Research Group of Prof.Gregor Eichele. At the end of April and the beginning of May 2022, he was a guest researcher at the Ruhr University in Bochum, Germany, for one week. In 2019 - 2023, he specialized in "Anatomy, histology and cytology" at the Medical University - Varna and in 2023, he acquired a specialty. From 2019 to the present, he has been a participant as a junior researcher of neural stem cells in the TRANSTEM project of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. Actively participates in the freely elective course in Neurobiology at the Department of Anatomy and Cell Biology. He is a member of the Bulgarian Anatomical Society and the Society of Molecular Biology and Evolution.

The PhD thesis on the topic "Proliferation and differentiation of progenitor cells in the subventricular zone from the telencephalon of adult primates" is written in 147 pages and divided into sections as follows: *Contents* – 3 pages, *Dedication* - 1 page, *Abbreviations* – 3 pages, *Introduction* – 2 pages, *Literature review* – 18 pages, *Aim and objectives* – 2 pages, *Material and methods* – 28 pages, *Results* – 51 pages, *Discussion* – 15 pages, *Disadvantages of the present study* – 2 pages, *Conclusion* – 1 page, *Implications* – 1 page, *Reference to the contribution of the PhD thesis* – 1 page, *Publications related to the PhD thesis* – 1 page, *Reports associated with the PhD thesis* – 1 page, *Bibliography* – 14 pages, including 179 literary sources, of which 178 are in Latin and 1 in Bulgarian. The dissertation is illustrated with 59 figures (5 of the figures are in the "Literature review", eight are in the "Materials and Methods" section, and 46 in the "Results" section) and three tables in the "Materials and Methods".

The topic of the dissertation is interesting, relevant and very well chosen. Globally, cerebral ischemia is a disease leading to disability or death. Ischemia-induced neurogenesis is a poorly studied process, which limits the possibilities for the endogenous or exogenous effects of neurogenesis and the subsequent integration of newly formed neurons. A significant challenge is to find ways to increase the levels of neurogenesis effectively and, accordingly, of newly formed neurons capable of integrating into existing neuronal networks. Many of the processes required for full neurogenesis have been studied in rodents. The availability of reliable markers used to represent cell subpopulations found in neurogenic zones in rodents is mainly unsuccessful and has no application in the study of neurogenesis in primates. This necessitates using primate models (non-human primates) to study cell differentiation, proliferation and integration because the primates are phylogenetically closer to humans.

The **Introduction** is short, concisely written and introduces the reader to the subject of the dissertation.

The **Literature review** is detailed and provides, in thematically separated subsections, extensive information on neuronal stem cells and the concept of neurogenesis, anatomy of the

subventricular zone in rodents and primates, the mechanisms for activation of neurogenesis as a result of cerebral ischemia, alteration of the transcriptome in the subventricular zone. Of the cited literary sources, 172 (95% of all) were published after 2000, proving the PhD student's good literary awareness and the relevance of the developed topic.

The **Purpose** of the research is clear and precisely defined. The **Tasks** are correctly formulated and, as will be understood from the subsequent exposition, are entirely feasible with the help of the methods used. Genes with significant changes in major components of the stem cell niche in the anterior horn of the lateral ventricle (aSVZ) were investigated. The PhD student did a comparative analysis of the degree of expression of the selected genes in ischemia compared to their expression in healthy individuals.

The **Material and Methods** section is detailed and comprehensive. The necessary ethical requirements for working with animals and the positive ethical evaluation that precedes the work with human tissue samples have been observed. The statistical methods used in processing the obtained results are clearly explained.

The **Results** section is divided into separate, descriptively formulated subsections and developed in a sequence following the chronologically set tasks. Candidate genes upregulated after brief global cerebral ischemia in the subventricular zone of non-human primates were investigated. The condition of cerebral ischemia induces an increase in the proliferation and differentiation of neural stem cells, which is a way to screen for candidate genes involved in regenerative and neurogenetic processes. Their topographic localization along the lateral ventricle (rostrocaudal) is visualized, and their phenotype under normal conditions is shown. Because of the potential for pharmacological treatment, detailed immunohistological phenotyping in normal adult human brains was also performed for APLNR. Dr. Martin Ivanov's dissertation presents a quantitative analysis of the proliferation and differentiation of neural stem cells in adult primates. Results from non-human primate and human phenotypes may lead to discovering more effective ways to activate neuronal regeneration and their subsequent use to treat neurodegenerative or psychiatric diseases.

The **Discussion** is very well written. It shows the PhD student's skill in discussing his results, comparing them with the known facts in the literature, and interpreting them skillfully to draw valuable conclusions about the established candidate genes for identifying neural stem cells.

The **Conclusions** reached by the PhD student are ten and are a logical sequence of the in-depth and adequate analysis of the results. There are five formulated **Contributions,** mainly of a scientific and scientific-applied nature.

The **Abstract of the dissertation** is prepared according to the requirements and adequately reflects the state of the researched problem, the set goals and objectives, the methods used for their

practical implementation, the results obtained, their analytical description and interpretation of the own data, as well as the author's conclusions and contributions.

The present PhD thesis is the work of Martin Nikolaev Ivanov, MD, as proof of this finding are the scientific publications and reports presented by him on the subject. The doctoral student presents a list of two publications on the topic of the dissertation, one of the publications has an impact factor of IF=3.5, and in the second publication he is the first author. Research results have been widely promoted and reported at 3 national and 5 international forums.

In **Conclusion**, the PhD thesis of Martin Nikolaev Ivanov, MD, is a thorough and complex study showing the increased expression of four genes with the potential to mark progenitor cells. It is well thought out, precisely methodically grounded, and accurately executed and illustrated. The obtained data are discussed and contribute to expanding the knowledge of the phenotypic characteristics of the genes selected for study in monkeys. One of these genes was also investigated for presence and phenotype in human tissues.

The study's results make an original and significant theoretical-applied contribution to the ability of the investigated candidate genes to identify neuronal stem cells. The work presented meets the legal requirements for obtaining the scientific-educational degree. Based on all of the above, I confidently express a **positive opinion** about the developed PhD thesis and, in my capacity as a member of the Scientific Jury, I give my positive vote for awarding the educational and scientific degree "Doctor of Philosophy" to Martin Nikolaev Ivanov, MD.

Prepared the statement,

Assoc. Prof. Dimitrinka Atanasova-Dimitrova, PhD

20.12.2023г.

Stara Zagora