STATEMENT

By: Prof. Dr. Anton Bozhidarov Tonchev, MD, DSc - Head of the Department of Anatomy and Cell Biology, Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

On: Dissertation for obtaining scientific degree “Doctor of Science” for Higher Education in the field 7. Health and Sport, Professional Field 7.1. Medicine, Scientific specialty: "Gastroenterology", on the topic "Serum expression of microribonucleic acids in patients with chronic inflammatory bowel disease"

Candidate: Assoc. Prof. Dr. Antonia Yordanova Atanassova, MD, PhD, Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

By a Decision of the Faculty Council of the Faculty of Medicine No. 43/ 11.05.21 and an Executive Order of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna No. P-109-217 from 17.05.2021, I have been appointed as a member of the Scientific Jury evaluating the dissertation of Assoc. Prof. Dr. Antonia Yordanova Atanassova, MD, PhD, for acquiring the scientific degree "Doctor of Science". By Protocol 1/31.05.2021 from a meeting of the Scientific Jury I was assigned to prepare a Review of the thesis.

I. Biographical information on Assoc. Prof. Dr. Antonia Yordanova Atanassova, MD, PhD

Antonia Yordanova Atanassova was born on 16 June 1961 in Varna. She started high school in Varna in 1975 and graduated in 1980. Antonia Atanassova was admitted and studied medicine at the Medical University-Varna (then HMI-Varna) in the period between 1980 and 1986. After obtaining a master's degree in Medicine, she worked

as a resident doctor in a hospital in Shumen. After successfully passing the exam for assistant physician, Dr. Atanassova began working at HMI- Varna and at the Clinic of Gastroenterology at MHAT "St. Marina" in the period 1987-1994. From 1994 to 2015, Dr. Atanassova worked as a senior and chief assistant at the same Clinic. In 1994 Dr. Atanassova acquired the specialisation "Internal Medicine", and in 1996 she acquired the specialisation "Gastroenterology". Dr. Atanassova went through a number of qualification courses; some of the most recent are:

- 1-st Ultrasound Workshop 02.2014 Copenhagen, Danmark;
- 35-th ECCO Educational Workshop 06.2014 r.;
- Leading change in IBD. Optimizing IBD care: practical approaches to implement changes. Athens, Greece,20-21. 06.2015;
- IBD Center of Excellence-Growing best practice in IBD Units Semmelweis University, Budapest, Hungary, 24-25.09.2015;
- Speaker Training Budapest, Hungary 2-3.02.2016;
- 3-rd ECCO-ESGAR Ultrasound Workshop-17.03.2016, Amsterdam, The Netherlands;
- The Importance of continuous clinical response in UC control Regional Expert Input Forum, Belgrade, Serbia, April 1st, 2016;
- 4-th ECCO Ultrasound Workshop, Barcelona, Spain 15.02.2017;
- Transforming Standards of care in IBD 13-14.10.2017 Brussels;
- 5-th ECCO Ultrasound – MRI Workshop 14.02.2018;
- IBD School: IBD Via clinical cases workshop, Moderator, 03.2018;
- IBD Academy: IBD Via- work group headed by A. Atanassova, Varna,31.05.2018
- IBD Academy, Sofia, 10.2018;
- Targets For Optimal UC Care- 15-16.06.2018, Brussels, Belgium;
- 26-th United European Gastroenterology Week – October 20-24, 2018 Vienne, Austria. Part I +Basic Course: “Basic and Introduction in abdominal Ultrasound

examination";

- 26-th United European Gastroenterology Week –October 20-24, 2018 Vienne, Austria. Part II+B: Advance Course: "Pathology and special topics in abdominal Ultrasound examination";
- 6th ECCO-ESGAR Ultrasound Workshop – Advanced- March 6-9, 2019, Copenhagen, Denmark;
- IBD practice June 20-21.2019, Rome, Italy;
- IBD Peer Preceptorship Meeting, Oxford, UK, 4-6 September 2019;
- IBD Peer Preceptorship Meeting, Prague, Czech Republic, 5-6 December 2019;
- 2-nd ECCO-ESGAR Basic Imaging Workshop, 12, February, 2020 Vienna, Austria.

In 2014, she defended a dissertation entitled Clinical Evaluation of Patients with Ulcerative Colitis-Modern Approach. With this, Dr. Antonia Atanassova acquired a PhD in the scientific specialty "Gastroenterology", and since 2015 she has been an associate professor at the Department of Anatomy and Cell Biology, Faculty of Medicine at MU - Varna.

Antonia Atanassova speaks Russian, English and French.

Summary: Assoc. Prof. Antonia Atanassova has a career of more than 34 years of clinical and scientific work, including 34 years of teaching Gastroenterology.

II. Overview of the dissertation of Assoc Prof. Antonia Atanassova

Assoc. Antonia Atanassova's dissertation is spread over 179 standard printed pages (excluding the pages of the bibliography). The dissertation has the following chapters:

1. Abbreviations
2. Introduction

3. Literature review

4. Aim, objectives and hypothesis

5. Methodology

6. Results

7. Discussion

8. Implications

9. Conclusion

10. Original contributions of the dissertation

11. Publications related to the dissertation

12. Bibliography

Summary: The obtained data and the performed analyses are illustrated with 55 figures and 59 tables. The bibliography contains over 500 titles by Bulgarian and foreign authors.

III. Evaluation of the significance of the topic of the dissertation

The topic of the dissertation is inflammatory bowel diseases (IBD) - Crohn's Disease (CD) and Ulcerative Colitis (UC). Despite the advances in medicine, these two diseases remain of unclear aetiology and pathogenesis. Genetically predisposed individuals suffering from CD and UC develop an inflammatory process as a result of the interaction between a defective immune response of the intestinal wall, involving the intestinal microbiome, epigenetic factors and environmental factors. There is evidence of a link between inflammatory bowel disease and small ribonucleic acids (RNAs), known as microRNAs. MicroRNAs do not encode proteins, but are able to regulate the expression of coding genes, including entire constellations of coding genes. A number of studies have focused on altered miRNA expression in IBD and their important role as regulators and diagnostic biomarkers. The dissertation of Assoc. Prof. Dr. Antonia Atanassova is dedicated to this exact

topic, and deals with the study of serum levels of miRNAs in IBD patients. This study is relevant because most such studies use tissues and / or cell cultures as a starting sample. At the same time, there are relatively few studies that quantify the expression of microRNAs that circulate in the serum of IBD patients. The use of serum as a subject of study is particularly relevant given the low invasiveness for obtaining such samples and the correlation between serum and tissue levels of miRNAs, which leads to the isolation of new molecular markers and potential therapeutic targets in CD and UC.

<u>Conclusion:</u> I assess the dissertation topic as highly significant and up-to-date.

IV. Evaluation of the sections "Introduction" and "Literature review"

The introduction clearly motivates the need for research in the field of the dissertation. The main scientific data at the moment on the topic and the availability of empty fields, which deserve the original research and analysis of the author, are considered in summary. The literature review, spreads over 46 standard pages, shows the good awareness of the author of the available literature sources on the topic of miRNAs and their connection with IBD. In fact, the literature review itself can serve readers as a compendium of microRNAs expressed in intestinal and immune cells. The fact that the bibliography cites over 500 sources (many of them from the recent years) in itself is indicative of the need to deal with **an avalanche of information during the work on the dissertation - something that, in my opinion, Assoc. Prof. Atanassova has successfully achieved.**

V. Evaluation of the Aim and the Objectives of the dissertation

The aim and the objectives are clearly formulated. The main goal is to study and evaluate the serum expression of miRNAs in IBD patients. The tasks present in detail the steps for achieving the chosen aim, and contain a comprehensive list of miRNAs -

the object of study. **Given the presented methodologies, I assess the aim as feasible.**

VI. Evaluation of the methods

The criteria for the selection of patients with Crohn's disease and ulcerative colitis, the control group, the methods for laboratory analysis of samples, and the statistical evaluation of the data are clearly presented.

Conclusion: The materials and methods are described in detail, in a way that would allow the experiments to be repeated by independent researchers.

VII. Evaluation of the results

The results are spread over 76 standard pages, illustrated with 55 figures and 59 tables. The analyses are illustrated with 55 figures and 59 tables. Serum miRNA levels were analysed in CD patients (*Hs_miR-28_1; *Hs_miR -29c_1; *Hs_miR -96_1; *Hs_miR -191_1; *Hs_miR -451_1; *Hs_miR -142-5p_1; *Hs_miR -199a_1; *Hs_miR -363_1; *Hs_miR -144_4; *Hs_miR -142-3p_2; *Hs_miR -155_2; *Hs_miR -16_2; *Hs_miR -1228-3p_1) and in UC patients (*Hs_miR-28_1; *Hs_miR -29c_1; *Hs_miR -96_1; *Hs_miR -191_1; 84 3 *Hs_miR -451_1; *Hs_miR -142-5p_1; *Hs_miR -199a_1; *Hs_miR -363_1; *Hs_miR -144_4; *Hs_miR -142-3p_2; *Hs_miR -155_2; *Hs_miR -16_2; *Hs_miR -1228-3p_1). The expression of the abovementioned miRNAs was compared between patients with Crohn's disease and those with ulcerative colitis.

Of particular interest is the correlation between serum microRNA expression and vitamin D levels. Such an analysis is new worldwide and has serious potential for discovering new mechanisms in the development of Crohn's disease and ulcerative colitis.

The author's data shows the presence of a disease-specific (Crohn's disease or ulcerative colitis) miRNA "signature" (a constellation of miRNAs). This will contribute to a more accurate diagnosis of these diseases, and some of the miRNAs are new targets for pharmacological treatment and/or markers of prognostic and predictive significance in Crohn's disease and ulcerative colitis.

Conclusion: The presented results are of extremely high quality and support the theses of the dissertation.

VIII. Evaluation of sections "Discussion", "Contributions", and "Conclusion"

The discussion is over 35 pages long and again demonstrates the good literary awareness and analytical thinking of Assoc. Prof. Atanassova. The original results are commented in the light of an in-depth synthesis of the known data in the literature.

The contributions are formulated in 11 points, reflecting the more important results achieved by the author of the dissertation. I accept the contributions presented in this way as corresponding to the results.

The introduction of a separate chapter *Conclusion* helps to summarize the results and opens the door to new, future research. Assoc. Prof. Atanassova focused on the established new miRNA "signatures", which the study has established in Crohn's disease or ulcerative colitis and the presence of a correlation between the vitamin D levels and the miRNAs miR-142-5p, miR-96, miR- 199a in IBD patients.

Conclusion: The presented "Discussion", "Contributions", and "Conclusion" correctly and precisely discuss and summarize the main points of the dissertation.

IX. Evaluation of the original contributions of the dissertation

As the main contribution of the dissertation, I evaluate the presented data for establishing miRNA molecular "signatures" in Crohn's disease or ulcerative colitis, as well as the presence of a correlation between the vitamin D levels and microRNAs in IBD patients. There are 7 more contributions of the dissertation with theoretical and applied character, which I accept.

Conclusion: I consider the contributions are sufficient for awarding the scientific degree "Doctor of science".

X. Assessment of the science metrics of Assoc. Prof. Atanassova

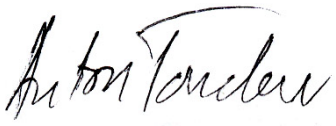
A reference is attached from the Library of MU-Varna, showing the science metrics of Assoc. Prof. Atanassova cover the requirements of the Bulgarian Law on the Career Development of the Academic Staff, as well as the local regulations of MU-Varna in this respect.

Overall assessment and conclusion on the dissertation

The dissertation of Assoc. Prof. Dr. Antonia Yordanova Atanassova, MD, PhD, Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna is dedicated to an extremely current subject. The author of the dissertation has presented a number of original results prepared as a result of the application of clinical and laboratory (molecular) methods. The dissertation is very interdisciplinary and includes original scientific data from at least several fields of medicine, including gastroenterology, immunology, molecular biology. I conclude that the presented dissertation completely fulfils the Bulgarian Law on the Career Development of the Academic Staff and local regulations of MU-Varna in this respect for acquiring the scientific

degree "Doctor of Science". That is why I will vote <u>in favour</u> for awarding her with the Degree and recommend the same to the other members of the honoured Scientific Jury, appointed with an Executive Order No R-109-217 from 17.05.2021 of the Rector at Medical University-Varna.

Reviewer:



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