STATEMENT

From Assoc. Prof. Eleonora Georgieva Dimitrova-Gospodinova, MD, PhD

Head of the Department of Oncology, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

In accordance with the Order of the Rector of the Medical University – Varna № P-109-148/ 13.03.2025, I have been appointed as a member of the Scientific Jury and according to Protocol № 1/ 25.03.2025r, I have been assigned to prepare a statement for the procedure of awarding the educational and scientific degree "Doctor" in the doctoral program "Hematology and Blood Transfusion", in the professional area 7.1. Medicine, in the field of higher education 7. Healthcare and Sports.

Regarding the dissertation titled: "Role of Selected Plasma microRNAs as Diagnostic and Prognostic Biomarkers in Myelodysplastic Syndrome"

Author: Dr. Svilena Angelova Atanasova, full-time doctoral student at the Second Department of Internal Medicine, Hematology Sector, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

Scientific Supervisor: Prof. Ilina Micheva, MD, PhD

1. Significance of the Problem, Formulation of the Aim and Objectives

Myelodysplastic syndrome (MDS) represents a heterogeneous group of hematopoietic disorders with variable clinical course and prognosis, for which accurate diagnosis and risk stratification remain a challenge in daily clinical practice. The identification of novel biomarkers with potential for early diagnosis, risk assessment, and prognosis is essential for improving the individualized approach to patients with MDS.

MicroRNAs (miRNAs), as regulators of gene expression, are attracting increasing interest as minimally invasive biomarkers in a number of hematological diseases, including MDS. The investigation of their plasma expression may contribute to expanding existing knowledge on the pathogenesis of the disease, as well as to the development of new diagnostic and prognostic algorithms.

All these facts lead to the definition of a specific aim of the present study: "to evaluate the expression levels of five selected microRNAs — miR-22, miR-144, miR-16, miR-451a, and let-7a — in patients with MDS; to conduct a comparative analysis of the results between high-risk and low-risk MDS groups, as well as between MDS patients and healthy controls; and to determine their prognostic significance."

In relation to the stated aim, nine specific objectives have been clearly formulated.

2. Dissertation structure

The presented dissertation consists of 176 pages, including: Introduction – 2 pages, Literature Review – 46 pages, Aim and Objectives – 2 pages, Materials and Methods – 7 pages, Results – 49 pages, Discussion – 21 pages, Conclusions – 1 page, Contributions – 1 page, Scientific publications related to the dissertation – 1 page, and References – 38 pages. The dissertation is illustrated with 29 tables and 19 figures. The bibliography includes 290 sources from international authors.

The structure of the dissertation is well-balanced and well-organized. It complies with the requirements of the Development of Academic Staff in the Republic of Bulgaria Act.

3. Awareness of the existing scientific literature

The literature review presents data on the epidemiology, diagnostic challenges, and current classifications of the disease, including the new WHO classification from 2022. The role of key pathogenetic mechanisms, such as genetic and epigenetic alterations, is analyzed, and the importance of non-coding RNAs—with a focus on microRNAs—is discussed. Their biogenesis, physiological and pathological role in hematopoiesis, as well as their significance as potential diagnostic and prognostic biomarkers in MDS, are presented.

A comprehensive overview of the existing data on the relationship between the expression of specific microRNAs and the clinical course of the disease is provided, including progression to acute myeloid leukemia and response to therapy. Special attention is given to the potential of plasma microRNAs as minimally invasive biomarkers.

4. Study Design and methodology

The results are based on a prospective study conducted at the Clinic of Clinical Hematology, University Hospital "St. Marina" – Varna. A total of 50 participants were included: 40 patients with confirmed myelodysplastic syndrome and 10 clinically healthy controls. The selection was carried out according to predefined inclusion and exclusion criteria.

Plasma was isolated from venous blood, and the five selected microRNAs were extracted and quantitatively analyzed using a commercial kit and Real-Time PCR. The statistical methods used for data analysis are described in detail.

5. Alignment between aim, results and conclusions

There is a clear alignment between the stated aim of the study, the formulated objectives, and the achieved results. The data obtained from the study are illustrated in tables and figures. The results demonstrate that four of the analyzed microRNAs—miR-144, miR-16, miR-451a, and let-7a—are significantly downregulated in patients with MDS compared to healthy controls. ROC analysis indicates good to excellent discriminatory ability of these microRNAs, with miR-451a showing the highest diagnostic accuracy. Logistic regression and LASSO analysis further confirm that miR-451a and miR-22 possess the highest predictive value. In terms of risk stratification, let-7a stands out as a significant predictor of both high risk according to R-IPSS and the presence of MDS with increased blast percentage. Correlation analyses reveal significant associations between some microRNAs and laboratory parameters such as LDH,

ferritin, and erythropoietin, supporting their role in metabolic and hematopoietic regulation. The prognostic analysis did not identify any microRNA with an independently significant association with overall survival. In conclusion, the study results demonstrate the diagnostic potential of the selected microRNAs in patients with MDS.

6. Analysis of the Conclusions and Contributions

Six main conclusions have been formulated, summarizing the most important results of the study. The conclusions are specific, clearly structured, and reflect the consistency between the obtained data and the objectives of the research.

Contributions of both theoretical and applied scientific value have been identified. Among the key contributions of the dissertation are the following:

- For the first time in Bulgaria, a study of plasma microRNAs (miR-16, miR-144, miR-22, miR-451a, and let-7a) has been conducted in patients with myelodysplastic syndrome (MDS), and their diagnostic and prognostic value has been evaluated.
- For the first time, the relationship between the expression of selected microRNAs and key biochemical parameters (LDH, ferritin, erythropoietin), reflecting metabolic processes in MDS, has been analyzed.
- An integrated statistical approach has been applied, including simple and multivariable logistic regression, LASSO analysis, and ROC analysis, to assess the individual and combined predictive value of the microRNAs.
- It has been confirmed that miR-16, miR-144, miR-451a, and let-7a are significantly downregulated in patients with MDS compared to healthy controls, which aligns with international data on their diagnostic role.
- It has been demonstrated that let-7a is associated with bone marrow blast percentage and R-IPSS risk stratification, highlighting its potential as a prognostic biomarker.
- A diagnostic model based on miR-22 and miR-451a has been developed, demonstrating exceptionally high diagnostic accuracy in distinguishing MDS patients from healthy controls.
- The potential role of plasma microRNAs as a minimally invasive, supplementary tool for diagnosis and risk assessment in MDS has been emphasized.

The doctoral candidate has presented four scientific publications related to the dissertation. The author's abstract comprises 128 pages and covers all main sections of the dissertation.

7. Conclusion

The dissertation of Dr. Svilena Angelova Atanasova represents an in-depth scientific study dedicated to MDS, one of the major challenges in modern medicine and clinical hematology, and to the ongoing search for reliable biomarkers for diagnosis, risk assessment, and prognosis in hematological disorders. The topic is both current and extensive. The methods applied in the study are appropriate for the stated aim and objectives, the results are unambiguous, and the

conclusions are clearly formulated. The dissertation presents contributions of both theoretical and applied scientific value.

The dissertation titled "Role of Selected Plasma microRNAs as Diagnostic and Prognostic Biomarkers in Myelodysplastic Syndrome" meets the criteria for awarding the educational and scientific degree "Doctor". It complies with the requirements of the Development of Academic Staff in the Republic of Bulgaria Act and its implementing regulations.

Based on the above, I give a positive evaluation of Dr. Svilena Atanasova's dissertation and recommend to the esteemed members of the Scientific Jury that she be awarded the educational and scientific degree "Doctor".

7.05.2025