

Opinion Statement

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**External Member of the Scientific Jury for the awarding of the educational
and scientific degree "Doctor"**

REGARDING: the dissertation for the award of the educational and scientific degree "Doctor" in the field of Higher Education Area 7. "Healthcare and Sports", in the professional direction 7.1. Medicine; doctoral program in the scientific specialty "Hematology and Blood Transfusion", code 03.01.39, on the topic: **"BIOMARKERS FOR A PERSONALIZED APPROACH IN THE SELECTION OF TREATMENT FOR PATIENTS WITH MULTIPLE MYELOMA"**,

authored by **Radi Evgeniev Lukanov M.D.**, a full-time doctoral student at the "Second Department of Internal Medicine, Hematology Unit," Faculty of Medicine, Medical University – Varna.

Relevance of the Problem: This dissertation addresses a clinically significant issue in contemporary hematology—the role of microRNAs (miRNAs) in the clinical progression of patients with Multiple Myeloma (MM), the second most common hematological malignancy in adults. MM is characterized by substantial clinical heterogeneity stemming from variability in cytogenetic and molecular profiles, which results in unpredictability regarding therapeutic responses. Recently, research efforts aimed at clarifying this disease heterogeneity have increasingly focused on biological processes through the identification of biomarkers that can assess prognostic risk at diagnosis, thus facilitating personalized therapeutic approaches. In this context, studies investigating the involvement of miRNAs in mechanisms regulating gene expression, specifically concerning cellular proliferation and apoptosis, are undeniably timely and of substantial practical significance.

Structure and Formatting: the dissertation presented by Radi Lukanov M.D. fully complies with the core requirements set forth in Article 27 of the Regulations for Implementation of the Law for Development of Academic Staff in the Republic of Bulgaria (State Gazette No. 75/24.09.2010, amended SG No. 30/2018) and the Regulations on the Terms and Conditions for Acquisition of Scientific Degrees and Holding Academic Positions at the Medical University – Varna:

1. The dissertation comprises a total of 137 pages, structured according to specific requirements, including: a title page; contents and abbreviations used (10 pages in total); introduction (1 page); main body structured into the following sections – literature review (33 pages); aim and objectives (1 page); materials and methods (10 pages); results, illustrated with 34 figures and 15 tables (37 pages); discussion of results (11 pages); conclusion presented as summarizing findings from the conducted research (2 pages); contributions (1 page); appendices (6 pages); publications (1 page); and bibliography, including 214 references cited in Latin alphabet (14 pages).

Additionally, a thesis summary is provided, succinctly outlining the essence of the dissertation, the aims and objectives defined, the obtained results from the conducted studies and their interpretation, conclusions, and a summary of the scientific contributions. The clinical study was conducted following approval by the Ethics Committee for Scientific Research at Medical University "Prof. Dr. Paraskev Stoyanov" – Varna (Decision No.129 dated 06.04.2023), in compliance with the principles set forth in the Declaration of Helsinki.

2. The dissertation contains original scientific and applied research results, confirming the candidate's deep theoretical knowledge in their specialty and demonstrating their capability for independent scientific research.

2.1. The dissertation includes a comprehensive Literature review containing clinical and epidemiological data on multiple myeloma, with an analysis of the genetic abnormalities involved in the etiopathogenesis of the disease. The doctoral candidate synthetically analyzes information about the role of microRNAs in epigenetic regulation of transcription and translation, citing studies that highlight the link between microRNA dysregulation and the pathophysiology of malignant neoplasms. A separate section examines the role of circulating miRNAs in the diagnosis of malignant neoplasms and their potential as therapeutic targets. This part clearly demonstrates the doctoral candidate's thorough knowledge regarding

the involvement of microRNAs in cellular differentiation, neoplastic proliferation, and apoptosis. Additionally, the clinical significance of microRNAs in MM patients is analyzed in relation to their diagnostic and prognostic relevance, emphasizing their predictive value for chemoresistance to certain drugs included in standard therapeutic regimens. This section of the dissertation shows the candidate's detailed familiarity with the existing literature, ability to select relevant sources, creatively structure the information, and critically interpret the data.

2.2. Based on a comprehensive literature review, the candidate logically and convincingly defines the dissertation's primary objective: to investigate the expression levels of selected circulating miRNAs in patients with newly diagnosed MM and healthy controls, with the main aim being to demonstrate specific miRNA expression at diagnosis and after 6 months of treatment. Fourteen clearly and precisely formulated tasks support this objective, adequately defining the scope and aligning with the dissertation's aim.

2.3. The candidate has precisely detailed the *Materials and Methods* used, appropriately selected in accordance with the dissertation's primary objective and tasks. The research was conducted as a prospective non-interventional, single-center clinical trial from 2022 to 2024, involving 56 newly diagnosed MM patients meeting inclusion criteria without exclusion criteria, who received standard therapy according to the Velcade, cyclophosphamide, dexamethasone protocol, and 12 healthy volunteers.

- Participants underwent comprehensive cytological, biochemical, immunological, and genetic analyses. Modern molecular biology techniques, including polymerase chain reaction using the miRNeasy Serum/Plasma Kit (Qiagen), were employed to analyze 100 samples (56 from newly diagnosed patients, 32 from treated controls, and 12 from healthy volunteers). The methods employed are well-suited to the tasks at hand. Additionally, the statistical methods used to analyze the obtained data are comprehensively described

2.4. The dissertation presents a substantial volume of scientific results, very well illustrated with figures and tables, described precisely and logically, aligned with the established tasks. The exposition allows identification of several research directions:

- 1) **Decreased expression levels of miR-126-5p** – the concentrations of miR-126 decrease with advanced clinical stage, increased tumor burden, and markers associated with poor prognosis and aggressive disease. Post-treatment control studies show miR-126 expression returning to levels comparable to healthy controls in patients achieving complete remission (CR) and very good partial response (VGPR).
 - 2) **Decreased expression levels of miR-199a-5p in MM patients** – ROC analysis identified miR-199a-5p as the biomarker with the highest sensitivity for differentiating MM patients from healthy controls. Lower levels correlate with pronounced bone disease, while higher levels correlate positively with higher hemoglobin and inversely with beta2-microglobulin.
 - 3) **MicroRNAs as biomarkers differentiating MM patients from healthy controls** – ROC analysis highlights miR-126-5p and miR-199a-5p as promising diagnostic biomarkers due to their high AUC values and significant statistical relevance.
 - 4) **Prognostic value of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p expression as predictive biomarkers for overall survival (OS)**. Significant statistical results identify miR-214-3p and miR-497-5p as predictive markers for OS and risk of early death.
 - 5) **Prognostic value of miRNA expression for progression-free survival (PFS)**. Analysis underscores miR-214-3p and miR-497-5p as significant predictive biomarkers, with elevated levels associated with up to a 38.1% increased risk of disease progression. Higher miR-497-5p levels correlate with more than a twofold increase in progression frequency.
3. The discussion of the results is presented separately, although for such a complex topic, understanding would have been facilitated by a brief analysis of individual results following each chapter. The candidate's profound knowledge and analytical thinking enable skillful interpretation of the obtained results within the context of existing studies, effectively summarizing and drawing well-supported conclusions.

The dissertation provides grounds for formulating the following principal contributions, clearly and precisely reflecting the results obtained:

1. **Scientific-Theoretical Contributions:** The conducted research offers data on the expression levels of circulating microRNAs in healthy controls and newly diagnosed multiple myeloma patients, assessing their diagnostic and predictive value for disease progression and early mortality. The dynamics of miRNA expression levels during treatment and their impact on therapeutic response were analyzed. The observed low expression levels of miR-126-5p and

miR-199a-5p at diagnosis emphasize their potential as diagnostic markers and indicators of therapeutic response. The normalization of miR-126-5p levels following effective therapy further establishes its potential as a biomarker for monitoring therapeutic efficacy. Additionally, the unfavorable prognostic significance of miR-214-3p and miR-497-5p was confirmed, as their elevated expression correlates with shorter overall survival.

2. Scientific-Applied Contributions: The significant correlation between low levels of miR-126-5p and miR-199a-5p at diagnosis provides an opportunity for their application in clinical practice within diagnostic algorithms and risk stratification for newly diagnosed multiple myeloma patients. From a clinical perspective, examining the microRNA expression profile at diagnosis can further refine risk assessment in MM patients, supporting individualized therapeutic approaches.

Scientific Publications: The doctoral candidate has three publications related to the dissertation: one in the Bulgarian journal Hematology and two in international, peer-reviewed journals (EHA Library - PB1686 and American Journal of Hematology Volume 99, Issue S2 Supplement-2024). These articles have not been used in previous procedures, and the candidate is the primary author in all three publications.

The dissertation demonstrates high scientific merit, presenting Radi Lukanov M.D. as a dedicated young researcher in the field of hematology and molecular biology. I recommend to the Scientific Jury to award the educational and scientific degree "DOCTOR" to Radi Evgeniev Lukanov M.D.

07.05.2025 г.

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
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/Assoc. Prof. E. Hadjiev, MD, PhD/

