

# OPINION

**By Prof. Dr. Nikolay Conev**

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**Regarding the dissertation titled:**

**BIOMARKERS FOR PERSONALIZED TREATMENT APPROACH IN PATIENTS  
WITH MULTIPLE MYELOMA**

**Author: Dr. Radi Evgeniev Lukanov**

*Pursuant to Order No. R-109-149/13.03.2025 of the Rector of the Medical University – Varna, and on the basis of Article 24, para. 6 and Article 30, para. 3 of the Rules for the Implementation of the Act on the Development of Academic Staff in the Republic of Bulgaria (ADASRB), and Article 68, para. 1 of the regulations of the Medical University – Varna, I have been appointed as a member of the Scientific Jury and hereby present my opinion on the dissertation of Dr. Radi Evgeniev Lukanov.*

The doctoral thesis is well-structured in terms of sequence and scope of the sections. It features excellent and modern visualization using appropriately selected graphical tools – figures, graphs, tables.

The dissertation is prepared in accordance with the legal requirements for acquiring academic degrees. It comprises 134 pages, divided as follows: introduction – 2 pages, literature review – 22 pages, objectives and aims – 2 pages, study groups and methods – 8 pages, results – 37 pages, discussion – 11 pages, conclusions – 1 page, contributions – 2 pages, bibliography – 23 pages.

The bibliography includes 214 sources.

The abstract consists of 71 pages and fully corresponds to the content of the dissertation.

### **Relevance of the Topic**

Multiple myeloma is the second most common malignant hematological disease, characterized by clonal proliferation of plasma cells in the bone marrow and numerous systemic manifestations. Despite significant advances in diagnostics and the development of more effective therapeutic regimens over the last decade, multiple myeloma remains a disease with high morbidity and mortality. Traditional diagnostic approaches rely on a combination of morphological, cytogenetic, immunological, and imaging methods. However, many challenges remain — from the lack of sufficiently sensitive markers for early detection and risk stratification to monitoring treatment response and timely detection of relapse.

Over the last two decades, numerous proteins, biomolecular, and genetic markers have been intensively studied. Despite many published recommendations for the inclusion of novel prognostic markers, a clear consensus on most newly studied indicators is still lacking, and their integration into routine practice has been slow.

MicroRNAs (miRNAs) are small RNA molecules, about 21–25 nucleotides in length, that do not code for proteins but regulate gene expression. They were first described in 1993. MicroRNAs possess specific properties that make them suitable as biomarkers:

- Their expression is often dysregulated in cancer;
- miRNA expression is tissue-specific;
- miRNAs exhibit high stability in formalin-fixed tissue samples;

- They are also stable in serum and plasma due to protection from degradation by endogenous RNases through incorporation into microvesicles, exosomes, and other microparticles;
- They can be detected in other body fluids such as urine and saliva;
- miRNAs can even be detected in feces.

## **Objectives and Tasks**

The dissertation's main objective is clearly defined:

To examine the expression levels of selected circulating miRNAs in newly diagnosed MM patients and healthy controls. The patient group is also tested after six months. The main goal is to demonstrate specific expression of the preselected miRNAs at diagnosis and after six months of treatment. The results are compared with treatment response, changes in clinical status, and laboratory parameters.

Section 3.2 defines 14 specific research tasks.

## **Study Methodology**

Research sites:

- Clinical Hematology Clinic, University Hospital “St. Marina” – Varna
- Clinical Immunology Laboratory at the Department of Medical Genetics, Medical University – Varna

Study type: A prospective, non-interventional, single-center clinical trial.

Period: 2022–2024

Participants: 56 patients meeting the inclusion criteria and 12 healthy volunteers.

A medical file was created for each participant.

Laboratory method: RNA isolation and real-time PCR using the miRNeasy Serum/Plasma Kit (Qiagen).

Statistical analysis: IBM SPSS Statistics and GraphPad Prism software.

## **Evaluation of the Dissertation and Contributions**

Dr. Radi Lukanov has presented results, conclusions, and contributions across several sections:

Conclusions (6 main points):

- Significantly higher levels of miR-126 and miR-199a were found in patients with multiple myeloma compared to healthy controls;
- A significant inverse correlation was established between miR-126-5p and miR-199a levels and beta-2 microglobulin;
- A moderate positive correlation was observed between miR-199a-5p expression and hemoglobin levels;
- Significantly higher levels of miR-126-5p were recorded in treated patients (CR+VGPR), approaching levels found in the control group;
- miR-126-5p and miR-199a-5p showed significant diagnostic potential in ROC analyses;
- Elevated levels of miR-214-3p and miR-497-5p were associated with a higher risk of disease progression and early death.

## **Main scientific contributions:**

For the first time, plasma levels of miR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p were studied in a large cohort of patients with a rare hematological malignancy like multiple myeloma.

For the first time, the relationship between these miRNAs and a number of clinical-laboratory and molecular-genetic factors was examined in newly diagnosed MM patients.

For the first time, the dynamics of serum miRNA levels during the therapeutic process were analyzed in relation to treatment response.

This is the first study in Bulgaria assessing the diagnostic role of these miRNAs in MM patients.

This is also the first study in Bulgaria evaluating the predictive value of these miRNAs for disease progression and early mortality in MM patients.

## **Conclusion**

The dissertation contains scientific and applied research results with original contributions to science and meets all legal requirements of the Act on the Development of Academic Staff in the Republic of Bulgaria, its implementation regulations, and the regulations of the Medical University – Varna. The submitted materials (dissertation, publications, scientific reports) comply with the specific requirements of the law.

The dissertation demonstrates that doctoral candidate Dr. Radi Evgeniev Lukanov possesses deep theoretical knowledge and professional competence in the scientific field of clinical hematology, showing ability and skills for independent scientific research.

Based on the above, I give my POSITIVE assessment of the conducted research and recommend that the esteemed scientific jury award the educational and scientific degree “Doctor” to Radi Evgeniev Lukanov.

Prof. Dr. Nikolay Conev

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