To

The chairman of a scientific jury,

Determined by Order No. R109-82/21.03.2024

by the Rector

Of Medical University

"Prof. Dr. Paraskev Stoyanov "-Varna

Prof. Dr. Dimitar Raykov, MD,PhD.

Attached I present: Statement on the procedure for acquiring an educational and scientific degree "PhD" in the Doctoral Program “Genetics”, professional field “4.3. Biological Sciences”.

Reviewer: Assoc. Prof. Dr. Antonio Ivanov Antonov, DM

Scientific specialty: 03.01.39 - Hematology and blood transfusion

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Statement

By Assoc. Prof. Antonio Ivanov Antonov, MD,PhD

Head of the Hematology Clinic

UMBAL “St. Marina - EAD, Pleven

Concerning: Procedure for acquiring a PhD's scientific and educational degree

Higher education area: 4. "Natural Sciences, Mathematics and Informatics"

Professional field: 4.3 "Biological Sciences".

Doctoral Program “Genetics”

By Order No. P -109-82/21.03.2024 of the Rector of Medical University “Prof. Dr. Paraskev Stoyanov ”-Varna I am appointed as an external member of a scientific jury whose first meeting I am elected to prepare a statement.

The structure of the dissertation: The presented dissertation work is completed in accordance with the generally accepted requirements. It is formed in nine sections in proportional ratios. The content covers 103 pages with 12 tables included and 15 figures. The tables are configured and explained very well. The figures clearly reflect the necessary information. The bibliography is sufficient, with a total of 201 literary sources, 5 of which in Cyrillic and 196 in Latin.

Themed relevance of the dissertation work: Acute myeloid leukemias are undoubtedly the most serious and fatal pathology in the modern hematology. The huge progress in the diagnostic process has made it clear that behind this generally accepted diagnosis lies a large heterogeneous group of diseases of different etiology, similar pathophysiology and individual clinical course. A huge number of scientific developments explored the intimate mechanisms of the genesis of these diseases. They brought the acquired genetic disorders as a major factor modeling their pathogenesis. The huge variety of significant genetic changes and their combination explained the observed differences and led to the introduction of new classifications. In practical terms, this necessitated the introduction of new genetic methodologies, a new risk stratification and a remodeling of the existing therapeutic strategies. The evolution of methods of genetic analysis transformed the molecular-genetic studies in an integral part of the modern diagnostic process that upgraded the classic cytogenetic analysis. The sampling of the multiple ligation-dependent amplification (MLPA) as a molecular methodology that expands and complements information from cytogenetic analysis is an interesting and rational approach.

The topic is extremely up-to-date, controversial and enables the accumulation of valuable practical experience with a modern molecular-genetic method. Similar researches and studies in our national medical literature are limited.

Literary review: The presented review covers 41 pages, illustrated with 4 figures and 6 tables. It is made up of three parts. The first begins with a review of the traditional FAB classification. Later, the genetic characteristics of the WHO Classification /2022 /were described in thorough but synthesized form. The International Consensus Classification (ICC) is also considered, commenting on the main differences with the previous one. This part ends with a review of the factors determining the risk distribution by ELN criteria. The second part describes the main molecular methodologies with an analysis of the capabilities, advantages and disadvantages in practical aspect. Finally, a well -formulated summary that targets the main goals and tasks is shaped.

The structure of the review corresponds to the topic of the dissertation work. In it, the doctoral student has presented and systematized the contemporary scientific achievements on the problem.

Purpose and tasks: The main purpose is formulated clearly and specifically. 5 research tasks have been derived, which implements various practical aspects of scientific development.

I accept the formulated purpose and research tasks for optimally structured.

The dissertation methodology: The methods applied are well described in detail. It is very important to note the lack of criteria reflecting the general condition and expected survival in the formation of the patient group. These criteria would prevent inclusion of patients in a very aggravated condition, whose further development could change the end result at a later stage. The selection of the healthy control group was conducted accurately and precisely.

The methods are modern, high -tech, described with a good practical knowledge of the work. A total of 61 patients with AML were examined for the period from 02.2022 to 05.2023 at the Hematology Clinic of St. Marina Hospital and 21 clinically healthy volunteers.

Results: The description of the results follows the consistent approach of the tasks set. They are exposed and visualized very well. They are quite interesting in practical terms and provide some unexpected information. Comparison with the classic cytogenetic analysis as a success rate and as genetic information is well represented. The ELN 2022 risk group is also reassessed, depending on the results of the MLPA, and in some cases the role of the new methods is fundamental. It is continued by determining the average survival and the final distribution of patients under WHO 2022.

I take the results described as true and adequate to the goals and tasks.

Discussion: The results in the context of similar studies were initially discussed. However, the actual results obtained when using MLPA is the main interest. The starting point is the limitations of the methodology to the requirements of the modern diagnostic guidelines. Comparisons have been made with the available literary data from the limited publications with this methodology. The relatively restricted contingent patients have been reasonably noted. The success rate of both MLPA and routine cytogenetics and the combination of them, including inappropriate patients for more aggressive studies, has been monitored.

The possibilities of more modern methodologies incl. NGS and OGM were pointed out, outlining the problems preventing their wider spread.

I find the discussion as well -formed, objective and taking into account the practical realities.

Conclusion and prospects for future work: The benefits of combining MLPA and classic cytogenetic analysis such as initial screening and foundation for primary risk stratification and target therapy are here in a very synthesized form. In theoretical terms, a considerable interest is the idea of using for the biological examination material from both peripheral blood and bone marrow with a subsequent interpretation of the results and the comparison with NGS methods but in a more significant number of patients.

Conclusions: The conclusions are well structured, clear and give a good starting point for forming a good practical approach.

Contributions of the dissertation: the contributions are shaped into 3 groups.

Original contributions highlight the pioneering role of the study and emphasize the high success rate of the combination of classic cytogenetics and MLPA.

Confirmative contributions are basic and outline the need to upgrade the diagnostic process

Contributions of applied nature synthesizes the practical recommendation of the scientific development.

Generally, I accept both the contributions formulated by the author and the offers of autoreferat, which meets the necessary requirements.

Conclusion:

The introduction of the multiple ligation-dependent sampling amplification (MLPA) as a molecular methodology, expands and supplements information about the genetic changes obtained from the routine cytogenetic analysis in patients with AML. The combination of the two methodologies with some restrictions is a practical, rational and reasonable approach. It significantly changes the risk stratification of patients and determines the therapeutic approach.

The work is up -to -date and promising. It meets the scientometric criteria in accordance with the Rules for the Academic Development of the Medical University of Varna for the award of a PhD's scientific education degree.

I recommend to the scientific jury to award the PhD degree in Scientific Specialty 4.3 Biological Science doctoral programme "Genetics" to Dinnar Ali Yaha,MD.

Date: 06.06.2024

Assoc. Prof. Dr. A Antonov