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To The Chairman of the Scientific Jury,
On the Basis of Protocol №1 /21.07.2021

STATEMENT

From

PROF. LYUDMILA BONCHEVA ANGELOVA, MD, PhD,
Head of the Department of Medical Genetics, MEDICAL UNIVERSITY-VARNA

Regarding

The dissertation of **Merlin Erol Efraim, MD, PhD** student of independent training at Department of Internal Diseases II, Faculty of Medicine at the Medical University "Prof. Dr. Paraskev Stoyanov " – Varna

On the topic

CLINICAL-BIOLOGICAL AND GENETIC MARKERS IN RISK STRATIFICATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

Scientific Supervisor: Assoc. Prof. lina Dimitrova Micheva, MD, Ph.D.

For awarding the educational and scientific degree **'Doctor'**, in the field of higher education 7. Healthcare and sports, professional field 7.1. Medicine, doctoral program in 'Haematology and Blood Transfusion'.

I am determined to present the current statement based on a decision of the Chairman of The Scientific Jury and according to an Order of the Rector of MU-Varna **№ 109-303 /16.07.2021.**

The documents for the dissertation for awarding the educational and scientific degree 'doctor; have been prepared in accordance with the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations of its application and the Regulations for the development of Academic Staff of Medical University – Varna. I declare that I do not have any conflict of interest with the author of the dissertation.

The dissertation of d-r Merlin Erol Efraim has been prepared at the Second Department of Internal Diseases of Medical University – Varna and the Clinic of Clinical Haematology of 'St. Marina' University Hospital -Varna.

Short details of the applicant D-r Merlin Efraim was born in 1983 in Shumen, where in 2002 she graduates her secondary education. In 2008 she graduates with Master's degree from 'Prof D-r Paraskev Stoyanov' Medical University – Varna and works two year in the Emergency department in the town of Provadia. Since October 2010 she works in the Clinic of Clinical Haematology of 'St. Marina' University Hospital -Varna and during that period (2015) she acquired specialty in hematology. Since 2015 she has been appointed as an assistant in Medical University – Varna, and since April 2019 she has been registered as a student of independent training for a period of 3 years (until April 2022).In her curriculum vitae there is no information of specialisations and courses in Bulgaria and abroad, participations in scientific projects.

The dissertation is **structured** according to the accepted requirements: volume 178 non-standard pages (211 pages, including the references) and well -illustrated with 28 tables and 82 figures. The references list 234 sources, of which 92 (37.8%) from the last 10 years (2011-2020). The dissertation consists of: Introduction, Literature Review; Aim, tasks and hypothesis, Research Material and Methods, Results, Discussion, Conclusion, Contributions, Scientific Publications on the Topic, References, Appendix of the clinical card for the participants in the research – 1 pages.

There are no sources in Cyrillic in the references.

In the **title** `Clinical-biological and genetic markers in risk stratification in patients with myelodysplastic syndrome` the genetic factors have been separated even though these markers are biological (more precisely „Clinical and biological markers"). I assume that their separation is due to their significance in the risk stratification scales. My motives for this are the well-known fact that the cytogenetic profile is one of the 3 basic indicators in the risk assessment as well as the presence of a summary in the literature review „ The biological factors – cytogenetic and molecular profile, status of methylation and microRNA profile" is one of the three main subgroups of prognostic factors, related to the disease.

The dissertation refers a current topic related to haematological disease, which is distinctive with peculiar diversity in "clinical presentation, cytogenetic disorders and its course" and consequently variations in patient's survival. From this point of view, prognostic factors are very important to determine the risk of progression, survival and transformation into AML.

The **literature review** has an impressive volume (60 pages, this is 1/3 of the total volume of the dissertation with a brief summary) in which the author correctly systemises and presents the scientific information related to the topic of the dissertation, sometimes with unnecessary details. In my opinion, the work would win if investigations from our country used for stratification of MDS and/or references in Cyrillic were commented / cited in the review. Bulgarian dissertation for the clinical and prognostic significance of the evolution of the cytogenetic disorders and clonal evolution in the risk assessment (with 105 patients with MDS and 248 with AML) would have supported the doctorial hypothesis. ("Addition, to the approved prognostic scales and analysis and some additional clinical and biological factors can play a key role in the risk stratification of MDS for the more accurate risk stratification, survival and risk of transformation").

The **aim** of the current dissertation is „to investigate and analyse the impact of the factors, related to the disease (clinical-biological and *genetic* in its full text in the dissertation) and with the patient (age, ECOG and comorbidities), for risk stratification, survival and the risk of transformation into AML.

The main **tasks** are six. Of particular interest to me are tasks 2 and 6 related to the role of genetic factors for the survival (prognosis), and to certain extent tasks 3 and 4, related to the hypothesis for contribution of additional factors „comorbidity" and „frailty" as an option enhancing risk stratification for MDS. *In this respect, I consider that, characterisation of patients (task 1) can be presented with the cytogenetic and molecular-genetic indicators (1.6 and 1.7).*

Task 1. To **characterise** patients with MDS according to:

1.1 demographic data;
1.2 classification systems;
1.3 risk assessment scales;
1.4 the clinical "frailty" scale and comorbidity indices;
1.5 basic laboratory parameters;

Task 2. To analyse **survival** according to**:**

2.1 demographic data;
2.2 classification systems and risk assessment scales;
2.3 laboratory parameters;
2.4 cytogenetic parameters;

Task 3. To assess and analyze survival according to the **comorbidity and "frailty" scales** and to compare them with the classification **systems and risk stratification scales** for MDS.

Task 4. To assess the **correlations** between risk stratification scales and comorbidity and "frailty" scales in patients with MDS.

Task 5. To study and analyze the risk of **transformation of MDS into AML** and to assess patient survival before and after transformation.

Task 6. To derive **factors with favorable and unfavorable prognosis** in terms of survival and transformation in AML.

Materials and methods have been presented in limited volume of 5 pages. In my opinion the investigated number of patient (219), diagnosed during a period of 10 years is sufficient for the *retrospective* research. The selection has been adequately described by inclusion criteria. In this respect, an individual patient card has been prepared (Appendix 1). It should precede the References chapter and could be structured by groups of indicators to allow its easy use in clinical practice for investigations with patients with MDS. Such an order, *structuring* by significance and *specific number* of tested patients applies for each specialised laboratory test (bone marrow biopsy is only a manipulation for the extraction of bone marrow for the purpose of morphological and immune-histochemical tests). Genetic tests require brief information on the relevance and effect from studying cytogenetics and mainly somatic mutations like *JAK V617F* and internal tandem duplication in *FLT3* gene. The presence of amplification *c-MYC, MLL and AML* genes in the karyotype of patients with MDS and AML and trisomy 8,11,21 in the evolutional event with prognostic significance are evaluated in Bulgarian patients with MDS.

The investigation methods (clinical, laboratory, statistical) point the *multidisciplinary character* of the dissertation with significant participation of *highly qualified team* of laboratory specialists and equipment for reaching the aim. The laboratory methods are well known, systematically used and established in the medical practice. The number of the statistical methods in the different investigations is impressive (7) which is a prerequisite for the statistical authenticity of the researched/obtained data.

The **results** (73 pages) are presented in details and illustrated with tables and figures in accordance with the set tasks with many results, some of which have the value of conclusions that could find a place in the official section. Important and statistically significant correlations were found both between the individual studied indicators and between the diseases themselves. The presented data of immunological / immuno-histochemical and genetic tests is quite relevant, though karyotype disorders and prognostic factors for survival (task 2.4 and task 6) are a bit schematic. Risk stratification is an important assessment - independent / accompanying aberrations, initiating, evolutionary clones. An understandably short place is given to the application of molecular genetic research as a prognostic factor - reporting the results of the study of 17 patients for mutational load - Jak2V617F status is artificial and does not allow for statistical processing with subsequent discussion, similar to FLT3 status in 29 patients.

The results obtained after precise statistical processing are theoretically and practically significant, even in cases where the expected significant difference is not established.

In the **Discussion section** (23 pages) the doctoral student gives a reasoned explanation of the analyzed indicators, showing knowledge in the analysis of laboratory test data and emphasized contribution to the comparison of rating scales and search for the importance of additional factors to improve stratification of patients with MDS and prognosis. It would be relevant to compare the data to that in the dissertation I quoted, where "chromosomal abnormalities in the karyotype were found in 40/105 (38.1%) of Bulgarian patients with MDS" with 1st main conclusion "comparative analysis of the frequency and structure of chromosomal aberrations in newly diagnosed patients with MDS and AML is consistent with data from multicenter studies in Europe".

The doctoral student does not focus only on the statistical data from the research, but makes a logical interconnection of all components of the set tasks.

The ten **conclusions** are in concordance with the obtained results. They correspond to the set tasks and reflect the essence of the achieved results. The aim of the study is not only to summarize and evaluate generally accepted criteria for stratification of Bulgarian patients with MDS, but also to include additional factors from clinic by analyzing all scales of comorbidity / "frailty" and their correlation to risk stratification. The way of presenting "Conclusions" is acceptable. I consider it would be more clear and appropriate to present them in accordance with the tasks (patient characteristics, survival, comorbidity and "frailty" scales, risk of transformation into AML, prognostic factors for survival and transformation). Conclusion 1 could be enriched by actually obtained results; consideration of the 2nd, 3rd, partly 4th and 9th conclusions could be presented in the context of one task - the risk of transformation into AML; conclusions 10 needs revision.

The **contributions** are presented in two groups. The contributions (4) of original character are with a serious impact: for Bulgaria (3) and for the world (1). In my opinion, contribution 1 "For the first time in Bulgaria an analysis of a large group of patients with MDS of demographic, clinical-laboratory and cytogenetic indicators" is not completely accurate due to the above scientific work with serious epidemiological, laboratory and especially cytogenetic indicators in MDS and AML. I assume that the reason is the insufficient computer programs and ability for reference and review the Bulgarian scientific database. I am not competent to comment on contribution 4 "For the first time in the world, a parallel analysis of all scales for determining the comorbid index in patients with MDS and their correlation with the classification and risk stratification systems", which would give an extremely high value as a personal work and merit of the doctoral student.

There are other 4 *confirmatory* contributions, the last "The need to assess the comorbidities associated with the risk of disease progression...." being actually the most original and additional factor according to the working hypothesis of the doctoral student.

According to the attached **research on the thesis topic**, Dr. Efraim has presented 3 publications in the following journals: Scripta scientifica medica (2018); Varna Medical Forum (2021) and Medinfo (2021), and in the last two she is the first author. As additional publications on the topic (outside the mandatory minimum number of full-text publications for review), the author lists 1 article "Role of comorbidity and index of "frailty" in the prognostic assessment of MDS" (in press in the Journal of Hematology) and 1 abstract from participation at the IX National Congress of Hematology in Pravets, 2012 concerning "A new approach in the assessment of cytogenetic risk in patients with MDS".

The **abstract** is 100 pages long, fully meets the requirements of the Act, and objectively and adequately reflects the main results and scientific contributions of the dissertation.

Critical remarks and recommendations - comments are presented in the relevant sections.

Conclusion: The dissertation demonstrates the doctoral student's knowledge not only in the field of clinical hematology (where she is admitted as a highly qualified specialist), but also in the statistical analysis of classifications and database of biological markers for the purposes of their practical application. The dissertation is up-to-date; the publication activity meets the requirements of the Academic Staff Development Act in the Republic of Bulgaria and the Rules for the Academic Staff Development in the Medical University of Varna for awarding the scientific-educational degree "Doctor". This gives me reason to recommend to the honorable members of the scientific jury to vote positively and to award the degree "Doctor" in the scientific specialty "Hematology and blood transfusions" to Dr. Merlin Erol Efraim.

12.08.21 **Member of scientific jury:**

Prof. Lyudmila Angelova, MD, PhD