TO

THE CHAIRMAN OF THE SCIENTIFIC JURY

IN ACCORDANCE WITH ORDER №P-109-303/16.07.21

OF THE RECTOR OF 'PROF D-R PARASKEV STOYANOV'

MEDICAL UNIVERSITY – VARNA

PROF. DR. VALENTIN IGNATOV, PH.D.

**OPINION**

**From**

**Prof. Gueorgui Balatzenko, M.D., Ph.D.**

**Head of Diagnostic and Consulting Unit,**

**National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia**

**Regarding:** The dissertation for awarding the scientific and educational degree 'Doctor' on topic 'CLINICAL-BIOLOGICAL AND GENETIC MARKERS IN RISK STRATIFICATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME' from **D-R MERLIN EROL EFRAIM,** self-study doctoral student in area of education – '7. Healthcare and sport', professional field – '7.1 Medicine', scientific specialty 'Hematology and Blood Transfusion' of Medical University – Varna, Faculty of Medicine, Department of Internal Diseases II, educational and scientific sector- Hematology and Clinic of clinical hematology „Sveta Marina" University Hospital – Varna, unsubscribed with order № P-109-303/16.07.2021 of the Rector of Medical University – Varna.

## RELEVANCE OF THE PROBLEM

The dissertation is dedicated to one of the relevant problems of contemporary hematology – importance of the clinical-biological and genetic markers in risk stratification in patients with myelodysplastic syndrome (MDS). MDSs include a heterogeneous group of clonal diseases of the pluripotent hematopoietic stem cell which are characterised by cytopenias, unilineage and multilineage dysplasia, ineffective hematopoiesis, the presence of recurrent genetic anomalies and an increased risk of transformation into acute myeloid leukemia (AML). The frequency of the newly diagnosed cases of MDS is around 3.2-4.9/100 000 on a yearly basis, while the morbidity varies widely depending on the age group of the analysed patients from 0.2/100000 for patients under the age of 40 to over 50/100000 for patients above the age of 80. The clinical evolution of the disease and the overall survival vary to a significant extent – from several months to 8-10 and more years. Numerous factors which have proven or assumed clinical significance have been described in relation to the prediction of the clinical course of the disease and its overall prognosis. The complex interaction between them have not yet been fully clarified. In this respect, *studies on the various factors associated with the disease and/or the patient, which can contribute for a better*

CLINICAL-BIOLOGICAL AND GENETIC MARKERS IN RISK STRATIFICATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME, irrespective the fact that this data could be used as a basis of future research in this field.

5. The analysis of the survival as per the results of the cytogenetic tests informs that the shortest survival for 2 (0.9%) of the patients with del(9q) (2 months), as well as among 13 (5.9%) of the patients with missing fit for analysis metaphase plates (5.1 months) – the survival is even worse compared with patients with complex modifications of the karyotype (8.7 months), which are defined with very bad prognosis. del(9q) is a rare anomaly of MDC with frequency < 1%. Irrespective the fact, that as per IPSS-R, patients with MDS and with isolate del(9q) fall under the group with intermediate cytogenetic risk, according to Haase et al. Blood, (2007), 110(13):4385-4395 and Schanz et al. Blood, (2008), 112(11):2688 it falls under the category of 'favourable' with unreached median survival. On the other side, unsuccessful cytogenetic investigation for patients with MDS often does not have any relevance to the biology of the disease and does not exclude the presence of various chromosomal anomalies. There are not any comments in the discussion with regards to these findings.
6. Total of 234 references have been presented in the references chapter and none of them is Bulgarian, despite the fact that there are series of publications and researches conducted in this field.

## CONCLUSION

Based on the above described I consider that despite my critical remarks, the presented dissertation by d-r Merlin Erol Efraim complies fully in type and volume to the specific requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Development of the Academic Staff of Medical University – Varna. The doctoral student possesses in-depth theoretical knowledge with regards to the various factors which have relevance for the risk stratification for patients with MDS, shows capability for creative interpretation of the data, not only the routine laboratory, but also the highly specialised tests, and establishment of scientific hypothesis, thanks to the obtained scientific and scientifically applied results, which are an original contribution.

On the basis of the above I give my positive assessment for the dissertation and I propose the honourable scientific jury to award the educational and scientific degree 'DOCTOR' to d-r Merlin Erol Efraim.

Sofia

19.08.2021

Prof. Gueorgui Balatzenko, M.D., Ph.D.

*prognostic stratification of the patient and in this way individualization of the therapeutic strategy are indisputably relevant and with scientific and practical significance.*

## BRIEF INFORMATION FOR THE PROFESSIONAL DEVELOPMENT OF THE DOCTORAL STUDENT

D-r Merlin Erol Efraim graduates with a Master's degree from 'Prof D-r Paraskev Stoyanov Medical Univesrity' Varna in 2008 (Diploma №000118/05.11.2008). Her professional development started in February 2008 in the Emergency Department in the town of Provadia where she worked as a doctor until May 2010. During the period October 2010 – May 2015 she was trainee in Clinical Hematology at the Clinic of Hematology of 'Sveta Marina' University Hospital – Varna. In April 2011 she was appointed as physician in the same clinic. She acquired medical specialty in Clinical Hematology (Diploma №-019375/20.07.2015). In May 2015 she was appointed as doctor-haematologist assistant in the clinic of Clinical Hematology of 'Sveta Marina' University Hospital – Varna, where she continues to work. Since April 2019 she has been registered as a self-study doctoral student at the 'Prof D-r Paraskev Stoyanov' Medical University – Varna with base at the clinic of Clinical Hematology of the 'Sveta Marina' University Hospital – Varna for the development of the current dissertation.

## STRUCTURE AND LAYOUT OF THE DISSERTATION

The dissertation has been laid out in accordance with the normative requirements spreading in 211 pages, visualised with 82 figures, 28 tables and 1 appendix and it includes the generally accepted chapters as follows: **Title Page** (1 page); **Contents** (4 pages); **List of Abbreviations** (3 pages); **Introduction** (2 pages); **Exposition:** *Literature Review* (58 pages); *Aim, tasks and hypothesis* (2 pages); *Research materials and methods* (6 pages); *Own Results for the Research* (73 pages); *Discussion* (24 pages); *Conclusion* (2 pages); *Conclusions* (2 pages); *Contributions* (1 page); *Scientific Publications on the topic* (1 page); *Acknowledgements* (1 page); *References* (30 pages) and *Appendices* (1 page).

References contain 243 sources in Latin, 9.5% (n=23) from the quoted literature was published in the last three years, and 16.9% (n=41) in the last five years.

The Abstract consists of 100 pages and corresponds to the dissertation.

**THE LITERATURE REVIEW** includes a large number of references and is well structured. The presented issues cover the heterogeneity of MDS and the variations in the clinical evolution, issues relevant to the diagnostics, prognostic stratification and their treatment. The pathogenesis of MDS has been considered in detail and the involvement of various genetic and epigenetic abnormalities, as well as the modifications in the regulation of the apoptosis and the immune system, which are the base of the biology of the disease and determining the emergence of ineffective haematopoiesis, the development of a pathological clone with signs of dysplasia and damaged function, modified differentiation and genomic instability. A series of risk factors have been presented for the development of MDS among which – environmental factors, preceding cytotoxic therapy, some genetic syndromes and others. The evolution of the classification approaches and the definition of new entities, reflect on the heterogeneity of MDS and the enhancement in the knowledge of the biology of the disease and the increased diagnostic power. The different risk stratification scales for MDS have been presented in detail (IPSS, IPSS-R, WPSS, MDAPSS), including principles, main factors the prognostic difference of the different groups and their clinical application. The

various predictive and prognostic factors for MDS have also been presented in detail, differentiated in two main groups: (1) Factors related to the patient – age, general condition, frailty index (CFS), pre-existing health conditions and comorbidity indices (CCI, HCT-CI, MDS-CI and ACE-27); (2) Factors relevant to the disease – particulars of the specific subtype of the disease according to the contemporary classifications, modifications in the clinical and laboratory parameters and the established biological factors. It makes an impression that in the literature review special attention has been brought to the significant number of molecular abnormalities, which are common for MDS, but which unfortunately have not been included in the experimental research of the current work and have not been investigated in the context of their prognostic significance which is the focus of the dissertation Clinical-biological and genetic markers in risk stratification in patients with MDS. The literature review emphasises the heterogeneity of the patients in relation to the clinical course, the spectrum of chromosomal aberrations and the clinical evolution of the disease, which establishes the work hypothesis that the analysis of some additional clinical and biological factors may have relevance on the risk stratification and their incorporation into the approved prognostic scales and classifications for patients with MDS may facilitate the more accurate risk stratification, prognosis of the survival and the risk of transformation into AML.

**The Aim** of the dissertation is clearly formulated "To investigate and analyse the impact of the factors, relevant for the disease (age, ECOG and comorbidities), for the risk stratification, survival and the risk of transformation into AML.

For achieving the aim **6 tasks** have been formulated which are logically connected: (1) To characterise patients with MDS according to demographic indicators, classification systems, risk assessment scales, clinical frailty scale and comorbidity indices and the main laboratory parameters. (2) To analyse survival depending on the demographic parameters, classifications and risk assessment scales, laboratory and cytogenetic parameters. (3) To assess and analyse survival depending on the scales of comorbidity and frailty and to compare that with the systems of classification and the scales for risk stratification for MDS. (4) To assess the link between the risk assessment scales and the comorbidity and frailty scales for patients with MDS. (5) To investigate and analyse the transformation of MDS into AML and to assess the survival of the patients before and after the transformation. (6) To derive factors with favourable and unfavourable prognosis in relation to the survival of patients with MDS.

**MATERIALS AND METHODS** – the study includes retrospective analysis of the demographic data, clinical-biological markers, and systems of classification and risk stratification, frailty scales and comorbidities for 219 adult patients with MDS, diagnosed and treated in the clinic of Clinical Hematology of 'Sveta Marina' University Hospital – Varna. The retrospective research is performed by analysing the available medical documentation, including medical history information, physical examination findings, current and past diseases, laboratory tests and treatment. For this purpose an individual medical card has been prepared for each patient. For each patient the following has been done: retrospective analysis of the demographic data; ECOG status; comorbidity indices (CFS, CCI, MDS-CI, HCT-CI, ACE-27); FAB classification, WHO (2008) and WHO (2016); risk groups stratification according to IPSS, IPSS-R and WPSS; routine laboratory tests (blood test; biochemical indicators; indicators for the iron metabolism) and the specialised laboratory tests – sternal puncture with myelogram; conventional chromosome banding analysis; immunophenotyping via flow cytometry; bone marrow biopsy with immunohistochemistry; investigation of the somatic mutations JAK V617F and FLT3-ITD gene. The obtained results are analysed statistically by using software product SPSS Statistics v.20.0 and

a wide spectrum of approaches and analysis – dispersion analysis; variation analysis; correlation analysis; regression analysis; ROC curve; comparative analysis and others.

**The RESULTS** are arranged in logical order, correctly presented and illustrated with numerous figures, graphics and tables.

**A comprehensive characteristic of the included patients with MDS has been made.**

It has been established that the average age of the patients is 70.7±10.2, with some prevalence of the males, which is more clearly seen in the de novo MDS, which constitutes above 90% of the cases, while the secondary MDS is prevailing among women. The distribution of the different subtypes of MDS is determined by using classification approaches [FAB, WHO (2008), WHO (2016)]. The results of the prognostic stratification of the patients by means of the most widely used risk assessment scales have been compared and according to IPSS and IPSS-R patients with intermediate risk prevail, while according to WPSS – those with high risk. The distribution of the patients according to the ECOG status, scale for clinical frailty (CFS) and the scales for determining the comorbidity indices (CCI, HCT-CI, MDS-CI, ACE-27) have also been examined. It has been found that the prevailing number of patients are with ECOG=0; the unfrail according to the frailty scale CFS; with CCI=0 points; with intermediate risk according to HCT-CI/MDS-CI (1–2 points) and those without existing health conditions according to ACE-27.

Relative portion of the cases with pronounced anemic syndrome, leukopenia, thrombocytopenia, high levels of LDH, bone marrow fibrosis and others has been determined and the clinical significance of series of laboratory parameters to the systems of classification and risk stratification has also been proven. The presence and type of chromosomal aberrations have also been analysed and it has been established that above 50% of the patients are with normal karyotype, followed by a group of complex karyotype and that with isolated del(5q). A small number of patients have been tested for the mutational status of FLT3-ITD (n=29) and JAK2 V617F (n=17), which subsequently have not been further analysed in relation to their prognostic significance.

**The survival of patients with MDS has been analysed in relation to demographic indicators, classification, risk stratification, clinical-biological and cytogenetic parameters.**

Average survival for the whole group of patients with MDS has been established to be 18,4±21,9 months without significant differences between de novo MDS and secondary MDS. Distinctions in the survival depending on the sex and age, subtypes of MDS according FAB and WHO (2016) classifications, with the risk group established by IPSS, IPSS-R and WPSS as well as some laboratory parameters, among which levels of haemoglobin, leukocyte and platelet count, levels of LDH, number of dysplasias, percentage of myeloblast in the bone marrow and the levels of serum iron have been established. Differences in the survival depending on the presence and type of proven cytogenetic anomalies have also been advised.

**The survival has been analysed in relation to the scales of comorbidity and frailty and has been compared with the systems of classification and scales for risk stratification for MDS.**

The absence of significant differences in survival has been found among the different groups related to the ECOG status, the clinical frailty scale and HCT-CI while prolonged survival has been found among patients with low risk according to MDS-CI and without comorbidity. At the same time series of correlations and significant differences have been determined in the overall survival depending on the FAB, WHO (2008) and WHO (2016) classifications and comorbidity and frailty scales ECOG, CFS, CCI, HCT-CI, MDS-CI and ACE-27. It is informed that the survival within

the same subtype of MDS for the respective classification can vary and with worsening of the respective parameters (increase of the scores/indices/points related to ECOG, CFS, CCI, HCT-CI, MDS-CI and ACE-27), which are reflection of the general condition, the existing diseases and others lead to decrease of the survival. The connection between the scales for risk assessment and comorbidity and frailty scales has been investigated for patients with MDS. No link has been found between IPSS and comorbidity and frailty scales, as well as difference at the extent of risk in relation to comorbidity. A slight correlation dependence is observed between IPSS-R and CFS where by increasing the extent of CFS, the risk in relation to IPSS-R also increases. Differences in risk in relation to the analysis WPSS and MDS-CI have also been found where all patients with very low risk are part of group MDS-CI=0. Increase in the number of patients in groups from 1 to 3 according to MDS-CI has been determined with the increase of risk as per WPSS.

**The cases of transformation of MDS to AML have been researched**

The frequency of transformation of MDS into AML has been established (22.4%), the average period of time for transformation from diagnosis to its occurrence is 16.3±19.8 months, as well as the survival of the patients after the transformation, which is 6 times shorter than that before the transformation. Series of differences between patients with and without transformation into AML have been found in relation to the average age, prevailing subtype of MDS in accordance with the different classifications and the risk group according to the prognostic scoring systems (high risk by WPSS, intermediary-2 risk by IPSS and very high risk by IPSS-R). Significant differences have not been found between the patients with and without transformation according to the comorbidity and frailty scales. Significant differences in some laboratory parameters have been established among the patients with transformation – lower average count of leukocytes and ANC, higher percentage of lymphocytes and lower values of creatinine and the β2-microglobulin.

**The main prognostic factors for patients with MDS have been analysed** by pointing the main profiles of patients with MDS from the evidence of the established available favourable and unfavourable factors.

In, **the discussion**, the obtained results from the demographic parameters, the distribution of the different subtypes of MDS according to the different classifications and prognostic scoring systems, the frailty scale and comorbidity indices, as well as the established laboratory results have been compared with the data from other similar studies and D-r MERLIN EFRAIM creatively summarises the results, clarifies the probable causes of the inconsistencies and creates well-founded hypothesis. The theoretical significance of the obtained results is clearly outlined and the practical aspects of the analysed dependences. The heterogeneous course of the different MDS has been pointed as well as the variations in relation to the clinical course and the outcome of the disease, which defines the necessity of grouping the patients according to the approved classification systems and scales of risk stratification, with the purpose of determining the prognosis and the risk. Despite this, none of the numerous approaches on its own is capable to define the clinical course of the disease for each patient. All this imposes differentiated strategy for the prognosis of risk for each patient and defines the advantage of the complex approach, which combines the systems for classification and risk stratification, and the comorbidity indices, as well as some clinical-biological markers as a promising option for précising the risk and survival for the different patients respectively better therapeutic strategy.

The conclusions in the dissertation originate from the obtained results and correspond to the tasks of the dissertation: (1) Age is one of the negative prognostic factors and the established average

age of the newly diagnosed patients is 70 years. (2) According to the FAB classification, patients with RA prevail, followed by RAEB. The highest risk of transformation and the shortest survival is for patients with RAEB and RAEB-t; (3) As per the WHO-2008 and WHO-2016 patients with RCMD/MDS-MLD prevail, followed by RAEB-1 and RAEB-2. The highest frequency of transformation and shortest survival is with RAEB-2. (4) As per IPSS and IPSS-R, patients with intermediary risk prevail, while as per WPSS – those with high risk. The survival is the shortest for patients form the groups of high and very high risk. (5) Significant difference in the overall survival has not been established among the different groups as per the comorbidity and frailty scales. (6) Negative moderate dependence is proven between ECOG, CFS and CCI and the survival according to the systems of classification and risk stratification. (7) Inversely proportional dependence is proven between the HCT-CI and MDS-CI risk groups and the survival according to the classification and risk stratification systems. (8) Weak positive dependence is proven between IPSS-R and CFS as well as differences in the risk analysis as per WPSS and MDS-CI. (9) One fifth of the patients with MDS transform into AML and the survival is six times shorter after the transformation. (10) The prognostic factors which have the biggest impact on the survival are age, leukocytes and platelets count, the number of dysplasias, the percentage of myeoblasts in the bone marrow, LDH and the levels of serum iron. (11) The leukocyte count, value of ANC, higher percentage of lymphocytes, lower values of creatinine and β2-microglobulin have been proven to have prognostic significance for the transformation into AML.

## SCIENTIFIC CONTRIBUTIONS

The scientific contributions of D-r Merlin Efraim are formulated in two main directions.

**Contributions with Original Character**

*№1. For the first time in Bulgaria an analysis was performed in a large group of patients with MDS of demographic, clinical-laboratory and cytogenetic indicators.*

I accept the contribution in relation to *"……performed analysis in a large group of patients with MDS of demographic and clinical-laboratory indicators"*, but I do not accept the contribution in the part "……cytogenetic indicators" due to the following reason. There are two successfully defended dissertations for acquiring the educational and scientific degree 'doctor'/candidate for medical sciencies on the topic of cytogenetic disorders with MDS by:

- D-r Stavri Asenov Toshkov (1989) – National Center of Hematology and Transfusiology – Sofia "Cytogenetic and Morphological Research of the Myelodysplastic Syndrome".
- Svetlana Aleksandrova Angelova (2013) - National Specialized Hospital for Active Treatment of Hematological Diseases – Sofia - "Clonal Evolution of the Karyotype of Patients with Acute myeloid leukemia and myelodysplastic syndrome: clinical significance".

*№2. For the first time in Bulgaria, an analysis was performed in patients with MDS against the comorbidity scales and the clinical "frailty" scale.*

I accept without any remarks.

*№3 For the first time in Bulgaria an analysis was performed in a group of patients with MDS of JAK2 V617F and FLT3 mutation status.*

I do not accept this contribution due to the following reasons – there are preceding scientific articles on this topic.

- Spassov B, Balatzenko G, Guenova M. "Acute myeloid leukemia associated with rapid acquisition of FLT3-internal tandem duplications, ecotropic virus integration site-1 and Wilms' tumor 1 genes overexpression 4 months after an intermediate- risk myelodysplastic syndrome diagnosis." Journal of Applied Hematology. 2015.6(2):82.
- Balatzenko G, Spassov B, Georgieva Y, Hrischev V, Guenova M. Low incidence of V617FJAK2 mutation in acute myeloid leukemia and myelodysplastic syndromes. Blood, 2015, 126(23):4957.

*№4. For the first time in the world, a parallel analysis of all scales for determining the comorbidity indices in patients with MDS and their correlation with the classification and risk stratification systems was conducted.*

I accept without any remarks.

**Contributions with Affirmative Nature**

*№1. The importance of the systems for classification and risk stratification as prognostic factors influencing the risk of transformation and survival in the Bulgarian population has been confirmed.*

*№2. The importance of age, leukocyte and platelet count, number of dysplasias, percentage of myeloblasts in BM, cytogenetic abberations and LDH for survival in the Bulgarian population was confirmed.*

*№3. The importance of comorbidities as prognostic predictors for Bulgarian patients with MDS has been established.*

*№4. The need to assess comorbidities associated with the risk of disease progression and risk-adapted therapy has been identified.*

I accept all four affirmative contributions without any remarks.

**ARTICLES RELATED TO THE DISSERTATION**

The attached list of scientific articles includes three full text articles in Bulgarian scientific journals/proceedings book of which:

Literature Review (1) – of which D-r Efraim is first author and it is dedicated to the issues of classification, prognostic stratification and genetic and molecular orders with MDS (Efraim M, Micheva I. New Biomarkers in the Diagnosis and Prognostic Assessment for

patients with MDS; Clinic of Hematology, 'Sveta Marina' University Hospital – Varna, Medinfo magazine IV. 2021; 100–104) – publication in not referred magazine with scientific review (30/2 = 15 points).

Original articles (2) in one of which D-r Efraim is the first author and in the second one the fourth author:

- Micheva I, Gerov, Dimitrova S, Efraim M, Gercheva L. Outcome after azacitidine treatment in patients with high-risk myelodysplastic syndrome and acute myeloid leukemia in the clinic of hematology at St. Marina university hospital, Varna. Scripta scientifica medica, 2018; 50 (1): 31–35 – publication in not referred magazine with scientific review (30/5 = 6 points).
- Merlin Efraim and Ilina Micheva; Socio-demographic Characteristics of the Survival Among Patients with Myelodysplastic Syndrome; Clinic of Hematology of 'Sveta Marina' Universit Hospital – Varna; 'Prof Dr Paraskev Stoyanov' Medical Univesrity – Varna. Varna Medical Forum, 2021, publication published in edited collective volumes (30/2 = 15 points)

Total number of points – 36 with necessary minimum of 30 points.

## CRITICAL REMARKS

1. Unification of abbreviations is missing in the text – for the same concept abbreviation in Cyrillic and Latin have been used. For example, the following is denoted in the used abbreviations: PAPC – for Refractory anemia with ringed sideroblasts in Cyrilic as well as RARS (Refractory anemia with ringed sideroblasts) in Latin.
2. As per the WHO (2016) classification there are two subtypes of MDS - MDS with excess of blasts-1 (MDS-EB-1) and MDS with excess of blasts-2 (MDS-EB-2). At the same time in the text for the same concepts Refractory anemia with excess blasts (RAEB) respectively RAEB1 and RAEB1 have been used, which is according to the classification of WHO (2008) and is missing in the WHO (2016).
3. In chapter Materials and Methods information is not provided for the methodology of investigation of mutation JAK V617F – it is described how DNA has been isolated from the leukocytes but the method of the investigation has not been described.
4. In chapter Results data is submitted for the established data has been presented for the presence of the FLT3-ITD among 2/29 (7%) of the patients and of $JAK2V^{617F}$ among 1/17 (6%). However, no information has been provided in regards to the mutational load among the patients, the type of patients it has been established among – subtype of MDS, age and other. The fact that the presence of the respective somatic mutations has been proved on its own does not provide any prognostic information. It is known that $JAK2V^{617F}$ can be found in people without any blood disorders, even in healthy individuals. On the other hand, data from literature regarding the prognostic significance of FLT3-ITD in the context of MDS is controversial. In this respect, the established presence is outside the context of the main aim of the dissertation -