

TO:  
THE CHAIRMAN OF THE SCIENTIFIC JURY  
APPOINTED BY ORDER  
№ P-109-377 / 06.10.2020  
OF THE RECTOR OF MEDICAL UNIVERSITY - VARNA  
PROF. KRASIMIR IVANOV  
city. Varna

O P I N I O N

From Prof. Margarita Lyubenova Genova, MD, PhD  
Specialized hospital for treatment of hematological diseases - Sofia

Subject:

OF A DOCTORAL THESIS

“PROGNOSTIC FACTORS: INTEGRATING A RISK ASSESSMENT AND TIME TO  
TREATMENT SCALE NAÏVE B- CLL PATIENTS IN CLINICAL PRACTICE”

For awarding the scientific and educational PhD degree Field:7."Health and Sports"  
Professional field: 7.1. "Medicine "Specialty : " Hematology and blood transfusion "  
Supervisor: Prof. Dr. Liana Gercheva- Kyuchukova MD PhD

**Dr. Vanya Slavcheva Popova**

PhD student at the Second Department of Internal Medicine, Faculty of Medicine, Medical  
University - Varna

DEAR MRS OF THE SCIENTIFIC JURY,

I bring to your attention an opinion on dissertation for awarding the educational and scientific  
degree "Doctor" by PhD student Vanya Slavcheva Popova according to the requirements of  
the Regulations for the development of the academic staff at the Medical University "Prof. Dr.  
Parashev Stoyanov" - Varna in connection with order № P-109-377 / 06.10.2020 of the Rector  
of MU-Varna.

### **I. Brief biographical data about the doctoral student:**

Dr. Vanya Slavcheva Popova graduated with a master's degree in medicine from the Medical Institute - Pleven in 1990. Acquires medical specialties of Internal Medicine ( Medical University of Sofia, 1997 ) and Clinical Hematology ( Medical University of Pleven, 1999).

Professional development of the PhD student include: doctor at the Polyclinic in the city Koynare ( 1990- 1995) , then - as an assistant / senior assistant in the Clinic of Hematology of the University Hospital "Dr. Georgi Stranski" ( 1996 to present ). Conducts classes for training fifth-year medical students in English and Bulgarian - lectures and exercises in the field of hematology.

Dr. Vanya Slavcheva Popova demonstrates her belonging to the active scientific community. Participates in several research projects, and so far two projects on B-chronic lymphocytic leukemia have been implemented, funded by the Medical University - Pleven. The results obtained in these projects are also included in this dissertation. Dr. Vanya Slavcheva Popova has published 22 articles in Bulgarian and foreign scientific journals, has participated with reports and posters at symposia and national conferences. She took part in clinical trials.

### **II. General evaluation of the dissertation:**

The dissertation is devoted to a long-standing scientific and medical problem, as chronic lymphocytic leukemia is the most common leukemia in adult patients, 90% of all chronic lymphoid leukemias and a total of about 7% of lymphoid neoplasms, with an annual incidence of an average of 4.2 per 100,000 people and the increasing of the frequency with age. The disease manifests itself in a wide range of asymptomatic course without the need for active treatment for a different period of time to a rapidly progressing treatment-resistant process. Although generally with an indolent clinical course, the disease poses serious medical, pharmaco-economic, and social problems, and remains virtually incurable with currently available therapeutic approaches. The average survival varies widely depending on the risk group. Numerous clinical and laboratory parameters are known, which are associated with the time to first treatment, as well as with the parameters of progression-free and overall survival. The study of their practical applicability in real medical practice is necessary for the implementation of sustainable models and practices of prognosis of the risk in CLL patients. In this regard, own studies on key biomarkers in comparison with the clinical parameters for the course of the disease in patients with chronic lymphocytic leukemia allow to define practically applicable risk groups, in order to optimize therapeutic behavior, which makes them undoubtedly relevant and promising. .

The presented dissertation by PhD student Vanya Slavcheva Popova corresponds to the basic requirements of the Regulations for application of the law for the development of the academic staff of the Republic of Bulgaria:

Presented scientific production in this competition fulfill the minimum national requirements corresponding to article of law 26 form LDASRB ( SN, N. 30 of 2018., with effect from 05.04.2018 yr. ) and article 1 of PP LDASRB ( SN, N. 56 from 2018., in force from 06.07.2018 ) for obtaining the educational and scientific degree "Doctor" in the field of education 7. "Health and Sports" Professional field 7.1 Medicine ( Table ) .

Group of indicators	Indicator	number of points for a “doctor”	number of points dr Vanya Popova
A	Dissertation for the award of educational and scientific degree “doctor”	50	50
	total for group G	30	30,75
G	7. Scientific publication and reports in scientific journals, referenced and indexed in world-famous databases with scientific information	60/n	22
	8. Scientific publication and reports in scientific journals in unreferred journals with scientific review or published and edited collective volumes	30/n	8,75

1.The dissertation is presented in the form and volume of a total of 123 pages, containing: content (1 page); used abbreviations ( 2 pages ); presentation, organized in the following parts - I . review ( 33 pages ); II . working hypothesis, goal and tasks ( 2 pages ); III . Research materials and methods ( 9 pages ); IV . Survey results ( 30 pages ); V . Discussion ( 19 pages ); main conclusions from the conducted research, conclusion and contributions ( 3 pages ); two applications ( Patient Information Sheet ; Questionnaire ); bibliography. The dissertation is illustrated with 49 figures and contains 30 tables. The bibliography includes 199 printed sources, 4 of which are in Bulgarian ; one Russian and 194 in English, 41.2% of which are from recent years (2015- 2020)

A 64-page abstract is attached, which reflects the main points of the dissertation and also contains (1) a list of publications related to the dissertation, which includes 4 publications in English, two of which are referenced in Scopus and Web of Science ; (2) a list of two participations in scientific forums in Bulgaria , (3) a reference to the original, confirmatory and applied contributions of the doctoral student.

2.The dissertation shows that PhD student Vanya Popova has in-depth theoretical knowledge in his specialty and ability to conduct research, the results of which represent a contribution to medical science and practice.

2.1. The dissertation contains a Review, which clearly shows the good awareness of the PhD student on the problems of chronic lymphocytic leukemia in a volume of 33 pages, structured in several main areas :

2.1.1. An overview of B-cell ontogenesis and the positioning of chronic lymphocytic leukemia in this process. Theories of the origin of the neoplastic branch are considered and the pathogenesis of the disease is discussed.

2.1.2.Clinical aspects of chronic lymphocytic leukemia. The focus of the review is on the possibilities for predicting the clinical course at the time of diagnosis on the basis of proven informative and practically applicable indicators in order to optimize the clinical approach and treatment.Classical clinical and laboratory, cytogenetic, molecular and flow cytometric markers are comprehensively presented. The concept of the need for a comprehensive risk assessment is presented.

The analysis of the data presented in the review allows Dr. Vanya Slavcheva Popova to formulate significant unresolved issues for our country, which should be included in the present dissertation. The working hypothesis is focused on the role of the molecular and genetic profile and the individual course of the disease and the possibility of applying surrogate, easily applicable methodological approaches to assess the underlying genetic disorders.

Dr. Vanya Slavcheva Popova shows skills for critical analysis and summaries of the cited scientific facts, based on which formulates the purpose of the dissertation - to study and analyze the impact of available clinical, laboratory, molecular and genetic indicators over time to the need to start treatment in untreated patients with B-CLL. To achieve it, the specific tasks are formulated: complex studies of demographic indicators, clinical stages and serum marker  $\beta 2$  – microglobulin as factors related to the time to treatment, as well as the study of the frequency of chromosomal aberrations, gene expression of LRL and ADAM29 in untreated patients.

2.2. PhD student Dr. Vanya Slavcheva Popova presented precisely used materials and methods in the research, which were selected in accordance with the goal and main tasks. The dissertation is based on the study of a total of 97 patients with CLL diagnosed on the basis of immunophenotypic constellation. Unfortunately, patients with atypical and immunophenotypic constellations were excluded from the analysis. A rich panel of clinical and laboratory parameters was used. The applied methods are described in detail and explained in the section in a way that allows a clear understanding of the nature of the performed tests: turbidimetric method for testing serum  $\beta 2$  – microglobulin; fluorescent in situ hybridization (FISH) to study the p53, ATM and DLEU 1 genes on interphase nuclei; reverse transcription multiplex polymerase chain reaction (RT - PCR) to study the expression of LPL and ADAM29 genes. A package of statistical methods was used for analysis of discrete and continuous quantities. The time to treatment was analyzed by the method of Kaplan and Meier, and the differences between the different groups were statistically assessed by log-rank test. The work would be very useful if the independent prognostic value of the individual parameters was evaluated simultaneously with the action of different factors through additional multivariate analysis.

2.3. The dissertation presents **Scientific results**, described correctly, illustrated with figures, graphs, photographs and tables, as follows:

- Studies of the demographic factors sex and age in terms of time to first treatment were conducted without identifying significant differences in the different groups.
- Studies of clinical indicators in terms of time to treatment have been performed. It is confirmed the significant difference between the clinical stages defined according to the criteria of Binet, including among patients with / without splenomegaly, which is one of the defining stage indicators. In this regard, it could be an analysis and the significance of the remaining components of the staging system (hematologic indicators, number of lymph groups affected), which could shed additional light on the relative severity of each of the indicators for a possible prognosis.
- The relationship between tumor volume and time to treatment was assessed. For this purpose, the results of the analysis are presented as a direct indicator of the tumor load - absolute lymphocyte count (a significant relationship was established with the treatment and the time to start it); and the indirect indicator - serum beta2 microglobulin, the elevated values of which significantly define a group with a shorter period before treatment, despite the high proportion of comorbidities and without the need for recalculation depending on renal function. In addition, a moderate correlation of beta2 microglobulin with patients' age and stage is shown.

- Studies of main molecular-cytogenetic markers were performed in 62 patients. The distribution of the most common, clinically significant chromosomal aberrations determined by locus-specific probes and FISH was established. As an independent finding in the dissertation are reported the frequency of aberrations, which explains the lower values compared to the data published in the scientific literature - del 13 q (42.6%), del 11 q (3.3%), del 17 p / p 53 (9.8%)., with differences depending on the stage of the disease. In 8.2% there is more than one aberration and clinical and laboratory indicators are provided to these patients. The recalculated frequency of individual aberrations, including in cases where there is more than one aberration, allow the generation of results close to the literature, as follows del13q (48.4%), del11q (9.7%), del17 p / p 53(12.9%). An analysis of the time to treatment was performed only with respect to the percentage of del13q without finding a dependence, but also without explaining how the discriminant value of 70% was determined. The work would benefit if the analysis covered all studied markers. I recommend Dr. Vanya Popova to publish a comprehensive earnest analysis of molecular cytogenetic data, as so far this is one of the most representative studies of their application to predict the time to treatment in patients with chronic lymphocytic leukemia in the country. In this regard, I would recommend in future publications to apply the approved nomenclature, to select appropriate photographic material presenting the findings in normal and pathology for each of the studied markers, to unify and refine the annotation of the figures.
- In the 48 patients was conducted a study of the expression of LPL and ADAM29 genes. The study is first in the our country and is used as a substitute IGVH status on the basis of literature data and defined in the "Materials and Methods" criteria "mutated IGVH status" ( in the presence of distinct amplification products comprising one band corresponding to ADAM29 expression or two products corresponding to ADAM29 and LPL ), "non-mutated IGVH status" ( in the presence of a distinct amplification product including one band corresponding to LPL ); "Undeterminate IGVH" ( in the absence of ADAM 29 and LPL amplification products ) . No own comparative studies have been conducted. The study identified in practice the equivalent of only "mutated" and "non-mutated" IGVH status. The so-called "mutated" status is characterized by a higher frequency of del 13q and no del 11q, while del 17p and the presence of more than one aberration are divided into both categories. Table №26 presents a significant difference between the two groups in terms of treatment need, without indicating whether this difference is statistically significant, as the difference in time to treatment is clearly demonstrated. The results of the doctoral student regarding the prognostic role of the biomarkers studied with RT - PCR are extremely interesting and with potential for practical implementation, but I would recommend the designation of gurip to be based on real findings, for example "ADAM29 (+) / LPL (±) "/" ADAM29 (-) / LPL (+) ", not " mutated IGVH status "/" non-mutated IGVH status ". The use of expression markers as substitutes for mutation status is well documented in the literature, but is not identical.
- The data are summarized in a modified prognostic scheme. Patients are divided into risk groups, with the median time to treatment significantly differentiating between the low-risk, intermediate-risk, and high- and very-high-risk groups.

2.4. In the presented section **Discussion of the results** Dr. Vanya Slavcheva Popova analyzes the data from her own research and those of other research groups found in the literature, which allows her to formulate summaries and **conclusions** regarding: (1) the distribution of patients according to the clinical stages at the time of diagnosis and

the relationship with the time to treatment. ( 2 ) the importance of serum levels of beta2 microglobulin in predicting the time to initiation of treatment ;(3) the prevalence of chromosomal aberrations, their relationship to molecular biomarkers corresponding to mutation status and the consequences for prognosis , (4) the benefits of an integrated approach to risk stratification

2.5. I generally accept the Contributions formulated by Dr. Vanya Slavcheva, divided into three main groups:

Original contributions: for the first time in our country the relationship between the expression of molecular biomarkers time to treatment was studied ; for the first time, these markers were used as a substitute for classical prognostic factor in the complex risk assessment in newly diagnosed patients with chronic lymphocytic leukemia.

Confirmatory contributions: the prognostic value of the clinical stages, serum levels of beta2 microglobulin and chromosomal aberrations established by molecular cytogenetic examination with respect to time to treatment has been confirmed.

Contribute applied nature: an approach for risk screening has been developed by preparing a questionnaire for practical application.

### **III. Recommendations and remarks.**

- Some of the conclusions are formulated without presenting in the section "Results" the relevant own observations of the doctoral student (5,6,8)and the impression remains that they are based more on literature data ; some of the conclusions sound like a finding (1,2).
- The results of the FISH study of molecular cytogenetic markers are not written correctly according to the approved nomenclature.
- In addition, the annotation of the microphotographs is incomplete ( fig. 36,38,40 - dissertation / fig. 22,24,26 - abstract ) . In case a figure shows the findings of the same patient, this must be clearly presented in the description. Fig. 36 ( dissertation ) / Fig. 22 ( abstract ) first, contains a description of only the findings with respect to TP53 as del(17 p) is demonstrated , but not the described monosomy ; on the other hand, there is no description of the ATM as the microphotograph is included.
- A uniform standard has not been observed when writing the literature sources, and not less than 26% ( n = 43) of the cited titles are incomplete or inaccurate.
- A significant part of the figures in the sections "literature review" and "discussion" are borrowed from other authors, although with clearly identified sources, which is not a good practice in the scientific literature. The doctoral student fully has the potential to develop his own modifications based on the high theoretical readiness he demonstrates in his work.
- These findings can for the most part be accepted as technical omissions and errors that do not change the work in substance.

### **IV. Conclusion.**

In conclusion, the presented dissertation work of Dr. Vanya Slavcheva Popova - PhD student at the Second Department of Internal Medicine, Faculty of Medicine, Medical University - Varna, on "Clinical application of prognostic factors and their integration into a scale for risk assessment and the time to treatment, in untreated patients with B-chronic lymphocytic leukemia ", is the doctoral student's own development on a current medical problem, structured and shaped according to the requirements. The dissertation with the attached scientific publications demonstrates the knowledge and opportunities for research of the doctoral student and meets the criteria of the Law on the Development of Academic Staff in

the Republic of Bulgaria and the regulations on the terms and conditions for acquiring scientific degrees and holding academic positions in MU.

Based on the above, I give a positive assessment of the dissertation and propose to the esteemed Scientific Jury to award the educational and scientific degree "Doctor" in field 7. Health and Sports, professional field 7.1. Medicine and scientific specialty "Hematology and blood transfusion" of PhD student Vanya Slavcheva Popova.

Prepared an opinion:

03.11.2020

Prof. Dr. Margarita Lyubenova Genova MD PhD  
SHATHD- Sofia