

OPINION
by Prof. Dr. Stefan Goranov, MD
Section of Hematology Faculty of Medicine / Medical University - Plovdiv

for awarding the educational and scientific degree Ph.D.
Field: 7. " Health and Sports" Professional field: 7.1. "Medicine"
Specialty : " Hematology and blood transfusion "

topic of the dissertation:

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

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Based on an order of the Rector of MU - Varna № P- 109-377 from № 6.10 .2020 for expulsion with the right to defense, as well as my appointment with the same order as a member of the Scientific Jury I was provided with a complete set of documents and dissertation procedure for awarding the educational and scientific degree "Doctor" in the field: 7. Health and sports, professional field: 7.1. Medicine and specialty "Hematology and blood transfusion"

1. General presentation of the procedure and the doctoral student. The necessary set of materials has been provided from the moment of enrollment until the decision of the Department Council and the Faculty Council for defense of a dissertation. The stages of the doctoral program have been observed, there is no change in the initial topic and the supervisor. The study was approved by the Committee on Scientific Ethics .

Dr. Vanya Slavcheva Popova is a graduate of the Pleven Medical Institute, where she graduated in medicine in 1990, and since 1996 has been an assistant at the Clinic of Hematology at the University Hospital "Dr. Georgi Stranski". She has specialties in Internal Medicine and Clinical Hematology. Dr. Slavcheva is a leading author and co-author of over 20 articles in the field of hematology, immunology and genetics, published in Bulgarian and foreign journals

I accept the procedure and all the documentation as fully compliant with the Regulations of MU Varna for the development of the academic staff and LRASRB .

2. Literature review With its slow evolution the chronic lymphocytic leukemia (CLL) is a classic example of the so-called indolent non-Hodgkin's lymphomas, to which primary hematogenous forms it belongs. In recent decades, many key pathogenetic mechanisms have been unraveled, which not only clarified the pathological signaling in the biology of the neoplasm pool, but also led to the creation of fundamentally new drugs. Despite well-defined indications for initiation of treatment, it is still extremely difficult to predict the quality of the therapeutic response and clinical response, regardless of new therapeutic options. The start of treatment and the choice of targeted or molecular therapy is directly dependent on modern risk-stratification. In this aspect, the topic of the dissertation - development a predictive or prognostic model for the specific and indefinite for CLL phase - "watch and wait" is not only relevant but also important scientific and practical significance. On the basis of adequately selected, modern and analytically presented literature data in the review a working hypothesis is formed: the early identification of factors at the time of diagnosing the process with already known unfavorable prognostic value with the formation of a score system for early initiation of therapy in high-risk CLL patients.

I accept the analytical and informative literature review, as well as the theoretically substantiated idea for the formation of the scientific hypothesis.

3 . Aim and tasks of the dissertation I accept the aim of the research as corresponding to the working hypothesis, and the set tasks as adequate and feasible in the realization of the goal.

4 . Material and methods 97 patients with B - CLL, over 18 years of age, diagnosed and observed in a university clinic were studied. The indicators of the disease, its activity and staged systems tested in a predictive aspect are generally accepted. Immunological and molecular genetic markers were developed in the Central Clinical Laboratory, Medical Laboratory of Immunodiagnostics of UMHAT Pleven and Laboratory of Molecular Biology and Cytogenetics at NSHATHD - Sofia. The methodology is described in detail, which gives the necessary representativeness. Statistical analysis includes the required panel methods of software product SPSS -19 at a significance level $p < 0.05$.

I accept the section without remarks

5 . Results . The obtained results were analyzed in a predictive aspect by the time to the start of the first treatment (TTFT), defined by default - the interval from diagnosis of the disease to the start of the first treatment, or the date of the last follow-up, or death (censored) . The median method of Kaplan Meier was used . The indications for treatment are in accordance with the recommendations of IWCLL - 2008 . (Hallek M et al , 2008). The median TTFT for the whole group was defined 48 months.

5.1. Demographics indicators. The author did not establish the rank of significance of sex and age for the start of first treatment. This result is quite interesting, given that age over 65 "managed" to become an independent prognostic factor among the 28 different indicators tested in over 3400 patients in the current International Patient Prognostic Index (CLL-IPI) in terms of however, of survival .

5.2. Binet staging system. The different stages according to *Binet staging system are* expected to predetermine significantly different TTFT, the result of which the author transforms and validates the prognostic value of the system in predictive of the start of treatment.

5.3. Splenomegaly . An elementary physical find, but also a very important part of CLL, has so far eluded significance in the various score systems, with the exception of the Rai staging system. The obtained data are ultimate - patients with splenomegaly start treatment five times earlier, respectively 23.8 ± 7 months. vs 115.4 ± 11 months for the group without splenomegaly ($p < 0.001$).

5.4. Tumor load. The absolute B lymphocyte count and β 2-M G above the reference values appeared with a shorter time to the start of treatment. Data were successfully derived from various intergroup combinations, including Binet stage (early A substage).

5.5 Comorbidity . The underlying conditions on which CLL develops is very well analyzed and tested with 8 groups of the most common co-morbidities in patients. The aim is to assess their effect on β 2-MG values .

5.6. Molecular genetic markers , surrogate markers (LPL , ADAM 29) and mutation status . It is a known fact that every second patient with CLL has a variety of chromosomal abnormalities. In 62 patients was performed FISH analysis using locus specific probes for: del13q , del11q , del17p / p53 and additional PCR analysis to determine the mutational status. Proven chromosomal aberrations were compared with the clinical manifestation of CLL, Binet stage and mutation status. Adverse prognostic factors del17p and unmutated status, which require earlier treatment, have been demonstrated in over 1/3 of patients at an early stage; the correlation between mutational status and the studied karyotype aberrations ($p = 0.035$); del11q and del17p , or the combination of more than one chromosomal aberration, including in combination with del13q, are associated with a shorter TTFT; the prognostic value of 13q- is different in cases where the latter is combined with unmutated status.

I accept the "Results" section with the presented data, processed with adequately selected statistical models.

6 . Discussion In general, the discussion is scientifically sound and competently made on the basis of the obtained results and the literature data on the problem.

7 . Remarks and recommendations . Consideration should be given to what part of the demographic data should go in the Patients and Methods section. The main data for the studied group are not results, but a characteristic of the observation. In the Results chapter, Figure 42 is a pure methodology and should be in the relevant section. Concomitant diseases can also be tested as indicators for starting treatment ...? When working with candidate - indicators for inclusion in a predictive for treatment or prognostic for survival model and especially with time (TTFT), for their evaluation multifactor Cox regression analysis is recommended, through which the established reliable results of the tested indicators, can reach the significance of "independent" by ultimately managing TTFT. Only one of them to acquire such a status is a theoretical and scientifically applied breakthrough. There are figures, tables and analyzes of literature data in the discussion, which would be more appropriate to be in the literature review. I consider it appropriate, although cited by the author, to make a table comparing one's own with other similar predictive indicators in the specialized literature. Regarding the conclusions - some of them represent derived results

Conclusion. The submit dissertation *Clinical application of prognostic factors and their integration into the scale for assessing risk and time to treatment in previously untreated patients with B-CLL* is the study of theoretical offerings unmatched in his native literature . All requirements for dissertation work in terms of structure and manner of presentation are met. I am convinced that I accept the development not only as a dissertation, but also with significant contributions of a scientific and applied nature. Based on the above, I give my positive assessment of the submitted dissertation and propose to the esteemed Scientific Jury to vote positively for the award of the educational and scientific degree "Doctor" to Dr. Vanya Slavcheva Popova .

Prepared the opinion

Prof. Dr. Stefan Goranov, MD

29.10.2020 / Plovdiv