STATEMENT

by Assoc. Prof. Milena Ivanova Belcheva, MD, PhD

Department of Pediatrics, Medical University "Prof. Dr. Paraskev Stoyanov", Varna

Regarding: Dissertation thesis for the award of the educational and scientific degree "Doctor"

Field of higher education: 7. "Healthcare and Sports"

Professional field: 7.1 Medicine

Doctoral program: Hematology and Blood Transfusion

Form of doctoral study: Full-time

Author: Dr. Yavor Anzhelov Petrov, Second Department of Internal Medicine, Faculty of Medicine, MU-Varna

Topic: *The role of lymphocyte populations after allogeneic hematopoietic stem cell transplantation*

Scientific supervisor: Prof. Dr. Ilina Dimitrova Micheva, MD, PhD

General presentation of the procedure

By Order No. R-109-332/28.07.2025 of the Vice-Rector of MU "Prof. Dr. Paraskev Stoyanov" Varna, based on the decision of the Faculty Council of the Faculty of Medicine (Protocol No. 43/21.07.2025), I was elected as an internal member of the scientific jury evaluating the dissertation thesis of Dr. Yavor Anzhelov Petrov on the topic "The role of lymphocyte populations after allogeneic hematopoietic stem cell transplantation", and was assigned to prepare an opinion. The set of materials submitted by the doctoral candidate in connection with the procedure for awarding the PhD degree is complete, available both in electronic and paper format, and complies with the Regulations for the development of the academic staff at MU-Varna, as well as the Law on the Development of the Academic Staff in the Republic of Bulgaria.

Brief biographical data on the doctoral candidate

Dr. Yavor Anzhelov Petrov graduated in Medicine at MU "Prof. Dr. Paraskev Stoyanov", Varna in 2013. In the same year he began work as a resident at the Clinic of Pediatric Clinical Hematology and Oncology at University Hospital "St. Marina", Varna. From 2014 to 2019 he specialized in Hematology at the National Specialized Hospital for Active Treatment of Hematological Diseases (SBALHZ), Sofia, where he was also Donor Search Coordinator. After obtaining his specialty in Hematology, until 2020 he worked as a specialist at SBALHZ. Since May 2020 to the present, he has been working as a specialist at the Clinic of Hematology, Stem Cell Transplantation Unit at University Hospital "St. Marina", which he has headed since September 2022.

Dr. Petrov has participated in several international projects and has been coordinator for JACIE accreditation both at SBALHZ and at University Hospital "St. Marina". He actively takes part in congresses, conferences, and training seminars on stem cell transplantation in leading European centers. In 2018/2019 he specialized at the National Institutes of Health/National Cancer Institute, Bethesda, Maryland, in the field of experimental transplantation and immunology. His scientific interests are in the area of transplantation. He is a member of the Bulgarian Medical Society of Hematology, the European Hematology Association, and the European and American Societies for Hematopoietic Stem Cell Transplantation.

The doctoral candidate possesses excellent theoretical training, rich practical experience, additional qualifications, and clearly defined scientific and practical interests in the field of his dissertation.

Relevance and significance of the topic

This dissertation is the first in Bulgaria to investigate immune reconstitution (absolute lymphocyte count and lymphocyte subpopulations) in patients with malignant hematological diseases after allogeneic hematopoietic stem cell transplantation and its relationship with various transplantation factors and complications.

The recovery of a balanced and functional immune system is a major determinant for the success of this therapeutic method and for patient survival. In the context of diverse transplantation modalities, conditioning regimens, therapeutic interventions, and post-transplant strategies, monitoring the kinetics and assessing the functionality of the immune system after allogeneic transplantation has the potential to personalize therapeutic interventions, reduce complications, and improve patient survival.

I find the topic of the dissertation to be important and useful for clinical practice.

Evaluation of the dissertation thesis

The dissertation is well-structured, following the classical layout with all main sections presented in a balanced manner.

Literature review

The literature review is comprehensive and demonstrates excellent knowledge of the scientific problem. The biological basis of allogeneic hematopoietic stem cell transplantation, donor selection criteria, potential stem cell sources, and the variety of conditioning regimens, as well as their role in immune phenomena and transplantation-related complications, are clearly and synthetically presented. A significant portion is devoted to immune reconstitution, with detailed discussion of the dynamics and timelines of recovery of lymphocyte subtypes and their relationship with immunological and infectious complications. The review justifies the need to study lymphocyte populations in the post-transplant period and their relation to donor characteristics, conditioning regimen, and complications.

Objectives (1 page):

A total of five objectives have been set, which are logical, clearly formulated, and consistent with the stated aim.

Design of the original study (5 pages):

The scientific research conducted is prospective. The analyzed group is sufficient in size and allows for statistical analysis. Among the patients included in the study, the predominant group consisted of those with acute myeloid leukemia, followed by patients with acute lymphoblastic leukemia, aplastic anemia, and non-Hodgkin's lymphoma. Transplantations were performed mainly with peripheral hematopoietic stem cells; fully matched related donors, fully matched unrelated donors, and haploidentical donors were used at comparable frequencies. The time points selected for monitoring the various lymphocyte subpopulations were consistent with the kinetics of immune reconstitution. No data were provided regarding the type and incidence of transplantation-related complications registered in the participants. The research methods are described clearly and consistently, outlining the approach to the problem. This allows for addressing the set objectives. The study was conducted with the approval of the Ethics Committee on Research at MU-Varna.

Results (18 pages):

The results of the study are presented in 19 tables and 52 figures, displayed sequentially and with clear text. The figures are of good quality and sufficiently informative, demonstrating statistically significant findings. The results are structured into four sections:

1.Monitoring of the absolute lymphocyte count on day 0, day +100, day +180, and day +270 post-transplantation showed a sustained positive dynamic. The same progression was confirmed for T-helper and activated cytotoxic populations, as well as NK and CD19+ cells, which were quantitatively assessed on day +100, day +180, and day +270.

2.Correlation analysis demonstrated the relationship between immune reconstitution and the type of underlying disease, as well as the influence of transplantation-related factors such as donor type and sex, conditioning regimen, and applied serotherapy. The B-cell population on day +100, as well as activated cytotoxic lymphocytes on day +100 and day +180, were significantly higher in patients with acute myeloid leukemia and non-Hodgkin's lymphoma compared to those with acute lymphoblastic leukemia. Patients transplanted from a geno-identical family donor showed the highest degree of recovery of absolute lymphocyte counts, T-helper, and B-lymphoblast populations compared to those transplanted from unrelated or haploidentical donors. Immune reconstitution was better in patients who received grafts from male donors. Delayed recovery of T-helper lymphocytes was demonstrated after conditioning with FluBuATG compared to BuCyATG, as well as a negative correlation between the recovery of T-helper, activated cytotoxic, and NK cell populations and the use of ATG.

3.In the third section, the relationship between lymphocyte subpopulation levels and transplantation complications was examined. An association was established between lower levels of T-helper lymphocytes on day +100 and day +180 and the occurrence of GVHD, relapse, infectious, and non-infectious complications. An inverse relationship was also found between low levels of activated cytotoxic lymphocytes on day +100, day +180, and day +270 and the occurrence of complications. NK and CD19+ cell levels were also significantly lower at all time points in patients with complications. Regression analysis demonstrated that the predictor coefficient for GVHD was highest in relation to CD3+CD4+ cells on day +100 and day +180 post-transplantation, and in relation to CD3+CD8+ cells on day +180 and day +270.

4.Finally, in a separate chapter, the relationship between the dynamics of immune recovery and overall survival was presented. Cut-off values for major lymphocyte subpopulations were established for day +100 and day +180 post-transplantation, which were associated with significantly better overall survival. The high sensitivity and specificity percentages highlighted these values as suitable biomarkers of prognostic significance.

Discussion (6 pages):

Consistently following the logic of the conducted study, Dr. Petrov presents in a synthesized manner a comparison of his scientific results with current scientific knowledge. The doctoral candidate demonstrates a solid familiarity with the literature and builds upon the information. A parallel is drawn with the results of previous clinical studies concerning all the analyzed parameters.

Conclusions, contributions, and summary (4 pages):

A total of six conclusions from the dissertation are formulated clearly, logically, and precisely, in accordance with the stated objectives. All of them are introduced for the first time within the national scientific context. Based on these conclusions, nine contributions of original, scientific-practical, and confirmatory nature are outlined.

Publications related to the research topic:

Dr. Petrov presents three publications (two articles in English and one participation in an international scientific conference). In all of them he is the first author, which confirms his personal contribution in the development of the scientific concept, the design of the study, the collection and synthesis of the results, and their publication.

Bibliography:

The bibliography includes 386 sources in Latin script. The analyzed scientific publications reflect current achievements in the relevant scientific field. The publications from the last five years demonstrate the ongoing research interest in the topic.

Abstract:

The abstract consists of 41 pages and structurally follows the content of the dissertation. It fully reflects the individual sections with the exception of the literature review, and includes all tables and figures with results. The abstract comprehensively presents the aims and objectives, methodology, results of the original research and their discussion, as well as the conclusions and contributions of the study.

Evaluation of the contributions of the dissertation:

The presented contributions are of original, scientific-practical, and confirmatory character. In essence, the dissertation represents the first comprehensive study in Bulgaria on the dynamics of recovery of lymphocyte subpopulations after allogeneic hematopoietic stem cell transplantation and the relationship between immune reconstitution and transplantation-related factors. Of particular value is the identification of specific cut-off values of the studied cellular populations as predictors of overall survival. The results of the dissertation provide a foundation for further scientific research and outline prospects for the early optimization of transplantation strategies in order to improve therapeutic outcomes. I accept the presented contributions.

Conclusion

The dissertation of Dr. Yavor Petrov is devoted to a topical scientific problem and contributes to the deepening of knowledge regarding the dynamics of immune recovery after allogeneic hematopoietic stem cell transplantation, the influence of transplantation-related factors, as well as the relationship between the reconstitution of individual lymphocyte populations and post-transplant complications. At the same time, the research identifies specific cut-off values of the studied cellular populations as predictors of overall survival, which outlines opportunities for optimizing therapeutic strategies.

In terms of structure, scope, and content, the dissertation meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, its Implementing Regulations, and the Regulations of MU "Prof. Dr. Paraskev Stoyanov" – Varna for the award of the scientific and educational degree "Doctor".

The dissertation reflects several years of in-depth work, carried out competently by a well-established clinician. It is an original work of the doctoral candidate and demonstrates his broad theoretical erudition, professional skills, and qualities for analysis and synthesis of scientific information.

I give my positive assessment of the dissertation on the topic "The role of lymphocyte populations after allogeneic hematopoietic stem cell transplantation" and propose to the esteemed scientific jury to vote in favor of awarding the scientific and educational degree "Doctor" to Dr. Yavor Anzhelov Petrov.

05.09.2025

Reviewer:

Assoc. Prof. Milena Belcheva, MD, PhD