TO THE CHAIRPERSON

OF THE SCIENTIFIC JURY

FOR THE COMPETITION FOR AWARDING THE EDUCATIONAL

AND SCIENTIFIC DEGREE "DOCTOR"

Order No. R-109-332/28.07.2025 of the

Rector of the Medical University
"Prof. Dr. Paraskev Stoyanov" – Varna

REVIEW

by

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External member of the scientific jury, appointed by Order No. R-109-332/28.07.2025 of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

Concerning:

Competition for the acquisition of the educational and scientific degree "Doctor" in the field of higher education 7. "Healthcare and Sports," professional field 7.1. "Medicine,"

by

Dr. Yavor Anzhelov Petrov – doctoral student, full-time training,
doctoral program "Hematology and Blood Transfusion," at the Second Department of Internal Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna,

with a dissertation entitled:

"The Role of Lymphocyte Populations after Allogeneic Hematopoietic Stem Cell Transplantation"

Supervisor: Prof. Dr. Ilina Micheva, MD.

I. Procedural issues

The candidate has submitted in both hard copy and electronic form the required documents and materials. Examination of the submitted set confirms that they have been prepared in accordance with the requirements of the Regulations on the Conditions and Procedures for the Acquisition of Educational and Scientific Degrees and for Holding Academic Positions at the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, as well as with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria.

Initially, the dissertation topic was *"The Role of Regulatory T Lymphocytes in Allogeneic Stem Cell Transplantation"* and was amended by rector's order in 2023. Both topics are consistent with the professional field "Medicine" and the doctoral program "Hematology and Blood Transfusion." The candidate is a full-time doctoral student, admitted to defense by rector's order.

Attached are the abstract, a list and copies of publications related to the topic, similarity check report, declarations of originality and authenticity, the respective enrollment and dismissal orders, the document for change of topic, as well as all other required documents under the law.

The candidate has been dismissed with the right to defense by rector's order No. R-109-332 of 28.07.2025.

II. Biographical data and analysis of career development

The doctoral candidate, Dr. Yavor Anzhelov Petrov, graduated from a language high school with English and Spanish, and subsequently obtained a Master's degree in Medicine from the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna (2013).

His professional activity began as a resident physician at UMHAT "St. Marina" – Varna (2013–2014), in the Pediatric Clinic of Hematology and Oncology. Between 2014–2019 he worked at the National Specialized Hospital for Active Treatment of Hematological Diseases – Sofia, focusing on adult hematology and hematopoietic stem cell transplantation. There he also served as donor search coordinator (2014–2020), was a member of the transplantation committee, and participated in the organization of educational sessions for patients and medical staff. In 2019 he obtained his specialty in Clinical Hematology.

Since 2020 Dr. Petrov has been working as a specialist physician at the Clinic of Hematology at UMHAT "St. Marina" – Varna, and since September 2022 he has headed the Department of Stem Cell Transplantation.

The candidate has completed a number of specializations at prestigious international centers, including: University Hospital in Zagreb (Croatia), University Hospital "George Papanikolaou"

Thessaloniki (Greece), as well as two consecutive training periods at the National Institutes of Health/National Cancer Institute – Bethesda, USA (2018–2019 and 2019–2020), where he worked in experimental transplantation, immunology and cell therapies (CAR-T, NK cells, TCR gene therapy).

Dr. Petrov is proficient in English and Spanish (B2 level, certified by international certificates), facilitating his participation in international scientific forums and professional collaborations. He is a member of the Bulgarian Medical Association, the Bulgarian Medical Society of Hematology, the European Hematology Association, and the European and American Societies for Hematopoietic Stem Cell Transplantation.

His scientific and clinical work is mainly focused on hematopoietic stem cell transplantation and its complications, including graft-versus-host disease (GVHD), post-transplant infections, and the application of novel cell therapies. This focus, combined with his active role in clinical and research activities, forms the practical basis for the chosen dissertation topic.

The analysis of his biographical data demonstrates purposeful development in hematology and transplantation, combining clinical practice, research, and international training. His leadership position in a university clinic reflects consistent professional growth and readiness for scientific and teaching activities.

III. Review of the dissertation

1. Introduction and general characteristics of the work, literature review

The dissertation addresses a highly relevant issue in clinical hematology and transplantation – immune reconstitution after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The introduction is clearly structured, highlighting the significance of the problem and the importance of allo-HSCT for treating various hematological diseases. The author examines multiple aspects of post-transplant immune recovery, analyzing the dynamics of lymphocyte subpopulations and the impact of different transplant-related factors and complications. The prognostic value of immune reconstitution for the final clinical outcome is emphasized. Indeed, immune recovery after transplantation is a decisive factor for the outcome, including in terms of GVHD, infectious complications, and relapses. The topic is therefore relevant and of scientific and practical importance.

The literature review is extensive, covering key aspects in the development of allo-HSCT: historical background, donor selection, stem cell sources and conditioning regimens, major immunological and infectious complications, and the dynamics of immune reconstitution across phagocytes, NK, T and B cells.

A particularly positive element is the inclusion of Bulgarian sources documenting the development of transplantation in Bulgaria, placing the research in a national as well as international context.

While the review is comprehensive, it could benefit from greater systematization and a stronger focus on the most recent findings, reducing the descriptive volume in favor of a more analytical approach. Highlighting controversial issues more explicitly would also better define the rationale for this research.

The introduction and literature review demonstrate solid knowledge of the subject, broad medical erudition, and the ability to compare national and international practice. They form a strong basis for defining the aims and tasks of the study, although the descriptive nature leaves room for a clearer statement of the author's own contribution.

The literature review is extensive and covers the main directions in the development of allo-HSCT. It systematically presents:

- the historical context — from the early attempts to the introduction of the method into clinical practice;
- methodological aspects of donor selection, sources of stem cells, and conditioning regimens;
- a review of major complications — immunological and infectious, which determine therapeutic results;
- the dynamics of immune reconstitution, including phagocytes, NK cells, T- and B-lymphocytes.

A particularly positive feature is the inclusion of **Bulgarian references** that trace the development of transplantation practice in the country. This is commendable, as it places the research in the context of national practice and not only international achievements (Bibliography — refs. 15, 177, 178, 179, 180, 181).

Nevertheless, the review could benefit from greater systematization and more emphasis on the most recent advances, reducing the substantial descriptive volume in favor of a more analytical approach. The topic of immune recovery after allo-HSCT has been extensively studied by numerous research groups over the years. A critical analysis of the achievements would have more clearly delineated the original contributions of the present dissertation compared to existing knowledge. Highlighting persisting controversies would also better justify the motivation for this study.

Overall, the introduction and literature review demonstrate **good knowledge of the subject, broad medical erudition, and the ability to compare national and international experience**. They provide a stable foundation for formulating the aims and tasks of the research, although the extensive descriptive nature leaves some room for a sharper outline of the author's personal contribution.

2. Aims and Objectives of the Research

The **aims** of the dissertation are clearly formulated and arise directly from the unresolved issues identified in the literature review. The main focus is on clarifying the **dynamics of immune reconstitution after allo-HSCT**, while analyzing the influence of different transplantation

factors (type and sex of the donor, conditioning regimen, serotherapy) and the role of post-transplant complications (GVHD, infections, relapses).

The **objectives** of the research are consistently structured and encompass:

- Monitoring the dynamics of lymphocyte subpopulations (T-, B-, and NK cells) at different time points after transplantation;
- Analyzing the influence of clinical and biological factors on the speed and quality of immune recovery;
- Investigating the relationship between immune recovery and patient survival;
- Determining cut-off values for lymphocyte subpopulations that have prognostic significance for clinical outcomes.

The formulation of the aims and objectives demonstrates a logical link **between the literature review and the author's own study. They are ambitious, yet reflect a clear effort to** build upon existing knowledge **with data from Bulgarian clinical practice.**

Some of the objectives are primarily descriptive and less reflective of specific hypotheses being tested. A clearer emphasis on the novel scientific aspect already at the stage of aims and objectives would highlight more distinctly the author's personal contribution.

In summary, the aims and objectives are appropriate to the research problem, correspond to the scope of the study, and follow logically from the literature analysis. They provide a solid foundation for presenting the materials, methods, and results.

3. Materials and Methods

The section *"Materials and Methods"* is thoroughly elaborated and provides a clear understanding of the study design, the patient cohort, the transplantation platforms applied, and the analytical approaches.

Patient cohort. The analysis includes **89 patients** over 18 years of age, allogeneically transplanted between 2017–2023 at the Department of Hematopoietic Stem Cell Transplantation, University Hospital "St. Marina", Varna. Demographic data are presented in detail, as well as the spectrum of underlying diagnoses – logically, the largest proportion consists of patients with acute myeloid leukemia (60.6%) – the most common acute hematologic malignancy treated with transplantation in adults, followed by acute lymphoblastic leukemia (18%) and several rarer nosological entities. This clearly defines the profile of the studied cohort and provides a good basis for interpreting the results. The number of included patients is representative for a single-center study and allows statistically significant analysis.

Donors and graft type. The distribution by donor type is precisely indicated: 10/10 HLA-matched related donor (30.3%), unrelated donor (32.6%), haploidentical donor (34.8%), and a single case with 9/10 match. The sex distribution of donors is noted, as well as the graft source (peripheral blood – 97.8% of cases). This section provides a solid overview of the diversity of

transplantation platforms and enables correlation between donor/graft type and immune reconstitution.

Conditioning regimens and immunosuppression. The various conditioning regimens are described in detail, with the exact number of patients for each type. Information is provided on the immunosuppressive prophylaxis used, as well as specific differences between related and unrelated donor settings. This comprehensive description is valuable as it allows precise assessment of the influence of individual factors on immune recovery.

Flow cytometry analysis. The core methodology for quantitative and qualitative assessment of lymphocyte subpopulations is described in detail. The mandatory technical specifications of materials and devices are correctly listed. The phenotypes of the main lymphocyte subpopulations are summarized in a table, in accordance with internationally accepted methodological standards and reproducibility criteria.

Statistical analysis. Data analysis was performed using the SPSS package, with methods for comparing independent groups (Mann–Whitney, ANOVA), correlation analysis (Spearman), regression models, and ROC analysis. The threshold for statistical significance was set at $p < 0.05$. The systematic presentation of statistical tools is in line with standards for similar clinical studies.

Overall, the *"Materials and Methods"* section is detailed, transparent, and provides sufficient information for reproducibility and credibility of the study. The number of patients and the follow-up checkpoints (day 100, 180, 270) are well justified and allow reliable evaluation of the dynamics of immune recovery. The methodological framework is one of the **strongest aspects** of the dissertation.

4. Results

In the presentation of the results, the text is structured into distinct subsections, each addressing the factors influencing the outcomes and the subsequent analysis. The findings are illustrated with **52 figures and 19 tables**, which provide a detailed visualization of the dynamics of immune recovery at different time points (days 30, 100, 180, and 270). A systematic approach in data collection and analysis is evident.

Overall recovery. The author clearly traces the restoration of absolute lymphocyte counts and individual subpopulations. The presentation is quantitatively substantiated and supported with graphical material, which facilitates the perception of dynamic changes.

Impact of transplantation factors. The influence of diagnosis, donor type and sex, as well as different conditioning regimens, has been analyzed separately. The results were statistically processed and clearly demonstrated specific dependencies, e.g., better recovery with fully matched related donors and less favorable recovery with haploidentical donors. The candidate demonstrates an analytical approach in identifying significant correlations.

Complications.
The relationship between immune recovery and the main complications – GVHD, infections, and other non-infectious issues – has been examined. Special attention is given to GVHD, where significant delays in the recovery of CD4+ and CD8+ populations were shown. This is an important contribution, as the data provide a local context consistent with international experience.

Survival. Through ROC analysis, cut-off values for ALC at day +100 and +180 were defined, associated with better overall survival. The results have practical applicability – enabling the identification of high-risk patients and offering the possibility for closer monitoring or early intervention.

The *"Results"* section is the most essential part of the dissertation. It presents a study with a sufficient number of patients (n=89), applying appropriate statistical methods and effective visualization. The author does not limit himself to descriptive data, but actively seeks relationships between parameters, thereby reaching practical equivalents. At certain points, the exposition is overly detailed, which hampers quick orientation – a clearer emphasis on the key results would increase its impact.

5. Discussion of the Results

The discussion is extensive, well-structured, and follows the logic of the presented results. The author provides an interpretation of the key relationships between transplantation factors (donor type, sex, conditioning regimens, immunosuppressive platforms), post-transplant complications (GVHD, infections), and the dynamics of recovery of the different lymphocyte subpopulations. The discussion is supported by a rich reference base and demonstrates good knowledge of contemporary advances in the field of allogeneic transplantation.

The author successfully compares and comments on his own results with those from international studies (e.g., Zhao et al., Servais et al., Ju et al., Espinoza-Gutarra et al.), which lends credibility and proves a careful and critical review of the relevant literature.

Clear comparisons are presented: for example, better recovery of ALC, CD4, and CD19 populations following transplantation from fully matched related donors compared to unrelated or haploidentical donors; better recovery with male donors; negative effects of certain conditioning regimens (e.g., FluBu) and immunosuppressors (ATG) on immune recovery.

Specific statistical correlations are reported, including Spearman's rho and ROC analysis, which strengthens the analytical value of the discussion.

All major clinical issues are covered: GVHD, infectious complications, risk of relapse, and the prognostic value of ALC.

In the concluding passages, the author outlines perspectives for future research – including innovative approaches such as adaptive T-cell transfer, allodepletion, and predictive models – which add completeness and a practical orientation.

In some places, the discussion is overly detailed and excessively descriptive, with long paragraphs and numerous citations that hinder easy comprehension. Some contradictory data from the literature (e.g., FluBu versus BuCy) are mentioned but not given more in-depth interpretation; the purpose of citing them remains isolated, without sufficient personal commentary. The prognostic value of the cut-off levels for lymphocyte subpopulations is convincingly stated, but broader discussion of their applicability in routine clinical practice is lacking (for example, limitations in different patient subgroups).

The discussion fulfills its role of summarizing and interpreting the results within the context of contemporary scientific data. The author demonstrates thorough knowledge and the ability to compare national and international experience. Despite a certain excess of descriptiveness, the discussion is scientifically well-argued and highlights clear contributions.

6. Conclusions from the Results

The conclusions are clearly formulated, numbered, and correspond to the results and the discussion. They summarize the most important relationships – the connection between the type of underlying disease and the dynamics of recovery, the role of the donor and their sex, the effect of conditioning regimens and serotherapy, as well as the impact of complications on lymphocyte recovery.

The formulated conclusions are concise, specific, and statistically substantiated. Their practical relevance is emphasized – improved overall survival in patients with lymphocyte populations above certain cut-off values. A balance has been achieved between laboratory findings (e.g., CD3+CD4+, NK, CD19+ populations) and clinical outcomes (overall survival, impact of complications).

Some of the conclusions overlap with the results and discussion, making them more of a summary rather than a synthetic interpretation. It would be useful to differentiate more clearly which conclusions are innovative and which confirm already known dependencies from the literature. There is a lack of emphasis on the limitations of the study (e.g., retrospective design, limited number of patients in some subgroups).

Overall, the conclusions are consistent and logically follow from the presented results. They successfully highlight the importance of immune recovery as a prognostic factor and have practical applicability.

7. Contributions of the Dissertation

Contributions with original character. The author explicitly states that for the first time in Bulgaria a systematic evaluation has been carried out on the impact of transplantation factors, complications, and lymphocyte recovery following allogeneic transplantation. This is an undeniable original contribution that places national practice within the context of international scientific trends.

Contributions with scientific-practical character. Monitoring lymphocyte subpopulations is substantiated as a clinical tool for predicting complications and outcomes. The importance of immune monitoring as a method for optimizing therapeutic interventions is emphasized. This makes the work useful not only for the scientific community but also for clinical practice.

Contributions with confirmatory character. The author clearly separates the observations that coincide with existing data in the literature (type of donor, role of GVHD, infections, etc.). This strengthens the reliability of the analysis and shows that the study is in line with global achievements.

Although the contributions are correctly formulated, in some places they sound too general. It would be useful to distinguish more clearly what is new for Bulgarian practice and what is a direct confirmation of international data. There is no discussion of the possibility of implementing the obtained cut-off values in clinical algorithms in Bulgaria.

The contributions are convincingly presented, showing clear originality and practical applicability. The dissertation undeniably expands knowledge in the field of allogeneic transplantation and lays the foundation for future national and international research.

8. Conclusion

The conclusion of the dissertation is clearly structured and synthesizes the main results of the study. The author succeeds in summarizing the significance of lymphocyte subpopulations in the process of immune recovery and their prognostic value for clinical outcomes in patients after allogeneic hematopoietic stem cell transplantation.

- The main findings are linked to specific clinical consequences (e.g., faster recovery in AML, better outcomes in related donors, negative effects of FluBu and ATG).
- Clear prognostic biomarkers are identified through the definition of cut-off values, which has practical relevance.
- The necessity of a personalized approach and the potential of immune monitoring as a tool for optimizing therapeutic decisions are highlighted.

The conclusion remains at a relatively high level of generalization and could contain a shorter, more focused synthesis of the innovative aspects. The limitations of the study (retrospective design, limited number of patients for some analyses) are not sufficiently emphasized. Such

clarification would enhance the critical value of the work. The perspectives for implementing the obtained results in national clinical guidelines or algorithms are not directly addressed, although the possibility is indirectly suggested.

The presented conclusion is well-argued, logical, and consistent with the stated objectives. It connects the individual results with the overall concept of the dissertation and highlights the importance of immune recovery as a key prognostic factor in transplantation practice.

9. Bibliography

The bibliography includes a total of 386 sources, which is evidence of the author's broad awareness and thorough study of the international context of the topic. Foreign publications of high rank predominate, ensuring the relevance and scientific weight of the dissertation. A particularly positive element is the inclusion of Bulgarian publications related to the development of transplantation activities in our country (works involving Bulgarian authors). In this way, the research is embedded not only within the international but also within the national scientific tradition. The arrangement and formatting of the bibliography are correct and in accordance with established standards.

10. Author's Abstract

The author's abstract has been prepared in accordance with the requirements and accurately reflects the content of the dissertation. It clearly formulates the objectives, tasks, materials and methods, the main results, the discussion, and the contributions of the research.

An essential advantage is that the abstract succeeds in presenting the extensive material in a synthesized manner, without losing connection to the key points of the dissertation. The exposition is concise, well-argued, and sufficiently comprehensive to orient the reader to the character and significance of the research. As a critical note, it may be pointed out that the style is at times more descriptive than analytical, which partly obscures the author's original contributions. Nevertheless, the abstract fulfills its intended purpose and complies with the required standards.

11. Publications Related to the Research Topic

Two publications in a peer-reviewed journal (*Scripta Scientifica Medica*) are presented—one presenting part of the main results regarding the impact of transplant complications on the recovery of lymphocyte subpopulations, and the other focusing on the evaluation of donor and conditioning factors in immune recovery. An abstract of a presentation at a prestigious international forum (EBMT, 2025) is also provided, demonstrating the scientific value and international significance of the research. The publications are appropriate, thematically connected to the dissertation, and confirm the author's original results. Particularly important is the candidate's participation in an international congress (EBMT), as this provides external validation and an opportunity for international discussion of the findings

IV. General Reviewer's Assessment

The presented dissertation is dedicated to a problem of exceptional relevance—immune reconstitution following allogeneic hematopoietic stem cell transplantation. The author, Dr. Yavor Petrov, demonstrates an in-depth knowledge of the international literature, as well as engagement with the national context through the inclusion of Bulgarian sources and practical observations from a leading center in our country.

The strengths of the dissertation include the choice of a clear object and subject of study, consistently formulated objectives and tasks, a well-selected and representative patient group, the application of modern methods of immunophenotyping and statistical analysis, linking the results to clinical practice, and the clear and convincing formulation of contributions.

Some nuances could be improved in the presentation of the research material—an overly descriptive character in the literature review, insufficiently detailed discussion of the study's limitations, and partial overlap between results, conclusions, and the final summary.

V. Conclusion

In conclusion, the dissertation fully meets all the requirements for a scientific work of this rank. It contains original, applied, and confirmatory contributions and expands knowledge in the field of hematopoietic stem cell transplantation. The research has undeniable practical significance for improving post-transplant monitoring and predicting clinical outcomes.

On this basis, I give a positive evaluation of the dissertation of Dr. Yavor Petrov and recommend to the esteemed members of the scientific jury that he be awarded the educational and scientific degree of **Doctor**.

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

05.09.2025 /Assoc. prof. Maya Yordanova/