**TO**
**ASSOC. PROF. TRIFON CHERVENKOV, MD, PhD**
**CHAIR OF THE SCIENTIFIC JURY**
**APPOINTED BY ORDER № R-109-332/ 28.07.2025**
**OF THE ACTING RECTOR OF MEDICAL UNIVERSITY – VARNA**
**"PROF. DR. PARASKEV STOYANOV"**

# REVIEW

By: Prof. Veselina Goranova-Marinova, MD, PhD
Head of Hematology Section, Faculty of Medicine, Medical University – Plovdiv
External member of the scientific jury according to Order № R-109-332/ 28.07.2025
of the Acting Rector of MU–Varna "Prof. Dr. Paraskev Stoyanov"

**Subject: Procedure for the acquisition 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"
**Author**: Dr. Yavor Anzhelov Petrov, Hematology Sector, Second Department of Internal Medicine, Faculty of Medicine, Medical University – Varna
**Form of PhD training**: Full-time
**Dissertation Title**: "Role of Lymphocyte Populations after Allogeneic Hematopoietic Stem Cell Transplantation"
**Scientific Supervisor**: Prof. Ilina D. Micheva, MD, PhD

**1. General presentation of the procedure**. The submitted set of materials, in both hard copy and electronic form, for the procedure for acquiring the educational and scientific degree "Doctor" at MU–Varna is in compliance with the Regulations of MU–Varna, Art. 68, para. 1 of the Higher Education Act; Art. 7, para. 1 of the Development of Academic Staff in the Republic of Bulgaria Act (ZRASRB); Art. 5, para. 1 and Art. 6, para. 1 of its Implementation Regulations, as well as the Regulations for the Development of the Academic Staff of MU–Varna. All required documents have been submitted, namely orders for enrollment and withdrawal of the doctoral candidate, protocols of extended departmental councils, and results of the doctoral minimum exam, reflecting all stages of the doctoral student's development, including the dissertation itself. A list and copies of publications related to the dissertation are attached. In total, 2 full-text publications in Scripta Scientifica Medica (official journal of MU–Varna) are presented. ***The doctoral procedure has been fully observed.***

**2. Presentation of the doctoral candidate** Dr. Yavor Anzhelov Petrov is a specialist in clinical hematology with more than ten years of professional experience, with a clear focus on hematopoietic stem cell transplantation (HSCT). His career began at University Hospital "St. Marina" – Varna, initially as a resident in the Pediatric Clinic. He then continued as a trainee in the Specialized Hospital for Active Treatment of Hematological Diseases – Sofia, where he gained substantial practical experience in the Transplantation Department and simultaneously served as a donor search coordinator, collaborating with national and international donor registries. He obtained his specialty in Clinical Hematology in 2019, after which he worked as a specialist primarily engaged in HSCT. Since 2020, he has continued his career at University Hospital "St. Marina" – Varna, in the Clinic of Clinical Hematology and the HSCT Department. At the same time, he was enrolled as a full-time PhD student in the doctoral program "Hematology and Blood Transfusion." In 2022, he was appointed Head of the HSCT Department, responsible both for the medical aspects of transplantation and for the overall organization of the process and team. His academic and clinical training includes specializations in transplantation, immunology, and innovative cell therapies. He specialized in HSCT at University Hospital – Zagreb, Croatia (2016–2017), and University Hospital "George Papanikolaou" – Thessaloniki, Greece (2018). From 2018 to 2020, he trained at the National Institutes of Health / National Cancer Institute – Bethesda, Maryland, USA, focusing on experimental transplantation and immunology. His work included novel conditioning regimens, management of early and late complications post-allo-HSCT, CAR-T and NK cell therapies, TCR gene therapy for solid tumors, and mechanisms of resistance and complications in cellular therapies.

He has a strong interest in new therapeutic approaches for graft-versus-host disease (GVHD) and the use of advanced cell technologies in hematological malignancies and solid tumors. His profile combines clinical competence in diagnosis, preparation, and follow-up of transplant patients with proven expertise in organizational and logistical aspects of donor search and international cooperation. He has professional fluency in English and Spanish, which supports international collaboration. ***Dr. Petrov has the theoretical knowledge, practical skills, additional qualifications, and well-defined scientific interests relevant to his doctoral work.***

**3. Relevance of the topic and knowledge of the problem**

The dissertation topic is timely and significant in modern transplantology, hematology, and clinical practice. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a well-established treatment for various malignant and non-malignant hematological disorders. Despite advances, the method remains associated with major complications, including

GVHD, infections (viral, bacterial, fungal), and non-infectious toxicities leading to organ and systemic dysfunction. Another major challenge is the slow and often incomplete reconstitution of the immune system post-transplantation.

The research problem is highly relevant, since immune reconstitution after allo-HSCT is a complex, long-lasting process, determining long-term treatment outcomes and survival. Lymphocyte subpopulations play a central role in immune regulation, and disturbances in their number and function may result in GVHD, disease relapse, and infectious or non-infectious complications. Monitoring and deepening the understanding of lymphocyte subpopulation dynamics post-transplant is key for optimizing therapeutic strategies and improving prognosis.

**4. Knowledge of the scientific problem**. The dissertation clearly addresses a knowledge gap, identified through thorough and analytical review. Although data exist on certain aspects of immune reconstitution post-allo-HSCT, limited knowledge remains about the overall dynamics of lymphocyte subpopulations and their associations with clinical outcomes. It is not fully elucidated which immunological parameters have the strongest predictive value for GVHD, relapse, or infections, nor how they can be integrated into personalized therapeutic approaches. ***Dr. Petrov demonstrates systematic, up-to-date, and critical mastery of the topic; he knows the problem in depth and is capable of formulating valid research questions and hypotheses.***

**5. Methodology of the research**. The dissertation is a single-center observational study conducted in the HSCT Department, Clinic of Clinical Hematology, University Hospital "St. Marina" – Varna, between 2017–2023. Eighty-nine adult patients (≥18 years) with malignant hematological diseases undergoing allo-HSCT were included. The cohort was stratified by demographics, diagnosis, donor type (HLA-matched related, HLA-matched unrelated, mismatched unrelated [MMUD 9/10], haploidentical), stem cell source (peripheral blood, bone marrow), conditioning regimens (FluBu, BuCy, FluCyATG, TBF, FLAMSA-Bu, CyATG, FluMelBCNUATG, RIC FluBu), and GVHD prophylaxis (calcineurin inhibitors or tacrolimus + mycophenolate mofetil ± methotrexate). Lymphocyte subpopulations were monitored at day +100, +180, +270 by immunophenotyping. Primary endpoints: dynamics of absolute and relative counts of lymphocyte subpopulations; secondary endpoints: associations between immunological parameters and outcomes (GVHD, infections, graft rejection).

**6. Characteristics and evaluation of the dissertation**

The dissertation is 105 pages long, includes 19 tables, 52 figures/graphs, and cites 386 references. The required sections are professionally developed and proportionately balanced. The text is clear, precise, and grammatically correct in Bulgarian.

### 6.1 Literature review

- ✓ ***Scope and depth***: covers allo-HSCT from historical data to current practice, addressing immunogenetics, donor selection, stem cell sources, conditioning, GVHD, infectious risks, immune reconstitution.
- ✓ ***Relevance:*** cites literature up to 2024, reflecting close monitoring of current publications.
- ✓ ***Critical synthesis***: compares competing models (MUD/MMUD/HD/UCB; MA/RIC/NMA; ATG-T vs ATG-F), highlighting pros/cons and clinical outcomes.
- ✓ ***Methodological awareness***: accounts for confounders (age, stem cell source, conditioning intensity, CMV serostatus) and uses precise definitions.
- ✓ ***Identification of scientific gaps***: emphasizes need for integrated models of lymphocyte dynamics, predictors for GVHD/relapse/infections, and HLA-related effects, especially in haplo-HSCT.

***The review ends with a well-motivated rationale for studying lymphocyte populations as determinants of allo-HSCT outcome***.

### 6.2 Aim and objectives

The aim is clearly defined, supported by 5 specific objective tasks, sequentially solved throughout the research.

### 6.3 Materials and methods

Presented on 6 standard pages. Conditioning regimens and GVHD prophylaxis follow international standards. Immunophenotyping with monoclonal antibodies (Becton Dickinson, USA) was conducted, with Sysmex XN-1000 analyzer for lymphocyte counts. Statistical methods (descriptive, comparative, correlation, regression, ROC) were appropriate and performed using IBM SPSS v.22.

### 6.4 Results

Presented on 19 pages, with logical structure and visualization. A key finding is the role of early immune reconstitution (esp. $CD4^+$ T and NK cells) as a strong prognostic factor for overall survival (OS). Significant cut-off thresholds were defined (CD4 day 100/180; NK day 100/180), with high sensitivity/specificity, distinguishing OS outcomes. Donor type, conditioning, ATG use, and donor sex influenced immune reconstitution. Lower CD4, NK, and CD19 counts were consistently associated with complications and poor outcomes.

### 6.5 Discussion

On 5 pages, the discussion integrates findings with literature, highlighting practical applications. The dissertation moves beyond descriptive epidemiology to propose validated immune thresholds with direct prognostic and clinical value.

**6.6 Conclusions**

Six in number, aligned with aims and objectives, emphasizing the prognostic value of lymphocyte subpopulations and modifiable factors in allo-HSCT.

**6.7 References**

386 sources cited, 22% from the last 5 years, indicating topicality and active research interest.

**7. Contributions of the dissertation**

Contributions include 3 original, 3 applied, and 4 confirmatory findings. These are accepted as stated.

**8. Candidate's personal contribution**

Dr. Petrov personally contributed to study conception, data collection, study design, statistical analysis, interpretation, conclusions, and publications. He is first author on related papers.

**9. Abstract**

The abstract (41 pages) reflects the dissertation well, including key tables/figures and sufficient data.

**10. Critical notes and recommendations**

The dissertation is the first of its kind in Bulgaria in transplantation hematology, based on 89 patients in a 5-year period. Limitations include:

1. Heterogeneous cohort (AML, ALL, lymphomas, AA), affecting stratification.

2. Day 180/270 analyses include only survivors, possibly overestimating prognostic value.

3. Confounding variables (e.g., ATG use, conditioning regimens) could bias results; multivariate Cox models are needed.

4. GVHD is both influenced by and influences immune reconstitution.

5. The candidate should increase publication activity on the subject.

These limitations do not diminish the scientific value of the dissertation but rather provide grounds for further development in this field.

**11. Conclusion**

The dissertation of Dr. Yavor A. Petrov meets the requirements of ZRASRB, its Implementation Regulations, and the Regulations of MU–Varna for conferring the degree

"Doctor." It represents an important study, profiling key lymphocyte subtypes ($CD3^+CD4^+$, $CD3^+CD8^+CD38^+$, NK, $CD19^+$) and their impact on allo-HSCT outcome. The identified thresholds serve as a measurable biomarker panel with direct clinical utility, enabling early risk stratification and personalization of therapy.

The dissertation demonstrates Dr. Petrov's ability to analyze and synthesize scientific data, formulate hypotheses, and apply knowledge in hematology and blood transfusion.

I give my positive evaluation of the dissertation entitled "Role of Lymphocyte Populations after Allogeneic Hematopoietic Stem Cell Transplantation" and propose to the respected scientific jury to vote IN FAVOR of awarding the educational and scientific degree "Doctor" to Dr. Yavor A. Petrov.



**PROF. VESELINA GORANOVA-MARINOVA, MD, PhD**

***Reviewer***