

## STATEMENT OF OPINION

of the dissertation thesis submitted by Dr. Martina Radoslavova Bozhkova,  
Assistant Professor at the Department of Medical Microbiology and Immunology,  
“Prof. Dr. Elissay Yanev”, Medical University – Plovdiv

entitled “ **Study of B-Cell Immune Memory in COVID-19 and Post-Vaccinal Immunity**”,  
for the award of the educational and scientific degree “Doctor”  
within the Doctoral Programme in Immunology, Scientific Field 7.1. Medicine,  
Scientific Supervisor: Assoc. Prof. Velizar Shivarov, MD, PhD

by Prof. Todor Kantardzhiev, MD, DSc

The dissertation thesis by Dr. Bozhkova comprises 160 standard typewritten pages, includes 7 tables and 29 figures, and is logically structured into 12 chapters, namely: Introduction, Background and State of the Problem with an extensive literature review, Materials and Methods, Results, Discussion, Conclusions, and Contributions.

The bibliography includes 361 literature sources, a substantial proportion of which are peer-reviewed journal articles published after 2020, reflecting both the topical relevance of the subject and a precise selection of the most recent and authoritative scientific sources. The doctoral candidate was provided with excellent instrumental and organizational conditions by the Medical University and the respective department for the successful completion of this work. All laboratory investigations were conducted at the Department of Medical Microbiology and Immunology “Prof. Dr. Elisei Yanev”, Medical University – Plovdiv, while the statistical analyses were performed at the Department of Social Medicine and Public Health, with the active participation of the doctoral candidate.

The research was funded by an internal university project (KOB-03/2021) and a national research project (KP-06-H53/1), which further underlines its institutional and national significance.

The topic of the dissertation is undoubtedly timely and fully aligned with the leading scientific and clinical challenges facing modern healthcare, arising from the COVID-19 pandemic, and is

of substantial theoretical and practical importance for contemporary medical science. The necessity for comprehensive investigation of B-cell immune memory as a critical component of long-term protective immunity following natural infection and mRNA vaccination is clearly formulated. At the time of the study's implementation, this topic represented one of the most intensively debated issues in the international scientific community and remains central today for understanding post-infectious and post-vaccination immune responses, assessing the durability of protection, and informing the need for adaptation of vaccination strategies and evaluation of collective immunity.

The dissertation is logically organized and consistently structured. The introductory section clearly outlines the context and significance of the research problem. The subsequent chapter, State of the Problem, is the most extensive (29 pages) and provides the necessary depth and scientific rigor. Fundamental aspects of B-lymphocyte development and functional differentiation are presented, with a detailed discussion of the organization of the humoral immune response and contemporary concepts of B-cell immune memory formation. The doctoral candidate demonstrates an ability to synthesize a broad body of information and to present it analytically, tracing international experience and scientific achievements in the field of COVID-19 immunology, including novel data on the dynamics of memory B cells and the impact of different vaccine platforms.

The Materials and Methods section is comprehensively described over 17 pages and represents one of the strengths of the dissertation. The methodological approach includes assessment of both humoral and cellular components of immunity through ELFA analysis of anti-RBD IgG antibodies, virus neutralization inhibition assays, B-ELISpot, and multicolor flow cytometry, allowing an in-depth investigation of immune responses. Particular attention should be paid to the use of S1 tetramers, which is methodologically challenging and rarely applied in Bulgarian scientific practice. The development and application of this technology testify to strong experimental training, competence in working with highly specialized equipment, and mastery of state-of-the-art immunological methods.

The flow cytometric panel includes a rich set of markers enabling detailed discrimination of B-cell subpopulations (naïve, transitional, IgD /IgM cells, class-switched memory B cells, non-class-switched memory B cells, plasmablasts, and plasma cells). The methods are described accurately, clearly, and with sufficient detail to ensure reproducibility.

The results are presented in a clear, systematic, and logical manner, supported by appropriate graphical and tabular materials. Data are provided on the dynamics of anti-RBD antibodies, B-cell activity assessed by ELISpot, and structural changes in the B-lymphocyte repertoire, including antigen-specific memory B cells identified using tetramers. This integrated approach allows for the delineation of a coherent and well-substantiated picture of the formation and persistence of B-cell immune memory following infection and immunization.

The interpretation of the data is accurate and consistent with contemporary scientific literature. Comparison with internationally published results is precise and demonstrates a strong understanding of the specific characteristics of different vaccine platforms and immune responses in convalescent individuals. The observed trends in B-cell immune memory provide novel data for the Bulgarian population and contribute to a more comprehensive understanding of long-term immunity to SARS-CoV-2.

The dissertation contains clearly defined original scientific contributions, fully corresponding to the level expected of a doctoral thesis, including:

- Development of a multicolor flow cytometry panel for B-cell heterogeneity, enabling simultaneous detailed analysis of multiple B-lymphocyte subpopulations (CD19<sup>+</sup> cells, memory B cells, CSMB, IgG<sup>+</sup> CSMB, NCSMB, transitional B cells, plasmablasts, and plasma cells). The panel is optimized through a clearly defined gating strategy, ensuring high reproducibility and sensitivity.
- First description in the Bulgarian population of S1-specific memory B cells using tetramers in flow cytometric analysis.
- Characterization of dynamic changes in various B-cell subpopulations in convalescent individuals and individuals vaccinated with two different mRNA platforms across four time points within a 12-month period following immunization/infection.
- Identification of quantitative and qualitative differences in B-cell immune memory between naturally acquired and vaccine-induced immunity, with distinct dynamic profiles for each study group.
- Demonstration of persistence of S1-specific memory B cells up to 12 months after vaccination or infection, serving as an indicator of long-term immune memory.

These findings have not only theoretical significance but also clear applicability in the fields of clinical immunology and public health.

## Conclusion

The presented dissertation is a completed, independent, and scientifically significant study, meeting all requirements for the award of the educational and scientific degree “Doctor (PhD)”, in accordance with the Act on the Development of Academic Staff in the Republic of Bulgaria and its implementing regulations at the Medical University – Plovdiv. The doctoral candidate demonstrates profound theoretical knowledge, excellent experimental training, and the ability to interpret complex immunological data.

In view of the above, I give my positive evaluation of the conducted research, the dissertation thesis, the abstract, the achieved results, and the scientific contributions, and I propose that the esteemed Scientific Jury award the educational and scientific degree “Doctor” to Dr. Martina Bozhkova in the doctoral programme in Immunology.

Prof. Dr. Todor Kantardzhiev, MD, DSc

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Заличено на основание  
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