

OPINION

**by Prof. Dr. Iliya Nikolov Iliev,
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Regarding: the doctoral dissertation titled "Investigation of B-cell Immune Memory in COVID-19 and Post-Vaccination Immunity" submitted for official defense by Asst. Prof. Dr. Martina Radoslavova Bozhkova from the Department of Medical Microbiology and Immunology "Prof. Dr. Elisei Yanev" at the Medical University – Plovdiv for obtaining the educational and scientific degree of "DOCTOR" in the professional field 7.1. Medicine; Doctoral Program in Immunology.

Received materials: Dissertation, draft of abstract, list of publications.

By Order No. P-7134/16.12.2025 of the Vice-Rector of the Medical University of Plovdiv, I have been appointed as a member of the scientific jury for the defense of a dissertation for the acquisition of the educational and scientific degree of "Doctor" by doctoral student Martina Radoslavova Bozhkova from the Department of Medical Microbiology and Immunology "Prof. Dr. Elisey Yanev" at the Medical University of Plovdiv for the acquisition of the educational and scientific degree "DOCTOR" in the professional field 7.1. Medicine; Doctoral program "Immunology."

The proposed dissertation is structured according to a standard Bulgarian model, written on 160 standard typed pages, and contains the following main chapters: Contents – 2 pages, List of Figures – 1 page, List of Tables – 1 page, Abbreviations Used – 2 pages, Introduction – 2 pages, State of the Problem – 28 pages, Aim and Objectives – 1 page, Materials and Methods – 16 pages, Results – 37 pages, Discussion – 19 pages, Conclusion – 2 pages, Summary – 1 page, Contributions – 1 page, References – 40 pages, List of Publications Related to the Dissertation – 1 page. The dissertation includes 29 figures and 7 tables for illustration. PhD candidate Martina Bozhkova has used a very large number of literary sources – 361, all in Latin script. The broad scope of the topic, which has significant social importance, necessitates the examination of a large number of sources and explains the cited literature.

The proposed dissertation combines a purely theoretical study and a discussion on the refutation over the past two decades of the two main paradigms of the definition of immune memory, namely its limitation to adaptive immunity and to vertebrate animals, along with the development of the understanding of protective immunity as a result of the presence of neutralizing antibodies, active effector cells, or other components of the immune system that provide immediate protection. On the other hand, the applied aspect of this theoretical framework developed in an unprecedented

way in the context of the COVID-19 pandemic, which led to the rapid clinical implementation of various vaccines against the backdrop of relatively widely accessible immunological analytical techniques, and allowed the acceleration of research on protective immunity and immune memory in recent years towards establishing a link between the post-translational modifications of protein molecules and a number of diseases. This is why the author quite reasonably builds her hypothesis, on the one hand, on the understanding of the immune response and the duration of immune memory in previous coronavirus epidemics as a crucial factor in the development of effective therapeutic strategies and vaccine prophylaxis against SARS-CoV-2. On the other hand, she relies on the thesis that understanding B-lymphocyte subpopulations – including naive, transitional, plasma, IgM⁺ and switched memory cells, as well as CD21^{low} and marginal zone B-lymphocytes – allows for more precise diagnosis of primary immunodeficiencies, assessment of vaccine response, monitoring of therapeutic effect, and identification of secondary immune dysfunctions.

The information in the literature review is very detailed, both in terms of the characterization of B-lymphocyte subpopulations and the immune response against viral infections based on the closely coordinated interaction between innate and adaptive immunity. The author also elaborates on the research methods used and their practical value.

As a result of the literature review, the dissertation author formulates the aim of the study, namely to assess the dynamics and duration of the humoral and memory B-cell immune response after recovery from infection and vaccination with mRNA vaccines against SARS-CoV-2, which is achieved by addressing five tasks.

The "Materials and Methods" section describes the main methods applied in carrying out the tasks set out in this study. During the development of this scientific work, As. Bozhkova has mastered a large number of both classical biochemical methods and modern immunological and immunoenzymatic methods, which is a basis to consider that she has acquired the necessary methodological experience in conducting a scientific experiment.

The results of the conducted studies are presented in the 'Results' section. In the first stage of her research, the doctoral candidate began with a description of the demographic characteristics of the study participants, which she appropriately divided into the four studied groups – vaccinated with BNT162b2 (n=157), with mRNA-1273 (n=18), recovered from COVID-19 (n=110), and healthy controls (HC, n=18). In the second chapter, the results of the study on the dynamics of the humoral immune response are presented, which was monitored by quantitatively measuring IgG antibodies targeting the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein. Dr. Bozhkova found that vaccination with both mRNA vaccines leads to a

robust humoral response with a high peak at T1, followed by a decrease in levels at T2 and T3. For the mRNA-1273 vaccine, a higher median and more sustained values at later time points were observed, whereas for the BNT162b2 vaccine there was a stronger decline and a larger proportion of participants with a waning response at 12 months. Individuals who have recovered from the disease show the widest individual variability, reflecting the nature of natural immunity. Individuals who have recovered from the disease show the widest individual variability, reflecting the nature of natural immunity. In addressing the second task of the dissertation, Dr. Bozhkova conducted an assessment of cellular immunity, specifically of antibody-producing B lymphocytes, which she considers essential for understanding long-term protection following vaccination or a past SARS-CoV-2 infection. A large amount of data from the conducted study is presented, which requires very careful examination to identify the corresponding correlation dependencies in the context of early diagnosis. Using the ELISpot method, the number of spot-forming units (SFU) recognizing the receptor-binding domain (RBD) of the virus was tracked. The doctoral candidate comments on her results, concluding that immunization with mRNA vaccines leads to a strong peak of B-cell memory, with greater amplitude in mRNA-1273. Regardless of whether they have been vaccinated using both vaccines or have recovered from the disease, individual heterogeneity is observed, which must be taken into account when evaluating the need for booster doses and the duration of immune protection. In carrying out the third task, Dr. Bozhkova evaluates the T-cell response using the T-SPOT assay, which measures the number of spot-forming units (SFU) in response to stimulation with peptides in two different panels – Panel A (S peptide) and Panel B (N peptide). In the experiments addressing the fourth task, the PhD student evaluates the relative distribution of B-cell subpopulations as a percentage of live lymphocytes in each studied group and time point, as well as an additional analysis of antigen-specific (S1) and class-switched memory cells using multiparameter flow cytometry. In carrying out the fifth task, Dr. Bozhkova obtained results supporting the thesis that the long-term memory response to SARS-CoV-2 is primarily mediated through the class-switched cells, while memory B lymphocytes without class switching (NCSMB) play a more limited role and are present as a small fraction in the overall pool of S1-specific B lymphocytes.

It is notable how thorough and extensive the discussion of the results is in comparison with those of other authors in search of a point of convergence in terms of reliability. The difficulty in such cases arises from the use of a large number of indicators as markers when looking for correlations in diseases that are epidemiologically widespread. The author formulates six main conclusions, which generally describe the results achieved in the experiments. I accept the contributions formulated by the doctoral candidate in principle, though I would suggest a clearer distinction of some of them from the conclusions. For example, in contribution 4, the scientific achievement is not clearly stated, and in contribution 5, more emphasis could be placed on the practical relevance of the contribution.

The proposed dissertation is written in a clear and informative scientific language and fully meets the objectives, tasks, and achieved results presented in it.

Data are provided regarding the publication of results in 3 scientific publications, two of which have an IF, thus fully meeting the minimum requirements for obtaining the academic title of "Doctor." One of the articles is published in a Q2 journal - Bozhkova M., Raycheva R., Petrov S., Dudova D., Kalfova T., Murdjeva M., Taskov H., Shivarov V. Humoral and Memory B Cell Responses Following SARS-CoV-2 Infection and mRNA Vaccination. *Vaccines* 2025, 13(8), 799; <https://doi.org/10.3390/vaccines13080799> Q2. Another article is in Q3 - Bozhkova M., Gardzheva P., Rangelova V., Taskov H., Murdjeva M. Cutting-edge assessment techniques for B cell immune memory: an overview. *Biotechnology & Biotechnological Equipment*. 2024;38(1):2345119, <https://doi.org/10.1080/13102818.2024.2345119>.

The PhD candidate has also submitted a draft of the abstract, which meets all requirements.

Conclusion:

My assessment of the presented doctoral dissertation by Asst. Prof. Dr. Martina Radoslavova Bozhkova, for obtaining the educational and scientific degree of "DOCTOR" in the professional field 7.1. Medicine, doctoral program in immunology, is entirely positive. I would like to once again highlight its merits – first and foremost, a scientifically grounded and precisely executed research study, with original research approaches, containing correctly interpreted and summarized results. The experimental work exceeds the required standard for this type of dissertation. The dissertation demonstrates pronounced scientific and scientific-applied contributions that could find application in clinical diagnostics of viral infections and in evaluating the effectiveness of vaccine administration.

The submitted dissertation fully meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) and its implementation regulations. The results achieved give me grounds to propose that an educational and scientific degree of "Doctor" be awarded to Assoc. Dr. Martina Radoslavova Bozhkova in the professional field 7.1. Medicine, doctoral program in Immunology.

23.02.2026

Plovdiv

Prepared by:

Заличено на основание
Чл.5 §1, 6, "В" Регламент (ЕС)2016/679

(Prof. Dr. Iliya Iliev)