

REVIEW

of the dissertation titled "TREATMENT OF A MURINE MODEL OF MYELOMA THROUGH
EPI TOPE-SPECIFIC TUMOR INHIBITION"

by Emilia Zakharia Stoyanova,
supervised by Professor Andrey Ivanov Chorbanov, PhD

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1. Professional Background

Emilia Stoyanova completed her secondary education in biology with English language instruction at a school offering a Natural Sciences and Mathematics profile. Since 2017, she has been an intern in the "Immunology" Department at the Laboratory of Experimental Immunology, Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences (BAS). In 2018, she obtained a Bachelor's degree in Biology from New Bulgarian University and later earned her Master's degree in Molecular Biology with a specialization in "Biochemistry" (Molecular Immunology track) from the Faculty of Biology, Sofia University "St. Kliment Ohridski." Her coursework and Master's thesis were graded with honors. Since 2021, she has been a doctoral student in the "Immunology" Department at the Laboratory of Experimental Immunology, BAS. From 2022, she has held the position of "Biologist" at the Clinical Laboratory of the Specialized University Hospital for Active Treatment in Oncology "Prof. Ivan Chernozemski."

Her professional trajectory demonstrates exceptional focus. The fields of molecular biology and immunology have consistently been her central areas of interest. There are no instances of aimless exploration in unrelated areas of biomedical science. Her conduct reflects that of a person who knows her interests, goals, and abilities.

2. Academic and Research Activity

Emilia Stoyanova has presented two publications indexed in journals with an impact factor of 5, where she is the first author. She has also delivered six presentations on the dissertation's topic, four in Bulgaria and two internationally.

Regarding educational activity, the candidate has participated in two specialized courses at the Institute of Experimental Morphology, Pathology, and Anthropology with Museum, BAS, which are directly relevant to the dissertation topic:

- "Theoretical and Practical Foundations of Classical and Modern Histological Methods"
- "Introduction to Cellular and Tissue Engineering"

A report from the Stefan Angelov Institute of Microbiology, BAS, indicates that Emilia Stoyanova has earned 204 credits from her doctoral research work and 170 credits from her educational activity. The minimum requirements of the Academic Staff Development Act and additional requirements of the Institute of Microbiology have been met.

3. Dissertation

The dissertation is 129 pages long and cites 134 literature sources. It is illustrated with 52 high-quality figures. The structure follows the classical format: Introduction, Literature Review, Aims and Objectives, Materials and Methods, Results, Discussion and Conclusion, and Findings.

The dissertation topic is highly relevant, addressing a form of immunotherapy applied to an experimental model concerning a disease of significant individual and social importance.

The literature review is intelligently and analytically written, offering a comprehensive examination of the issue. The reader is logically guided through discussions on malignant diseases, particularly malignant melanoma, the complex and contradictory interactions between the immune system and tumors, culminating in the specificities of the dissertation.

The focus on carbohydrate antigens as vaccines and the role of hemocyanins as adjuvants is well-presented. Combined with an explanation of murine experimental models, the framework is skillfully assembled. The review is masterfully written, engaging yet devoid of unnecessary detail or oversimplification. Nevertheless, I have three remarks:

- The description of T-helper subpopulations overlooks Th17 and Tregs, which play critical roles in the interaction between malignant tumors and the immune system.
- Galen was not a Greek but a Roman physician.

- The term "African American" is ideologically accepted but inaccurate when referring to racial comparisons.

The aims and objectives are clearly and succinctly formulated.

The "Materials and Methods" section thoroughly describes the rich methodological arsenal employed by the candidate. The conceptualization of two therapeutic approaches—one using two types of hemocyanins relying on their adjuvant effect and another using hemocyanins conjugated to the carbohydrate epitope GD2P4 as a constructed vaccine—is carefully detailed. The section describes everything from the isolation of hemocyanins and their conjugation with GD2P4 to the immunological methods used to demonstrate the effect. Experimental animals and the melanoma line are also clearly and comprehensively described. The "Results" section is another strong aspect of the dissertation. Through different time intervals, the effects of the standalone use of hemocyanins and the constructed vaccine of hemocyanins with GD2P4 have been investigated, yielding extensive data on tumor growth, survival rates of experimental animals, humoral and cytotoxic T-cell anti-tumor immune responses, macrophage polarization, cellular infiltration, cytokine secretion, histological findings, and more. The sheer volume of work is commendable, as is the meticulous analysis of the immune anti-tumor response, despite a more minimalistic approach being acceptable. My main critique, however, lies here: while the humoral and cytotoxic T-cell responses against the melanoma line were tested and found to be present, they were not tested against GD2P4. The responses were observed even with standalone hemocyanins. Thus, it cannot be definitively claimed that the immune response is epitope-specific.

The discussion is intelligently written, analyzing the obtained results. However, I have two remarks:

- Insufficient comparisons are made with results from other research teams on similar topics.
- The aforementioned limitation—lack of evidence for an epitope-specific response against GD2P4—should have been addressed and interpreted here. There are plausible scientific and technical explanations for these limitations, and discussing them within the context of the dissertation would have enriched the discussion.

The conclusions are well-formulated, clear, and precise, reflecting the results obtained, although they seem slightly more definitive than warranted by the data.

Conclusion

In summary, I find this dissertation to be exceptionally well-planned, executed, and described. It reflects both the candidate's high level of biological expertise and her personal intelligence directed toward addressing a significant scientific problem. While not flawless, my overall impression is of a valuable and in-depth theoretical and experimental analysis of an important research topic.

Based on the above, I recommend that the jury award Emilia Zakharia Stoyanova the academic and educational degree of "Doctor," a decision I also support.

Prof. Dr. Dobroslav Kyurkchiev, PhD, DSc**

27.12.2024

A handwritten signature in blue ink, consisting of a series of fluid, connected strokes that form a stylized representation of the name Dobroslav Kyurkchiev.