OPINION

by Corr. Member. Prof. Soren B. Hayrabedyan, MD, PhD, DSc

Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", BAS

of a dissertation for awarding the educational and scientific degree "*Doctor*"

Professional field "*4.3. Biological Sciences*", from the District Higher Education "*4. Natural Sciences, Mathematics and Informatics*"

PhD program in accredited scientific specialty "*Immunology*"

PhD student: *Emilia Zahariya Stoyanova*

Form of doctoral studies: Full-time

Laboratory: *Department of Immunology, Laboratory of Experimental Immunology*,

Institution: *Institute of Microbiology "Stefan Angelov" – BAS*

Topic: "*A novel approach for treating a mouse model of melanoma via an epitope-specific tumour inhibition*"

Supervisor: *Prof. Dr. Andrey Ivanov Chorbanov, PhD*

1. General Overview of the Procedure and the Candidate

The submitted materials by Emilia Stoyanova fully comply with the requirements of the Bulgarian Academic Staff Development Act (LDASRB), its Implementation Regulations, and the rules of the Bulgarian Academy of Sciences (BAS) and IMicB-BAS. The dissertation includes a detailed thesis, an abstract, and two articles published in peer-reviewed high-impact journals (rank Q1). The doctoral candidate is actively engaged in the scientific community, with participation in six forums (three international) and involvement in a funded research project. Emilia Stoyanova is emerging as a promising young researcher in the field of oncoimmunology, possessing extensive scientific preparation. She holds a master's degree in molecular biology from Sofia University "St. Kliment Ohridski" and has gained international research experience in laboratories in Germany and the Netherlands, where she deepened her expertise in antitumor immunotherapy.

2. Relevance of the Topic

The dissertation focuses on one of the most current aspects of oncoimmunology—immunotherapeutic approaches for aggressive cancers, specifically melanoma. The therapeutic application of epitope-specific tumour inhibition via conjugated hemocyanin vaccines is an innovative strategy that aligns with the global goal of developing more effective and less invasive methods for treating malignant diseases.

In the context of the increasing incidence of melanoma and its challenging prognosis, this approach holds significant scientific and practical relevance.

3. Understanding of the Problem

Doctoral candidate Stoyanova demonstrates a deep analysis of contemporary scientific literature and issues, reflected in an extensive literature review (spanning 35 pages) with numerous figures and examples visualizing key aspects of tumour biology, immunological mechanisms in malignant tumours, and the advantages of immunotherapeutic approaches. Particularly impressive is the discussion of tumour-associated carbohydrate antigens (TACAs) and their role in immunosuppressive mechanisms, contributing to a broadened understanding of melanoma pathogenesis and opportunities for intervention in the tumour microenvironment. The detailed review includes numerous publications from the past five years, confirming that the dissertation incorporates the latest trends in oncoimmunology. The dissertation spans 129 pages, illustrated with 52 figures (12 in the literature review) and eight tables. The results are systematically described over 45 pages, with the discussion covering 15 pages, enabling a comprehensive commentary and interpretation of the obtained data.

4. Research Methodology

The dissertation methodology is modern and well-structured. The experimental platform is built on classical and advanced methods in oncoimmunology. A mouse melanoma model (generated using the B16F10 cell line) was employed, and three vaccination strategies were investigated, based on conjugated hemocyanin vaccines (RtH and HaH) with the GD3P4 mimotope peptide. Each strategy is characterized by specific features:

- **First regimen**: Early vaccination for rapid activation of M1 macrophages and moderate tumour growth control.
- **Second regimen**: Combines prolonged immunostimulation and enhanced cytotoxicity, associated with increased IFN-γ and IL-12 secretion, leading to maximum survival extension.
- **Third scheme**: Delayed application strategy aimed at long-term protection, albeit with less pronounced effects on tumour control.

The methods for evaluating efficacy include ELISA for profiling pro-inflammatory cytokines, immunophenotyping for analysing key cell populations (cytotoxic T lymphocytes, M1 macrophages, regulatory T cells), histological analyses of the tumour microenvironment, and ELISpot for precise cytokine activity assessment at the cellular level. The overall experimental design is relevant and meets the requirements for systematically investigating the antitumor immune response.

5. Main scientific contributions

1. **Development of novel hemocyanin vaccines with antitumor effects in a murine melanoma model:**

Innovative conjugates of hemocyanins (RtH and HaH) with the GD3P4 mimotope peptide were developed and tested in three different schemes, each modulating the immune response differently (survival and immune response). The second scheme, combining vaccination with prolonged immunostimulation, led to a significant increase in CTL and pro-inflammatory cytokines, demonstrating the best results in tumour growth suppression, restoration of immune surveillance, and prolonged survival. This scheme achieves an optimal balance between activating key cell populations and ensuring long-term protection against tumour development.

2. **Identification of cytotoxic and humoral components in the antitumor response:**

The applied vaccines induced specific antibody formation and stimulated cytotoxic T lymphocytes (CD8+) and IFN-γ production, leading to effective melanoma progression limitation. The most pronounced benefits were observed with the second vaccination scheme.

3. **Monitoring cellular and cytokine dynamics:**

A significant increase in M1 macrophages and pro-inflammatory cytokines (IFN-γ, IL-12, TNF-α) was observed, particularly with combined and prolonged immunostimulation. These results clearly demonstrate the potential of the developed vaccines to restore immune surveillance and modulate the tumour microenvironment.

4. **Optimization of the vaccination scheme:**

Among the three investigated strategies, the most effective is the one with prolonged immunostimulation (the second scheme), which leads to the highest levels of cytotoxicity and extended survival. This finding provides a concrete direction for the future clinical application of such vaccines.

5. Evaluation of Publications and Personal Contribution of the Doctoral Candidate

Emilia Stoyanova is the first author of two high-quality scientific publications (impact factors 5.4 and 4.8), validating the international prestige of the presented results. Her independent role in designing and conducting experimental studies is clearly reflected, and her ability to publish in leading journals demonstrates a high level of competence and academic maturity.

7. Abstract

The abstract (71 pages, 52 figures, and 134 bibliographic references) accurately represents the structure of the dissertation, its objectives, and the obtained results. The doctoral candidate successfully summarizes the scientific contribution and practical significance of the developed methodology, aligning with the standards of academic publishing.

CONCLUSION

The dissertation contains ***scientific and applied results that represent an original contribution to science*** and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the Academic Staff Development Act and the Regulations of the Institute of Microbiology "Stefan Angelov" – BAS. The presented materials and dissertation results fully comply with the specific requirements adopted in connection with the Regulations of the Bulgarian Academy of Sciences and the Institute of Microbiology and the Bulgarian Academy of Sciences for the application of the LDASRB.

The dissertation demonstrates that Emilia Stoyanova possesses profound theoretical knowledge and professional skills in the scientific specialty "*Immunology*" as a full-time doctoral student, showing qualities and abilities for conducting independent scientific research.

In view of the above, I confidently give my **positive assessment of** the research carried out, presented by the above-reviewed dissertation, abstract, results achieved and contributions, and **I propose** to the honourable scientific jury to award the educational and scientific degree "*Doctor*" to *Emilia Zahariya Stoyanova* in the doctoral program in the scientific specialty "*Immunology*", in professional field 4.3. Biological Sciences, from the field of higher education 4. Natural Sciences, Mathematics and Computer Science.

09.01.2025 **Prepared by**:

Corr. Member Prof. Soren Hayrabedyan, DSc