

REVIEW

by Prof. Radostina Ivaylova Alexandrova, PhD (Department "Pathology", Institute of Experimental Morphology, Pathology and Anthropology with Museum - BAS) - member of the Scientific Jury according to Order No. I - 168/ 29.11.2024 of the Director of the Institute of Microbiology "Stefan Angelov" - Bulgarian Academy of Sciences (IMic - BAS)

Regarding the dissertation on the topic: "A new approach for the treatment of a mouse model of melanoma through epitope-specific tumor inhibition"

for awarding the educational and scientific degree "DOCTOR" in scientific specialty "Immunology", code 01.06.23, professional field "Biological Sciences" 4.3., field of higher education 4. "Natural Sciences, Mathematics and Informatics";

to PhD student Emilia Zaharia Stoyanova, IMic - BAS, Sofia, Bulgaria

with scientific supervisor: Prof. Andrey Chorbanov, MSc, PhD, Institute of Microbiology - BAS, Sofia, Bulgaria

1. Relevance and significance of the dissertation work

The relevance and significance of the presented dissertation work are beyond doubt. They arise from at least two circumstances: 1) The importance of cancer, and in particular malignant melanoma, as one of the greatest health and social challenges of our time; 2) The recognition of immunotherapy as one of the most promising approaches in oncology and the need for its development and improvement.

The fact that in our country there are trained specialists who successfully work in this field is a source of pride for Bulgarian science and places it on the map of the world's scientific elite.

The topic is in line with the objectives of the National Strategy for the Development of Scientific Research in the Republic of Bulgaria 2017-2030, as well as with the priorities of the EC's Horizon Europe program, as it contributes to improving health and increasing the quality of life, helping to solve global challenges, such as cancer diseases, and creating a prerequisite for achieving top scientific achievements.

2. Overview of the dissertation work

The dissertation is written on 119 pages and includes: Title page, Table of Contents (3 pages), List of abbreviations (3 pages), Introduction (1 page), Literature review (43 pages), Goals and objectives (1 page), Materials and methods (17 pages), Results (35 pages), Discussion (10 pages), Conclusions (1 page), List of publications and participation in scientific forums in the field of the dissertation (1 page), References (13 pages). It is illustrated with 52 high-quality figures.

The **Introduction** directly introduces the reader to the essence of the problem; the reasons why the doctoral student and the scientific supervisor have focused on hemocyanins isolated from gastropods; the results obtained in the preparation of the dissertation.

The **Literature review** can be divided into five parts:

The first part briefly describes the biology and behavior of tumor cells and the characteristics of cancer, in particular skin tumors and especially melanoma.

The second part is dedicated to tumor immunology and examines the role of the innate and acquired immune response.

The third part reviews the therapeutic approaches to melanoma, with attention paid to antitumor vaccines.

The fourth part contains information on natural products as antitumor agents, with special attention paid to the antitumor effect of hemocyanins.

The last part introduces readers to the mouse models used in experimental practice; the possibilities they offer, their strengths and the limitations that we must take into account.

The literature review demonstrates the excellent theoretical preparation of the doctoral student, her ability to work with literary sources. It is of interest to all who work in the field of experimental oncology and tumor immunology.

This section is illustrated with 17 figures.

Goals and objectives. The goal of the dissertation and the 4 tasks envisaged for its achievement are clearly and precisely formulated.

A wide range of suitable modern **materials and methods** have been selected for their implementation. They are described in sufficient detail and may be useful to anyone working in this field. They are illustrated with 8 figures.

Results. The course of the experimental work strictly follows the set goals and objectives. In the first part of the study, the effectiveness of the applied experimental therapy with hemocyanins RtH or HaH was investigated. It was found that with different immunization schemes it leads to suppression of tumor growth and prolongation of the life span of the treated laboratory animals; causes the appearance of high levels of IgM specific antibodies, formation of immune cell infiltrates in the tumors; synthesis of proinflammatory cytokines; appearance of tumor-specific cytotoxic T lymphocytes (CTL) and high levels of tumor-infiltrated M1 macrophages.

The second group of experiments monitored the consequences of the application of protein-engineered vaccines RtH-GD3P4 or HaH-GD3P4. It was found that all three immunization schemes reduce the incidence of tumors, suppress their growth and prolong the survival of the treated animals; infiltration of immunocompetent cells into the tumors, formation of tumor-specific CTL in the spleen, high levels of specific anti-B16F10 IgM antibodies in the sera of the experimental groups and significantly higher levels of tumor-infiltrated M1 macrophages were observed, compared to untreated control animals.

A huge amount of work has been done, the results are convincing and definitely deserve attention. The section is illustrated with 27 excellently crafted figures.

The "**Discussion**" section is also excellently prepared, which "makes sense" of the results obtained in the light of the currently available knowledge and outlines their significance and originality. The discussion is perhaps the most difficult part of writing a dissertation and is a kind of test of the scientific maturity of the doctoral student and the skills of the supervisor to teach - in this case, both of them coped successfully.

Two groups of **conclusions** have been made based on the data obtained from the applied experimental therapy with hemocyanins RtH or HaH and the therapy with the protein-engineered vaccines RtH-GD3P4 or HaH-GD3P4, which I fully accept.

The dissertation does not include **contributions**. However, the original results obtained are in an extremely rapidly developing and innovative field, and each of them is a contribution - starting from the experimental model introduced and moving on to the information revealing the potential possibilities for applying the hemocyanins tested and the vaccines developed as immunotherapeutic agents in malignant melanoma.

The list of **cited literature** includes 134 titles in Latin, 67% of which (90 publications) have been published since 2015.

3. Assessment of the correspondence between the Abstract of the PhD thesis and the Dissertation

The Abstract is prepared in full accordance with the dissertation and presents in a shortened version the essence of the study, the sequence of experimental work, the results obtained and the conclusions drawn on their basis.

4. Publications and participation in scientific forums of the doctoral student in the topic of the PhD thesis

Two articles on the topic of the dissertation have been published (in 2022 and 2024) in the authoritative international journal "Marine Drugs" (impact factor 5.4, Q1– 2022; impact factor 4.9, Q1, 2024). The new knowledge obtained and original results have been presented at 7 scientific forums in our country and abroad (Serbia, France).

5. Critical notes and comments, recommendations, questions to the PhD student

I have no substantive objections, but I noticed some technical omissions – here are some of them:

It would be easier for the reader to navigate if the terms included in the List of Abbreviations were arranged in alphabetical order.

Perhaps the place for information about where the dissertation was prepared and the source of funding is not on the title page.

The expression “infection” is not appropriate when it comes to inoculating tumor cells.

I noticed some minor technical errors (e.g., missing commas).

The noted omissions in no way diminish the quality of the dissertation.

6. Autobiographical data and personal impressions of the PhD student

Emilia Stoyanova graduated from the natural sciences and mathematics class "Biology with English" at 29 "Kuzman Shapkarev" Secondary School in Sofia. In 2018, she obtained a "Bachelor's" degree in "Biology - General and Applied", and in 2020 she became a "Master's" in "Biochemistry" at the Faculty of Biology of Sofia University "St. Kl. Ohridski". While still a student, she began training as an intern in the Laboratory of Experimental Immunology at the "Stefan Angelov" Institute of Microbiology - Bulgarian Academy of Sciences, where, after successfully after successfully passing a competitive exam, she was enrolled as a PhD student

on 01.01.2021. Since November 2022, she has been working as a laboratory assistant in the "Clinical Laboratory" department of the University Specialized Hospital for Active Treatment of Oncology "Ivan Chernozemski" in Sofia.

I know Emilia Stoyanova as one of my students in the "Pathobiochemistry" and "Tumor Immunology" courses at the Faculty of Biology of Sofia University "St. Kliment Ohridski". She impressed me with her high motivation to learn new knowledge, her responsibility, fresh ideas and willingness to present and defend her point of view in a well-founded manner.

7. Conclusion

The presented dissertation is dedicated to an important and significant topic, the goals and tasks set in it have been fully achieved, at a high scientific level, and the original results obtained are an undeniable contribution to the field of immunology, tumor biology and experimental oncology.

During the preparation, doctoral student Emilia Styanova has improved her biomedical knowledge, mastered a variety of modern and classical immunological, immunochemical, biochemical, cytotoxicity, histological methods and techniques, work with cell cultures and laboratory animals, has walked the path from a promising student and assistant to an independently thinking scientist, capable of meeting the challenges of research in the 21st century. She has actively participated in the planning and successful implementation of an ambitious research project, which with its original ideas and professional implementation meets the established European and world standards. And most importantly: the results obtained during the implementation of the dissertation work open the door to new possibilities for (immuno)therapy of one of the most aggressive tumor diseases - malignant melanoma. The work done by the doctoral student and the scientific supervisor and the final product obtained deserve high marks.

All this gives me reason to believe that the presented dissertation work fully complies with the Law on the Development of the Academic Staff in the Republic of Bulgaria, as well as the regulations for its application at the Institute of Microbiology "Stefan Angelov" - Bulgarian Academy of Sciences, for the awarding of the educational and scientific degree "Doctor". I confidently give my positive assessment and recommend to the members of the Honorable Scientific Jury to award Emilia Zaharia Stoyanova the educational and scientific degree "Doctor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics; professional field 4.3 Biological Sciences (Immunology).

10.01.2025 г.

.....
/Prof. R. Alexandrova/