OPINION

by Assoc. Prof. Dr. Nikolina Mihaylova Mihaylova, Laboratory of Immunohistochemistry and Immunopathology, Department of Immunology, Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences

Elected as a member of the scientific jury by Order No. I - 15/23.01.2025 of the Director of the Institute of Microbiology of the PhD dissertation for the award of the educational and scientific degree **"Doctor"**

In the field of higher education **4. Natural Sciences, Mathematics and Informatics**

Professional direction **4.3. Biological sciences**

Scientific Specialty **"Immunology"**

Author: Nikola Ralchev Ralchev

Topic: Suppression of antigen-specific B lymphocytes through protein engineering molecules in hypersensitivity reactions

Research supervisor: Prof. Andrey Tchorbanov, PhD

1. General Description of the Submitted Materials

The author of the dissertation is Nikola Ralchev Ralchev, a full-time doctoral student at the Department of Immunology, Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences (BAS), under the scientific supervision of Prof. Dr. Andrey Tchorbanov. The submitted set of materials, provided in both paper and electronic formats, complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), its implementing regulations, and the BAS regulations for its application. It meets the criteria set forth in the Regulations on the Conditions and Procedures for Acquiring Academic Degrees and Holding Academic Positions at the Stephan Angeloff Institute of Microbiology, BAS, for obtaining the educational and scientific degree "Doctor."

The doctoral candidate has submitted two publications related to the dissertation topic, along with supporting evidence and certificates of participation in scientific conferences. *His total scientific output includes 15 publications in international journals, cited 58 times, with an h-index of 3, according to Scopus data.*

2. Relevance of the Topic

The dissertation topic is highly relevant due to the increasing prevalence of allergic diseases both globally and in Bulgaria. Allergies affect a growing proportion of the population, particularly in industrialized countries, which is linked to pollution, lifestyle changes, and the hygiene hypothesis.

According to the World Health Organization, between 30-40% of the global population suffers from allergies, with the incidence of these conditions having doubled over the past two decades. In Bulgaria, an epidemiological study showed that the prevalence of bronchial asthma was 4.7% among individuals aged 20 to 44 years and 9.1% among those under 20 years old. The average prevalence of allergic rhinitis in the same study was 18.1%. The situation is particularly concerning among children. Allergic diseases affect between 30% and 35% of children, with their frequency continuing to rise in recent years. These statistics highlight the urgent need for enhanced prevention measures, early diagnosis, and effective treatment of allergic diseases in Bulgaria.

Moreover, increasing research indicates a link between allergies and autoimmune diseases, asthma, eczema, and other inflammatory conditions. Scientific advancements are shedding light on the genetic and epigenetic factors that determine susceptibility to allergies. Additionally, climate change, leading to longer pollen seasons and increased pollen concentrations, is contributing to the rise in seasonal allergies.

The development of new diagnostic methods (such as molecular allergy diagnostics) and innovative therapies—including biological drugs, immunotherapy, and monoclonal antibodies—is of critical importance both scientifically and for public health. This dissertation represents a highly relevant and valuable effort in the search for innovative therapeutic approaches.

3. Characteristics and Evaluation of the Dissertation

The dissertation follows the structure required by the Regulations for the Implementation of the Law on the Development of the Academic Staff at the Stephan Angeloff Institute of Microbiology, BAS. It consists of 105 pages, 29 figures, and a bibliographic reference list comprising 211 sources. The structure includes:

- ✓ **Title**
- ✓ **Table of Contents**
- ✓ **List of Abbreviations**

- ✓ **Introduction** – 1 page, concisely **presenting the main problem of the dissertation**.
- ✓ **Literature Review** – A 29-page section containing 9 illustrative figures and 2 tables.

The literature review demonstrates Nikola Ralchev's in-depth knowledge of current research on the topic. The dissertation elegantly introduces hypersensitivity reactions, transitioning from their etiology to the mechanisms of allergic responses. A separate subsection is dedicated to house dust mite (HDM) allergy, the central focus of the dissertation. The biological activity and functional characteristics of HDM allergens are thoroughly analyzed, specifically their interaction with TLRs, C-type lectin receptors, and NOD-like receptors. Modern therapeutic approaches for allergies are critically assessed, highlighting both advantages and limitations. The importance of experimental models for studying allergic diseases and developing new therapeutic strategies is emphasized. A separate subsection presents the previous research of the scientific team, showcasing continuity and systematic work on this topic.

- ✓ **Objectives and Tasks** – The dissertation's goal is clearly defined, supported by four logically connected tasks:

1. Construction and characterization of a murine Dp52-71 chimera.
2. Development of experimental models of HDM allergy:
   - Humanized Rag2-γc- model
   - BALB/c mouse model of chronic allergic inflammation.
3. Application of chimeric molecules in these models and analysis of their effect on allergen-specific antibodies.
4. Evaluation of the therapeutic effect of the chimeras on lung damage and immune response in both experimental models.

- ✓ **Materials and Methods** – 15 pages, 2 figures, describing a diverse set of molecular, immunological, and histological techniques. The dissertation employs two complex experimental models: a humanized mouse model and a chronic murine model of HDM allergy—both challenging but highly informative. Methods are categorized based on experimental models, making the experimental approach easy to follow. The applied methods allow for detailed characterization of both cellular and humoral immune responses, enabling the mechanistic understanding of the therapeutic effects.
- ✓ **Results** – The most extensive section, divided into two main subsections, reflecting the study's objectives. The dissertation presents 16 complex figures of high quality, illustrating the

significant volume of experimental work. In the humanized model, a reduction in allergen-specific IgE levels, as well as changes in total protein and β-hexosaminidase levels in bronchoalveolar lavage fluid, was observed. Human lymphocyte infiltration in mouse lungs was analyzed, revealing a decreasing trend in the therapeutic group. The overall lung inflammation was reduced in Dp52-71 chimera-treated animals. The second major results section describes the conjugation and characterization of chimeric molecules and their therapeutic effects in the chronic HDM allergy model. Notably, an increase in FcγRIIb expression on IgE-positive B cells was detected—an important finding for potential therapeutic applications.

- ✓ **Discussion** – 10 pages, providing a critical analysis of the results in the context of existing literature. The dissertation acknowledges the limitations of the experimental models and suggests possible solutions. New findings, such as FcγRIIb overexpression and correlations between anti-HDM IgG1 levels and allergic inflammation, offer new research directions.
- ✓ **Conclusions** – 1 page, clearly summarizing the study's key findings.
- ✓ **Contributions** – 1 page, outlining both fundamental and applied scientific contributions, which are well-justified.

Contributions with fundamental scientific significance

• It is shown for the first time that FcγRIIb is overexpressed on the surface of B lymphocytes, including IgE-positive B cells, isolated from the lungs of mice stimulated with house dust mite allergens.

• A correlation between serum levels of anti-HDM IgG1 antibodies and certain parameters of allergic inflammation has been discovered, which may help clarify the role of IgG1 antibodies in the pathogenesis of mouse models of HDM allergy.

Contributions with applied scientific significance

• Experimental humanized and chronic mouse models of house dust mite allergy have been developed, which can be used in studies related to the mechanisms of the disease, as well as new therapeutic approaches.

• The protein-engineered chimera technology used, aimed at selectively eliminating allergen-specific B cells, has the potential to be applied in the development of new approaches for targeted therapy.

- ✓ **Publications and Participation in Scientific Forums** – 1 page; two publications in indexed and reputable scientific journals (Q1 and Q2); 9 presentations at scientific forums on the dissertation topic – 7 oral presentations and 2 posters.
- ✓ **References** – 14 pages, 211 sources cited.

4. Evaluation of Publications and the Doctoral Candidate's Personal Contribution

Two articles related to the dissertation topic have been published in peer-reviewed and reputable international journals – *Scandinavian Journal of Immunology* (Q2) and *International Journal of Molecular Sciences* (Q1), with a total impact factor of 8.06.

During his doctoral studies, Nikola Ralchev has made numerous presentations at major international scientific forums. Notably, at two European immunology congresses, he was selected to give oral presentations. This alone is indicative of the high quality of the research, and the fact that Nikola, as a doctoral candidate, was approved for an oral presentation is an achievement that few can claim. It underscores both the importance and relevance of the dissertation's topic.

Personal Contribution of the Doctoral Candidate – The two publications in which Nikola Ralchev is the first author serve as proof of his direct involvement in the research. Additionally, I have personal observations of his work over the years. Throughout the entire dissertation period, he demonstrated dedication, initiative, and perseverance in overcoming the challenges that arose, which were by no means insignificant. I believe that this period and its difficulties have shaped Nikola Ralchev into a promising young scientist capable of tackling any scientific challenges that come his way. Based on this, I unequivocally acknowledge his personal contribution to the conducted research.

5. Critical Remarks, Recommendations, and Questions

I have no critical remarks regarding the conducted research.

6. Abstract

The abstract of Nikola Ralchev's dissertation reflects the main achieved results and meets all the standard requirements set forth in the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria (PPLDASRB).

CONCLUSION

The dissertation contains **scientific and applied scientific results that represent an original contribution to science and comply** with 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 LDASRB, and the Regulations for the Implementation of LDASRB at the Bulgarian Academy of Sciences (BAS). The presented dissertation findings **fully meet** the specific requirements of the regulations of the Institute of Microbiology – BAS for the application of LDASRB.

The dissertation demonstrates that Nikola Ralchev **possesses** profound theoretical knowledge and practical skills, showcasing his abilities for conducting independent scientific research.

Based on the above, I confidently give my **positive evaluation of the conducted research and recommend that the esteemed jury award Nikola Ralchev the educational and scientific degree of "Doctor" in the field of 4.3. Biological Sciences**.

February, 24 2025 Reviewer:.....

[Assoc. Prof. Dr. Nikolinka Mihailova]