

OPINION

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Regarding a dissertation submitted to a scientific jury, approved by order No. 196/30.06.2025 of the Director of the National Center for Infectious and Parasitic Diseases, for the acquisition of the educational and scientific degree "DOCTOR" in the professional field 4.3. "Biological Sciences", doctoral program "Virology"

Dissertation title: Molecular-virological analysis of the transmission clusters of introduced and prevalent HIV-1 subtypes in Bulgaria.

PhD student: Lyubomira Svilenova Grigorova

Scientific supervisor: Assoc. Prof. Dr. Ivaylo Aleksiev Ivanov

DECLARATION OF IMPARTIALITY

In compliance with the requirements formulated in Art. 4, para. 5 of the Act on the Development of Academic Staff in the Republic of Bulgaria, I certify that there are no joint scientific publications, scientific projects or other forms of academic cooperation between me and doctoral student Lyubomira Svilenova Grigorova that could give rise to a conflict of interest.

After having thoroughly reviewed the documentation submitted by the doctoral student, I conclude that all necessary elements fully comply with the current regulatory acts and procedural requirements for awarding the educational and scientific degree of "doctor". This gives me full reason to proceed with the preparation of this opinion.

1. Scientific validity and applicability of the research topic

HIV-1 infection remains one of the most serious and complex global threats to public health, despite the significant progress made in the development of antiretroviral therapies. The special ability of the virus to mutate, form recombinant forms and adapt to different population and geographical conditions poses a number of new challenges to researchers. In this sense, the topic of the dissertation work - **"Molecular-virological analysis of the transmission clusters of introduced and widespread HIV-1 subtypes in Bulgaria"** is extremely topical, scientifically significant and socially relevant. The topic is broad and brings together different

aspects of fundamental science, clinical virology and epidemiological practice. An advantage of the study is that it covers both the different geographical regions of the country and the main risk populations. An important aspect of the dissertation work is the comparison between the sequences of the Bulgarian virus isolates and international reference sequences, which contributes to the higher resolution of the phylogenetic classification and allows for a more accurate interpretation of the evolutionary processes and events that led to the emergence of new subtypes and recombinant forms in the country.

2. Purpose and objectives

The goal formulated in the dissertation is clearly defined and scientifically justified, and logically consistent with the hypothesis and structure of the study. The tasks are set in their logical sequence for the implementation of the goal and reflect the key stages of the research process.

3. Materials and methods

The “Materials and Methods” section is distinguished by scientific accuracy and systematicity. It not only fulfills its function of presenting the methodological tools used, which demonstrate a high level of competence and technical training, but also offers rich and precise information about the experimental setups, analytical approaches and work protocols. The three main groups of plasma samples used in the study are described in detail, covering a large number of genomic sequences, divided by time period and viral subtype. Special attention deserves the precise description of the optimization steps, which testifies to a high experimental culture.

The study was carried out within the framework of the National Reference HIV Confirmation Laboratory at the National Center for HIV Research and Development, and approval was obtained from the Ethics Committee. Funding through project BG05M2OP001-1.002-0001 under the OP “Science and Education for Smart Growth 2014–2020” further confirms the scientific value and significance of the study.

4. Results and interpretation

The results of the dissertation are presented clearly, analytically and logically arranged in three thematic sections, corresponding to the analyzed groups of samples. Each section ends with an interpretation of the identified transmission clusters, which helps to build a comprehensive scientific picture.

The data obtained provide important information about the most likely routes of HIV-1 introduction into Bulgaria, the dynamics of spread, regional specificity and vulnerable population groups. The analysis of samples from therapeutically naive patients is particularly valuable, which enriches the picture of the contemporary epidemiological situation in the country.

The phylogenetic methods used, including the construction of multiple evolutionary trees and detailed reconstruction of transmission networks, provide a complex and reliable molecular-epidemiological map of circulating HIV-1 variants. The analysis of subtype B is performed with particular precision and shows excellent mastery of bioinformatics tools and interpretation techniques.

5. Scientific conclusions and contributions

The doctoral student formulated six scientifically based conclusions and eight original contributions, all of which are directly supported by empirical results. The contributions are of high value both in scientific-theoretical and applied aspects – from enriching the data on the molecular characterization of HIV-1 in Bulgaria to opportunities for refining epidemiological surveillance and targeted interventions in public health.

6. Publication activity and scientific communication

In connection with the development of the dissertation, three scientific papers have been published in peer-reviewed scientific journals, with a total impact factor of 10.866, two Q1 and one in Q4. This fully meets the criteria for publication activity in accordance with the requirements for obtaining the ESD "Doctor".

Results from the experimental part of the dissertation have been presented in 18 national and 2 international scientific forums, which testifies to the active engagement with the scientific community and increased interest in the topic at the international level.

- Note

Certain terms and formulations in the "Literature Review" section are not sufficiently correct from a virological and molecular biological point of view.

7. Final assessment

The dissertation work of Lyubomira Grigorova is distinguished by a high scientific standard, methodological consistency and significant innovation. It represents a comprehensive,

complete and original study with a distinct applied value in the field of public health, epidemiology and molecular virology.

I note the great fruitful scientific support and methodological assistance provided by the scientific supervisor, Assoc. Prof. Dr. Ivaylo Aleksiev Ivanov, PhD, whose experience and expertise have undoubtedly contributed to the high quality of the study.

In view of all the above, I express my **positive opinion** and **propose that Lyubomira Svilenova Grigorova be awarded the educational and scientific degree "Doctor" in the scientific specialty "Virusology"**.

Author of the opinion:

/Assoc. Prof. Dr. Kalina Shishkova/