To the Members of the Scientific Jury, appointed by Order No. 276/06.10.2025.
of the Director of the NCIPD, Sofia

REVIEW

By Professor, Mariyana Stoycheva Vartigova, MD, DSc

Scientific specialty, infectious diseases

On a **dissertation for awarding a scientific degree**

DOCTOR OF SCIENCE

In the scientific specialty "Virology"

In the field of higher education: 4. Natural Sciences, Mathematics and Informatics; Professional field: 4.3. Biological sciences

Thesis topic: **Antiretroviral resistance and molecular epidemiology of HIV-1 in Bulgaria: integrated analysis of genetic diversity, phylodynamics and demographic correlations.**

Author: **Associate Professor Ivaylo Alexiev Ivanov**, Ph.D.

Relevance and significance of the topic.

The HIV/AIDS pandemic began in 1981 and has proven to be one of the most insidious and persistent in human history. According to UNAIDS data, more than 91.4 million people have become infected with HIV so far, approximately 44.1 million have died from AIDS-related diseases, and the number of people living with HIV is currently 40.8 million. Antiretroviral therapy (ART), and in particular combination ART introduced from 1995-1996, has led to a drastic reduction in mortality and limited transmission of HIV infection. AIDS from a deadly disease, into a chronic, controllable infection. Unfortunately, only 31.6 million (71.65%) of HIV worldwide have access to ART [UNAIDS, 2025], and the high genetic variability of HIV-1 and the increasing incidence of drug resistance is an additional, serious risk for therapeutic failure and delay in global elimination of the causative agent.

The rapid advancement of molecular epidemiology has provided opportunities for an in-depth understanding of the dynamics of the HIV-1 epidemic through the integration of phylogenetic analysis, genomic characterization and epidemiological data. Understanding the molecular epidemiology of HIV-1 has a direct impact on clinical practice and the formation of relevant public health policies. therapeutic regimens, and knowledge of the specific features of the HIV epidemic in Bulgaria is a reliable basis for making adequate management decisions to limit the spread of the virus and its resistance to antiretroviral drugs (ARVs).

The title of the dissertation reveals the complexity and relevance of the problem under consideration related to the study of antiretroviral resistance and molecular epidemiology of HIV-1 in our country, with an integrated analysis of genetic diversity, phylodynamics and demographic correlations.

These briefly presented notes outline the significance and relevance of the dissertation.

Structure of the dissertation.

The dissertation submitted to me for review is written on 310 pages and contains 58 figures and 43 tables. It is formatted according to the officially adopted scheme: title page, table of contents, abbreviations used, list of used figures, list of tables used; introduction - 3 pages; literature review - 87 pages; Purpose and objectives - 2 pages; materials and methods - 63 pages, including analysis of selected publications of the author; results – 92 pages, including results from selected publications of the author; discussion - 29 pages; conclusions – 4 pages; contributions – 3 pages; bibliography – 15 pages; and other appendices – 5 p.

The literature review is prepared on the basis of 342 sources, of which 6 are in Cyrillic and 336 in Latin. The presented significant amount of information shows an in-depth theoretical awareness of the author, which has been built up over many years. The review includes a detailed description of retroviruses, their classification, taxonomy of HIV, its occurrence, characteristics, evolution and life cycle. The three classes of antiretroviral drugs NRTI, NNRTI and PI, which were analyzed in the study period, are mainly considered. Their mechanism of action, as well as the effect of boosters (pharmacokinetic enhancers - Cobicistat and Ritonavir), the use of which is common in PI and other ARV drugs, are described in detail.

Drug resistance (transmission, acquired and primary) and the mechanisms of its occurrence in NIV-1 are discussed in detail. Its critical importance for both individual clinical outcomes and control of HIV infection at the population level is emphasized.

The overview is illustrated with 12 tables and 11 figures, which make it easier to comprehend this rather difficult to understand material.

The review demonstrates the author's excellent awareness of the problem and at the same time is written in a way that makes it interesting not only for specialists. It ends with an assessment of the author, regarding *the relevance and significance of the topic of the dissertation.* The analytical approach in its development facilitates the formulation of the purpose of the thesis

Purpose and objectives.

The goal is clearly formulated and fully corresponds to the title. There are 5 main tasks that outline the specific steps to achieve the goal. Within the framework of the main tasks, the detailed approaches and the volume of research for their implementation are defined.

The Materials and Methods **section** is written on 63 pages, informative enough, with all the necessary details about the persons studied and the methods used.

State-of-the-art methods were used to *(1) collect data:* routine genotyping of HIV-1, sequencing of critical regions PR (protease) and RT (reverse transcriptase) from pol genes (TRUGENE system ViroSeq system (Abbott)); andantiretroviral resistance analysis with the Stanford HIV Drug Resistance Database (HIVDB) algorithm Version 9.5.1; phylogenetic and phylodynamic analysis of resistant mutations; subtyping; recombinant analysis; multiple alignment; phylogenetic reconstruction and characterization of transmission clusters; and (2) *statistical methods of data processing.*

This section includes the results of six key publications of the author, in prestigious international journals, which demonstrate the evolution of research capabilities and the systematic elucidation of the complex molecular and epidemiological picture of HIV-1 in Bulgaria.
The section ends with *"Summary and significance of the methodological approach".*
Within the scope of my competence, I can say that the most modern, up-to-date, adequate molecular methods have been used, and detailed protocols, evaluation

scales have been presented for all of them, and their limitations and informativeness have been discussed. Examples of interpretation of the results are also proposed.
By itself, this section can serve as a manual for laboratory workers and clinicians - HIV specialists, with a view to the correct interpretation of laboratory data.

About **Results and Discussion**

Significant results have been achieved, presented and discussed on 121 pages, illustrated with 30 tables and 41 figures. The results are presented in 5 sections, which follow the order of the tasks.

Some of the results include:

It has been established:

- low incidence of primary resistance (5.7%) in untreated patients in Bulgaria, corresponding to the WHO criteria for controlled epidemic;
- high heterogeneity in resistant models, involving combinations of multiple mutations, with acquired resistance associated with the duration of therapy and the history of previous therapeutic regimens;
- high genetic heterogeneity has been established in the Bulgarian population with 26 different HIV-1 subtypes, circulating and unique recombinant forms, which reflects complex epidemiological interactions and the repeated introduction of viruses from different geographical regions;
- new or rare HIV-1 variants and recombinant forms have been identified and characterized, enriching the knowledge of global molecular epidemiology and highlighting the importance of Bulgaria as an epidemiological crossroads in Southeast Europe.
- The distribution of HIV-1 subtypes and resistant mutations shows significant differences by transmission categories, vulnerable groups and geographical regions in the country.
- In MSM, the highest incidence of resistance and predominance of subtype B was found, while CRF01_AE and CRF02_AG were more common in IDU.
- Almost half of all cases (47.9%) were registered in Sofia, where a higher incidence of resistance (29.0%) and concentration of subtype CRF01_AE were also reported. CRF02_AG dominates in Plovdiv, and subtype B is more evenly distributed in the country.

These differences highlight the need to apply a personalized therapeutic approach based on genotypic analysis in order to increase therapeutic success and limit the further development of resistance, as well as regionally adapted prevention and surveillance measures.
This section includes the results of six scientific articles by the author concerning the prevalence of antiretroviral resistance and HIV-1 subtypes isolated from PLWHIV in Bulgaria for the period 1989-2023.

The rich experimental material and the correctly conducted research ensure the reliability of the results obtained.
I would especially like to note that throughout the dissertation, the *practical applicability of the studies is sought and taken into account*. This trend is set in one of the main tasks *"Clinical Applications and Preventive Strategies", and* continues in results and conclusions, as well.

It is reported that the therapeutic approach based on genotypic analysis is a prerequisite for virological success and limiting the further development of resistance. Complex molecular epidemiological data have been generated, providing an empirical basis for updating the national strategy for prevention and control of HIV/AIDS and adapting interventions according to the identified epidemiological trends.

There are **5 main conclusions** that correspond to the tasks set and fully stem from the results obtained, with which I agree.
There are 11 scientific contributions of an original nature and 7 of a scientific and practical nature, with which I also agree.

The presented scientific contributions represent the most extensive and in-depth virological study of HIV-1 in Bulgaria, creating a solid scientific basis for clinical practice, public health and national policies, while enriching the international understanding of the molecular epidemiology of HIV-1 in the region of Southeast Europe. The complex sequencing and phylogenetic analysis of 1654 fragments of the HIV-1 gene, collected over a period of thirty-five years, created an extensive molecular database that is a fundamental resource for understanding viral evolution and epidemic dynamics in Bulgaria.

In connection with the dissertation, 12 publications are presented in prestigious peer-reviewed international journals with IF and one monograph. All publications of Assoc. Prof. Alexiev are collective, in cooperation with leading virologists, immunologists and infectious disease specialists from the country and

abroad. He has a leading role in their generation and he is the first author in six of them, with 7 being in Q1 and 1 in Q2.

I especially note the contribution of the monograph of Associate Professor I. Alexiev, PhD, State Volume "HIV. Antiviral drugs and resistant mutations", which fills an information need in the field of virology and HIV medicine in our country.

In the appendices to the dissertation, Assoc. Prof. I. Alexiev, Ph.D. presents information about successfully implemented 2 research projects in which he participates as a leader or contractor. Two of them are in international cooperation, one funded by the National Science Fund (project DN03/2 of 16.12.2016), as well as project BG05M2OP001-1.002-0001 under the Operational Program "Science and Education for Smart Growth 2014-2020", co-financed by the European Regional Development Fund. In addition, Assoc. Prof. I. Alexiev Ph.D presents 86 scientific publications, of which 52 are in journals with IF; citations – 363 and a total impact factor of scientific production is 213.9, H -index (Scopus, without self-citations) – 12, and numerous reports at national and international scientific forums. The impressive number of citations, the high IF and H index are proof of the scientific value of the publications.

The personal contribution of Assoc. Prof. Ivaylo Aleksiev Ivanov, Ph.D. In the development of the dissertation he is undoubted. He is a prominent and established, leading specialist in the field, and has been dealing with the problem for years. His opinion is sought after and respected by specialists at home and abroad.

The abstract is written in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (RASRB), the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations of the National Center for Academic Achievement in Sofia. It accurately reflects the main achievements of the dissertation.

The minimum national requirements for awarding the scientific degree DOCTOR OF SCIENCES have been met. From the attached table it is evident that Assoc. Prof. I. Alexiev, Ph.D., fulfills and in some indicators significantly exceeds the set requirements.

Indicator group	Required points	Points available
A (indicator 1)	50	50
B (Indicator 2)	100	100
D (Indicators 5-10)	100	240
E (indicator 11)	100	108
TOTAL	**350**	**498**

IN CONCLUSION

The dissertation is an extremely interesting, very well planned, thoughtful and precisely executed development with undoubted theoretical and practical contributions. The dissertation ***contains scientific, and applied results, which represent an original contribution to science*** and have great clinical significance. The dissertation **meets all** the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its Implementation and the Rules of the National Center of Infectious and parasitic Diseases, Sofia.

With pleasure and complete conviction, I give my ***positive assessment*** of the conducted research, presented by the above-reviewed dissertation, abstract, achieved results and contributions, and ***propose to the honorable scientific jury to award the scientific degree of "Doctor of Science"*** to Associate Professor Ivaylo Alexiev Ivanov, Ph.D., in the Professional field: 4.3. Biological Sciences, the scientific specialty VIROLOGY.

30.10.2025y. Reviewer:.....

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