Opinion

of the PhD thesis on the topic:

"A multi-omics approach in the analysis of the biological and clinical heterogeneity of some rare malignancies "

for the award of the educational and scientific degree "Doctor of Philosophy" in scientific field 4.3. "Biological Sciences"

PhD Student: ***Georgi Dimitrov Blazhev,*** *Dept. of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", with* ***scientific supervisor Assoc. Prof. Velizar Stefanov Shivarov***

Prepared by: ***Assoc. Prof. Svetoslav Gueorguiev Dimov****, chairman of the Dept. of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", elected as a regular member of the Scientific Jury by decision of the Faculty Council of the Faculty of Biology of Sofia University "St. Kliment Ohridski" according to Protocol No. 8 of April 16, 2024.*

Biographical data of the doctoral student. Georgi Blazhev was born in 1995. His professional career in the field of biological sciences began in 2014 when he was accepted as a full-time student BSc in "Molecular Biology" at the Faculty of Biology of the Sofia University "St. Kliment Ohridski". After graduating in 2018, he was accepted as a full-time student in MSc in "Genetic and Cellular Engineering", where he will graduate in 2020. In the same year, after a successful admissions competition, he was enrolled as a full-time doctoral student at the Department of Genetics, Faculty of Biology of the University of St. Kliment Ohridski" in Professional direction 4.3. – "Biological Sciences", scientific specialty "Genetics".

"Eligibility of the candidate for defending a doctoral dissertation for obtaining a PhD degree". Georgi Blazhev was enrolled as a regular doctoral student in the Department of Genetics, Faculty of Biology at Sofia University "St. Kliment Ohridski" under the scientific supervision of Assoc. Prof. Velizar Shivarov on July 20, 2020, by Order No. RD-20-1010/16.07.2020 of the Rector of Sofia University "St. Kliment Ohridski," with the doctoral program deadline being July 20, 2023. He was dismissed with the right to defend his dissertation as of July 20, 2023, according to Order No RD-20-1693/28.09.2023 of the Rector of Sofia University "St. Kliment Ohridski." In connection with the initiation of the defense procedure, the following documents were submitted: a declaration of originality and authenticity, a protocol for checking the originality of the dissertation work, an opinion on the procedure for preventing plagiarism in dissertation works, a statement of compliance with the minimum national requirements for a PhD degree in field 4 - "Natural Sciences, Mathematics, and Informatics," professional direction 4.3. "Biological Sciences," dissertation work, abstract in Bulgarian, abstract in English, and copies of diplomas for completing Bachelor's and Master's degrees. Additionally, copies of two scientific publications related to the dissertation work with quartiles Q2 and Q4 were also submitted, which, according to the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria, contribute a total of 32 points, exceeding the required minimum of 30 points. All this gives me grounds to admit the PhD thesis of candidate Georgi Blazhev for evaluation.

Scientific relevance of the dissertation work. For several reasons, investigating and studying rare malignant diseases is of paramount importance in the

field of medical research and healthcare. Firstly, although individually rare, collectively, these diseases affect a significant portion of the population, often with devastating consequences. Each case represents a unique set of challenges requiring special attention and innovative approaches to diagnosis, treatment, and management. Secondly, understanding rare malignant diseases provides invaluable insights into the fundamental mechanisms of cancer biology. These insights contribute to developing targeted therapies for rare diseases and have broader implications for understanding and treating more common forms of cancer. By unraveling the molecular and genetic complexity of rare malignant diseases, researchers can identify common pathways and vulnerabilities shared among different types of cancer. The significance of studying and researching rare malignant diseases is a multifaceted endeavor that benefits patients with rare diseases and enhances our understanding of cancer as a whole. Some of the most promising approaches in studying these diseases are omics technologies, which is why I consider the scientific significance of the dissertation work to be highly promising.

The overall structure of the dissertation work. The PhD thesis is developed within 110 pages, encompassing the following main sections: "introduction," "problem statement" (in place of “literature review”), "materials and methods," "results," and "discussion" (presented as separate sections). Additionally, sections for "research hypothesis, aims, and objectives," "conclusion and deductions," and "contributions" are provided separately. Finally, the dissertation concludes with a bibliography covering 173 literary sources, predominantly from recent years. Given the specificity of the research, which primarily relies on in silico analyses, I find the volume of work adequate.

The PhD thesis begins with a brief “**introduction”** section spanning three pages, briefly addressing the immediate medical needs in rare types of cancer—providing a definition of the term "cancer" while explaining which types of cancer should be considered rare. The focus of the research primarily on malignant pleural mesothelioma (MPM) is also justified. This section contains two figures of good graphical quality, which are not authored, but their sources are cited correctly.

Instead of a "Literature Review," a section entitled **"Problem statement – malignant pleural mesothelioma"** demonstrates the doctoral candidate's theoretical preparation on his dissertation topic. As indicated by its title, this section covers 25 pages focusing on MPM. The first part defines the diseases, identified as MPM, and discusses the prevalence of these diseases globally, determining frequencies, distribution by gender, and age distribution. The second part of the section focuses on the causes of MPM development, its pathology, and the carcinogenic mechanisms unlocked by asbestos fibers. The third part provides a detailed review of the molecular mechanisms underlying the development of MPM – primarily focusing on the role of the HMGB1 protein as a mediator of inflammatory processes and M2 phenotype macrophages. In the fourth part of the review, a successful attempt is made to characterize the genetic predisposition for the development of MPM as much as possible, considering the role of individual loci and their alleles, as well as structural changes in DNA such as induced point mutations, loss of heterozygosity, etc. The final part of the section reviews the development of omics technologies and their application in cancer research – genomics, epigenomics, transcriptomics, proteomics, and

metabolomics, with necessary attention given to managing and integrating data from various omics technologies.

The review section of the dissertation contains five figures of good graphical quality, which are not authored, but their sources are correctly cited. From the presentation of this part of the dissertation, I can find out that the doctoral candidate possesses broad scientific knowledge on the topic of the dissertation and is well acquainted with the latest research on a global scale in this area. It also indicates that he can skillfully interpret scientific literature from various sources and write highly scientifically. My only remark on this section is that, in my opinion, it would be better to have the part on omics technologies at the beginning of the section, considering the logical sequence of the presentation.

"Research Hypothesis, Aims, and Objectives". Georgi Blazhev's PhD thesis's primary research objective is based on the research hypothesis that it is possible to derive a new quantitative scoring system based on pre-existing omics data, which would have prognostic and predictive value for patients with MPM. To achieve this goal, nine research objectives have been set, which are entirely sufficient for its accomplishment, covering a rich array of research work and data interpretation, mastering which is necessary for obtaining a PhD degree.

"Materials and Methods". This section is developed within six pages. Given the specificity of the dissertation work, it includes bioinformatics methods aiming to create a database from omics studies, analysis of genes set enrichment, analysis of

profiles obtained with the CIBERSORT tool, drug sensitivity analysis, analysis of methylation profiles, as well as general statistical analyses. The section is illustrated with one figure of good graphical quality and one table. In my opinion, Figure 8, illustrating the used analytical approach, is a significant asset to this section, as it allows for a clear visualization of the process. For this reason, I would place it at the beginning of the section.

"The Results" section is developed within 34 pages, presenting the doctoral candidate's research results concisely with rich illustrative material – five author tables and 29 author figures of sufficiently good graphical quality. The section is well-structured with a logical sequence, allowing for easy assessment and analysis of the obtained results. It begins with identifying omics data from studies available in the PubMed database, extracting a dual-gene quantitative prognostic score for MPM based on two identified genes (*GOLT1B* and *MAD2L1*), and its validation. The second part of the section presents the results of gene set enrichment analysis in expression profiles. The third group of results includes the analysis of methylation profile data in patients with high and low dual-gene quantitative prognostic scores. Additionally, results from the use of the CIBERSORT algorithm on TCGA and Bueno datasets and their correlation with the dual-gene quantitative prognostic score are presented. At the end of the section, the candidate shows the results of the correlation of the prognostic ability of the dual-gene quantitative prognostic score with sensitivity to a series of drugs.

"The Discussion" section is notably developed within eight pages and does not contain illustrative material. Overall, the scientific language in which this section, as well as its content, convincingly demonstrate that the doctoral candidate skillfully analyzes and interprets his own results, comparing them with those of other scientific teams. I believe this ability is evidence of his maturity as a young scholar. The section begins with a skillful motivation of the necessity of the conducted research, followed by analysis and comparison of the candidate's own results with those available in the scientific literature, and drawing conclusions (clearly and concisely synthesized further in the "Conclusions" section), concluding with a brief paragraph on the significance and future directions of using omics technologies in studying MPM.

The second to last section of the PhD thesis is entitled **"Conclusion and inferences."** Five conclusions and four "exemplary rational directions for future research" are formulated. Conclusions are not explicitly formulated as such, though, in my opinion, the formulated conclusions essentially represent deductions and can be accepted. I have no remarks about them, as they clearly and concisely synthesize the scientific results of the dissertation work. As a note, I suggest that the formulated directions for future work, which I believe would have a better place in a slightly expanded form as a separate concluding section of the dissertation. However, it is commendable that contributions are not formulated as deductions (conclusions).

The PhD thesis concludes with a section titled **"Contributions,"** in which four original contributions and four confirmatory ones are formulated. I fully accept contributions from both groups without any remarks. It is commendable that deductions are not formulated as contributions.

The abstracts are presented in Bulgarian and English in accordance with regulatory requirements. The Bulgarian version spans 43 pages, while the English version contains 40. Both abstracts are identical in terms of content, and the volume differences are due to linguistic differences. They summarize the dissertation itself in a condensed form, including the main sections: "Introduction," "Materials and Methods," "Results," and "Discussion." Additionally, they present the "Research Hypothesis, Aims, and Objectives," "Conclusion and inferences," and "Contributions." They are illustrated with 29 figures and three tables. My only technical remark regarding both abstracts is that they are printed entirely in black and white, and some included figures cannot be analyzed this way. However, I note that the same figures in the dissertation are printed in color, eliminating this issue. Additionally, shortened bibliographic references are missing in them. For the Bulgarian version, I have an additional technical remark – the "Contributions" section and the publications related to the dissertation are presented twice.

General remarks. The dissertation presents impressively completed scientific research on a relevant issue using the most modern methods in the field of bioinformatics. The impression left is that the PhD student is a promising young scholar capable of conducting comprehensive scientific research in a specific area and interpreting the results adequately and substantively. One of my observations is that although the work is written in a highly scientific language, it contains foreign terms with a scientific equivalent in Bulgarian and instances of English word order. Another observation is about discrepancies in pagination between the figures and their

explanations, possibly due to formatting errors. However, these observations do not diminish the scientific merits of the work and do not change my excellent impression of it.

Scientific publications and contributions related to the dissertation work have been presented. There are a total of 2 scientific publications in international peer-reviewed specialized journals. The accumulated points according to the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria under Indicator Group "G" based on the quartiles of the journals are 32, exceeding the required 30 points for Professional Field 4.3. "Biological Sciences." Additionally, there is one participation in an international scientific forum.

Compliance with the educational and scientific degree requirements of "Doctor of Philosophy." Georgi Blazhev has acquired competence and compliance with the requirements for obtaining an educational and scientific degree. Through the execution of the theoretical and bioinformatic research work and the formulation of the dissertation itself, Georgi Blazhev has gained competence in terms of knowledge in the specific scientific field, skills in posing and solving specific research tasks, applying modern research methods in the specific field, and evaluating the obtained results.

CONCLUSION: The work presented for review represents a completed and comprehensive scientific study at a high level. Therefore, I will vote positively for

awarding the educational and scientific degree of "Doctor" to Georgi Dimitrov Blazhev, and I will recommend that my colleagues on the Scientific Jury do the same.

Sofia, May 21, 2024 Signature: