OPINION

on a dissertation on topic "**A multi-omics approach in the analysis of the biological and clinical heterogeneity of some rare malignancies**", presented by **Georgi Dimitrov Blazhev**, for achievement of educational and scientific degree "Doctor of Philosophy (PhD)" in professional field 4.3. "Biological sciences" (Genetics-Cancer Genetics), Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski"; with scientific supervisor **assoc. prof. Velizar Shivarov, M.D.–PhD**.

Opinion by **assos. prof. Sena Karachanak-Yankova, PhD** appointed as a member of a scientific jury by order RD-38-193/25.04.2024 of the Rector of SU "St. Kliment Ohridski"

The subject of the presented PhD thesis is the establishment and validation of a novel prognostic score, based on gene expression, by application of a multi-omics approach, which can be used for analysis of the biological and clinical heterogeneity of rare malignant diseases, in particular malignant pleural mesothelioma (MPM).

The PhD thesis is presented in 102 pages and is structured in a standard manner, including the following main sections: Literature review (problem statement), Materials and Methods, Results and Discussion. The additional sections are as follows: Introduction; Research hypothesis; Aims and objectives; Conclusions; Contributions, and recommendations. The dissertation work concludes with a bibliography covering 173 sources, primarily from recent years.

The PhD thesis begins with Introduction section spanning 4 pages, outlining the unmet medical needs to improve overall survival in rare types of cancer, focusing on the primary nosological unit of mesotheliomas, namely malignant pleural mesothelioma.

The "Literature Review" (Problem Statement) begins with the definition of malignant pleural mesothelioma and the epidemiology of this rare type of cancer. The description of the etiology and pathology of the disorder is followed by information about the mechanisms for carcinogenesis induction by asbestos and the subsequent molecular and immunological processes in the inflammatory microenvironment. Discussion of omics technologies (genomics, epigenomics, transcriptomics, proteomics, and metabolomics) is given, along with their

application in cancer biology, as well as the management and integration of omics data for extracting biologically relevant information.

The objective of the dissertation work is clearly formulated: deriving and validating a novel prognostic score based on gene expression in patients with MPM. Nine research tasks are outlined for the achievement of this goal.

"Materials and Methods". Three datasets from whole transcriptome analysis in MPM patients were used to introduce the binary prognostic score (2-PS). RNA-Seq data for 87 MPM cases from The Cancer Genome Atlas Program (TCGA) project were used as a training set, whereas RNA sequencing data from the Bueno study for 211 MPM cases and gene expression microarray data from the Blum study for 67 cases were used as validation datasets. 179 genes were selected from the DepMap project to build a model based on genes associated with survival. A binary score (high vs low) was defined based on the median of the continuous score for each cohort. Gene set enrichment analysis was performed using the standalone version of GSEA 4.0.3, with the molecular signatures database (MSigDB) used as a reference gene set; immune cell fractions were calculated using CIBERSORTx; drug sensitivity analysis was performed using data from GDSC1 and 2 cell lines; and integrated DNA methylation analysis was conducted using the COHCAP algorithm.

"Results". The derived model consists of two genes - *GOLT1B* and *MAD2L1*, which were validated using RNA-Seq data from Bueno and gene expression microarray data from Blum. Analysis of gene list enrichment in expression profiles revealed that 25 profiles were overexpressed with a high score in all three cohorts. Most of these profiles are related to DNA repair, response to DNA damage, and control of mitotic cell division. Using DNA methylation data obtained with the Infinium 450K bead chip (Illumina) for patients from the TCGA and Blum cohorts, it was found that in the first set, the methylation of CpG islands correlates inversely with the expression of the *SLC20A1* and *KIAA1949* genes. In both datasets, the continuous prognostic score showed a positive correlation with the CD8+ T-cell fraction, as well as with the fractions of macrophages M1 and M2. A correlation of 2-PS with response to treatment to cisplatin, gemcitabine, and vinblastine was established. The remarks on this section reffer to incorrect references to figures.

The results are further discussed in a separate section "Discussion," which describes the emphazis of the need for novel prognostic and predictive models for MPM as well as the role

of *MAD2L1* and *GOLT1B* genes in malignant pleural mesothelioma. Based on the conducted GSEA analysis, it is concluded that 2-PS reflects underlying characteristics of patients with high score compared to those with low score, as this reproducibility is not observed in the analysis of DNA methylation. The binary prognostic score correlates with the content of CD8+ T cells and M1/2 macrophages, consistent with the pathogenetic mechanism of chronic inflammation in MPM resulting from ineffective phagocytosis of asbestos fibers. In the analysis of drug sensitivity, the 2-PS score can predict higher sensitivity to cisplatin, while it may predict resistance to gemcitabine and vinblastine.

Five conclusions regarding the prognostic score are formulated based on the results from the doctoral thesis, as well as 4 exemplary rational guidelines for future research and implementation of 2-PS in clinical practice. A separate section contains: 5 original contributions regarding the developed prognostic model and the prognostic value of *GOLT1B* gene expression; and 3 confirmatory contributions.

Abstract. Abstracts of the thesis in Bulgarian and English are presented, each spanning 43 and 41 pages, respectively. The contents of both versions are identical, summarizing the dissertation work in a concise manner.

General remarks on the PhD thesis. Overall, the presented thesis makes a very good impression of a comprehensive scientific study on a relevant problem, utilizing data from various omics technologies in order to introduce and validate an oligogenic prognostic score for MPM. It aims to identify patient subgroups with similar expression profiles, to reflect specific profiles of immune cell infiltration, and to have predictive value regarding sensitivity or resistance to chemotherapeutic agents. My remark is of a technical nature and in no way diminishes the scientific merits and overall impression of the PhD thesis.

Scientific publications and presentations related to the dissertation work. A total of 2 scientific publications in international specialized journals with quartiles Q2 and Q4 are presented, in which the doctoral student is a co-author, as well as one participation in a national scientific conference. The points collected based on the quartiles of the journals are 32, exceeding the required 30 points in Professional Field 4.3 "Biological Sciences" according to the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria.

Conclusion. The presented PhD thesis is in a modern research field, it represents a completed scientific research carried out at a high level, which gives me the reason to vote positively for the awarding of the educational and scientific degree "Doctor of Philosophy (PhD)" to Georgi Dimitrov Blazhev in professional field 4.3. Biological Sciences (Genetics-Cancer Genetics).

22 May 2024 Assoc. Prof. Sena Karachanak-Yankova, PhD