

OPINION

Of Prof. Ivan Iliev, PhD, IEMPAM-BAS, appointed as a member of the scientific jury by

Order No. 121-OB / 27.05.2026 of the Director of IMB-BAS

About: the evaluation of the PhD thesis of Alexander Emilov Alexandrov, on the topic: "Synergistic induction of secretion of extracellular vesicles and DNA by autophagy inhibitors and DNA-damaging agents", for the educational and scientific degree "Doctor" in the scientific specialty "Molecular Biology", code 01.06.04.

The dissertation presents an original scientific study on the mechanisms for inducing the secretion of extracellular vesicles (EVs) and DNA by autophagy inhibitors and DNA-damaging agents. Specifically, the work covers the cultivation and treatment of various types of tumor cells with genotoxic agents and autophagy inhibitors. The levels of cytotoxicity, genotoxicity, apoptosis, necrosis, autophagy, secretion of EVs and nucleic acids were determined. Molecular biological, biochemical, biophysical and microscopic methods were used for qualitative and quantitative assessment of the studied biological processes. The relevance of the developed scientific problem is outlined by the diverse role of secreted extracellular vesicles in terms of intercellular communication and modulation of the microenvironment of secreting cells. In addition, EVs can be diagnostic and prognostic markers in a number of socially significant diseases (autoimmune, oncological, cardiovascular, metabolic, etc. diseases).

The dissertation submitted to me for evaluation contains 71 pages and is logically arranged, well illustrated and structured in the standard sequence with the necessary sections for a dissertation. The literature review is 17 pages and shows knowledge of the scientific issues. The goal is formulated briefly and clearly, which shows a high level of understanding of the problem. The tasks set are described in 9 points and are logically formulated in relation to the goal of the dissertation. In the "Materials and Methods" section, the cell lines, reagents and specific antibodies used are sequentially presented. The methods for isolation, purification and characterization of extracellular vesicles and nucleic acids, methods for determining cell viability, apoptosis, electrophoretic and microscopic methods are described. All described methods and equipment are

correctly selected for the implementation of the tasks set. In the "Results" section, which is on 26 pages, the results obtained are well described and appropriately illustrated with graphs, diagrams and microphotographs. The discussion is 8 pages, follows the presented data and is in good agreement with the described results and the used literature sources (total 99). The obtained results are summarized in 10 conclusions. As a result of the scientific work on the dissertation, two articles have been published in journals indexed in the world databases SCOPUS and Web of Science. The results have been presented at 3 scientific conferences.

The presented dissertation is indicative of precision, mastery of a wide range of biochemical, molecular biological and cytological methods and the ability to work in a team. These are prerequisites for building a promising young scientist.

Disadvantages: 1. The "Literature Review" section does not consider the influence of apoptosis on the secretion of EVs. 2. No original contributions or confirmatory contributions are formulated.

My recommendation is to correct the technical errors found in several places in the text.

The presented abstract in a volume of 51 pages is a short version of the dissertation and covers its main sections without the literature review and meets the requirements.

In conclusion, it can be summarized that the presented dissertation work is a valuable scientific study. The experimental tasks set were correctly implemented, which is evident from the results obtained. The two published scientific articles on the topic of the dissertation are evidence of the high level of the scientific research carried out.

I believe that all requirements according to the Academic Staff Development Act and the regulations for its implementation, which have been adopted for our country and the IMB-BAS, are covered and meet the required criteria for acquiring the PhD degree. With full conviction, I give a high assessment of the dissertation work on the topic "Synergistic induction of secretion of extracellular vesicles and DNA by autophagy inhibitors and DNA-damaging agents" and vote "YES" for awarding the educational and scientific degree "Doctor" to Alexander Emilov Alexandrov.

02.07.2026

Signed:

/Prof. Ivan Iliev/