

REVIEW REPORT

by

Prof. Milena Georgieva, PhD

Bulgarian Academy of Sciences, Institute of Molecular Biology "Acad. Roumen Tsanev",
Laboratory of Molecular Genetics, Epigenetics and Longevity, Member of the Scientific Jury
appointed by Order № 121-OB/27.05.2026 of the Director of IMB-BAS

Regarding the defence of the PhD thesis entitled:

**"Synergistic Induction of Extracellular Vesicle and DNA Secretion by Autophagy
Inhibitors and DNA-Damaging Agents"**

for the acquisition of the educational and scientific degree "**Doctor**" by

Aleksander Emilov Aleksandrov, MD

Field of higher education: **4. Natural Sciences, Mathematics and Informatics**

Professional field: **4.3. Biological Sciences**

Doctoral program: **Molecular Biology**

Scientific supervisor: **Assoc. Prof. Dimitar Borisov Iliev, PhD**

General Description of the Submitted Materials

By Order of the Director of the Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences, I have been appointed as a member of the Scientific Jury for the procedure related to the defence of the PhD thesis of Aleksander Emilov Aleksandrov for the acquisition of the educational and scientific degree "Doctor" in the professional field 4.3. Biological Sciences, doctoral program "Molecular Biology".

The submitted documentation has been prepared in accordance with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and its implementing regulations. The complete set of documents includes the doctoral thesis, abstract, curriculum vitae, a list of scientific publications and their copies, as well as all other materials required for the evaluation procedure. The documentation is complete, well prepared, and allows an objective assessment of both the dissertation work and the doctoral candidate's overall research activity.

Brief Biographical Data of the Doctoral Candidate

Aleksander Emilov Aleksandrov, MD, is a graduate of the Medical University of Sofia, where he earned his Master of Medicine in 2020. During his medical training, he

developed an early interest in experimental biomedicine and in 2018 completed an internship at the Department of Physiology at the Medical University of Sofia under the supervision of Prof. Lilyana Vitanova. In the same year, he received first place in an oral presentation at the preclinical Section of the International Congress of Medical Sciences (Sofia, Bulgaria), an early recognition of his scientific potential.

Since 2021, his professional development has been consistently associated with the Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences, Section "Regulation of Gene Expression". During the period 2021-2022, he worked as a biology specialist; from 2022 to 2026, he held the academic position of Assistant Professor; and since 2026, he has continued his work as a biology specialist in the same scientific unit. This professional trajectory reflects his sustained involvement in research within the Section and his accumulation of substantial experimental experience in molecular and cellular biology.

The scientific output of Aleksander Aleksandrov includes four publications in international scientific journals indexed in *Web of Science* and *Scopus*, all published within the last five years. Two of these publications are directly related to the doctoral thesis. The first study, published in the *International Journal of Molecular Sciences* (Q1, Impact Factor 5.6), presents original findings on the synergistic effects of chloroquine and temozolomide on the secretion of extracellular vesicles and extracellular nucleic acids in glioma cells. The second publication, in the *European Journal of Biology* (ESCI, JCR Q4, Impact Factor 0.9), demonstrates that ATM inhibition stimulates extracellular vesicle secretion by modulating the autophagic response, thereby complementing and expanding the scientific concept developed in the dissertation. The results of his research have been presented at international scientific forums through both oral and poster presentations. It should also be emphasized that the candidate has participated in several competitively funded research projects.

Relevance of the Research Topic and Knowledge of the Scientific Problem

During the last decade, extracellular vesicles (EVs) have become one of the most intensively studied subjects in modern Cell Biology and Molecular Medicine. Initially regarded merely as a mechanism for eliminating cellular waste, they are now recognized as key mediators of intercellular communication involved in numerous physiological and pathological processes, including inflammation, immune responses, neurodegenerative disorders, and tumour progression. Their role in therapy resistance, remodelling of the tumour microenvironment, and formation of metastatic niches has attracted considerable attention, making them promising diagnostic, prognostic, and therapeutic biomarkers. Against this background, investigating the relationship between autophagy and extracellular vesicle secretion represents one of the most rapidly evolving areas of contemporary cell biology. Increasing evidence suggests that these two processes are functionally interconnected and mutually regulated under different forms of cellular stress, including DNA damage. Despite significant progress, however, the molecular mechanisms governing this interaction and its implications for anticancer therapy remain insufficiently understood.

The present dissertation addresses precisely this important scientific challenge. The selected topic is both timely and highly relevant, with clear fundamental and translational significance. Investigating the combined effects of autophagy inhibitors and DNA damaging agents on the secretion of extracellular vesicles and extracellular nucleic acids helps elucidate the adaptive mechanisms by which tumour cells respond to therapeutic stress. The obtained findings expand current knowledge of the interplay between cellular homeostasis and intercellular communication.

Particularly noteworthy is the choice of *Glioblastoma multiforme* (GBM) as the experimental model. GBM is among the most aggressive human malignancies, characterized by pronounced therapeutic resistance and limited treatment options. In this context, identifying novel mechanisms that enhance tumour cell sensitivity to DNA-damaging agents is of considerable translational importance.

The literature review demonstrates an excellent understanding of the scientific field. The candidate systematically discusses current concepts regarding extracellular vesicle biogenesis and functional heterogeneity, autophagy, the role of ATM signalling in the DNA damage response, and the molecular characteristics of glioblastoma. Rather than providing a descriptive summary of published studies, the review integrates the available evidence into a coherent conceptual framework that convincingly justifies the rationale for the present investigation.

The candidate also demonstrates a thorough familiarity with the current literature and an ability to identify unresolved questions in the field. This provides a solid basis for formulating a clear scientific hypothesis and well-defined experimental objectives that address insufficiently explored aspects of the interaction among autophagy, DNA damage, and extracellular vesicle secretion.

Methodological Approach and Research Design

The dissertation addresses a timely and actively developing research area focused on the mechanisms by which cells respond to genotoxic stress by altering their secretory activity. The study aims to elucidate the molecular links between autophagy regulation, DNA damage, cell fate, and the release of extracellular vesicles and extracellular nucleic acids.

The methodological strategy is based on the contemporary understanding that extracellular vesicles are not merely passive cellular waste products but active mediators of intercellular communication, enabling cells to modify their microenvironment and transmit information about their physiological state. Within this conceptual framework, both the experimental model and the selected methodological approaches are scientifically well justified and suitable for investigating the complex interplay between intracellular processes and extracellular responses.

The study employs a comprehensive set of modern experimental techniques, including cell culture models, pharmacological induction of various types of cellular stress, assessment of cell viability and genotoxicity, analysis of mechanisms of cell death and autophagic activity, and characterization of secreted extracellular components. The combination of molecular biological, biochemical, microscopic, and biophysical methods provides a robust

experimental framework for reliable evaluation of the investigated processes and supports well-founded scientific conclusions.

A particular strength of the methodological approach is that the investigation goes beyond the description of phenotypic changes and seeks to elucidate the molecular mechanisms underlying the secretory response of cells under conditions of disturbed cellular homeostasis.

Evaluation of the Dissertation

The dissertation of Aleksander Aleksandrov presents an original experimental study addressing a highly relevant problem in contemporary cell and molecular biology, namely the mechanisms by which cells integrate different forms of stress and modify their intercellular communication.

The topic is particularly significant because extracellular vesicles (EVs) have emerged as key mediators of cell-to-cell communication, participating in both physiological processes and pathological conditions, including tumour progression. Within this context, investigating the interplay between autophagy, DNA damage-induced stress, and EV secretion represents an important contribution to understanding the mechanisms of cellular adaptation.

A major strength of the dissertation is the formulation of a research question that considers autophagy and the DNA damage response not as isolated biological processes, but as interconnected components of a complex cellular network. This approach reflects the current view of the cell as a dynamic system in which intracellular homeostatic mechanisms are closely integrated with extracellular signalling pathways.

The obtained results demonstrate that the combined action of autophagy inhibitors and DNA-damaging agents induces specific alterations in the secretory profile of tumour cells, including enhanced release of extracellular vesicles and extracellular nucleic acids. These findings contribute to a better understanding of how tumour cells respond to genotoxic stress and adapt their behavior under conditions of disturbed cellular homeostasis.

Another major strength of the study is its comprehensive evaluation of the cellular response by simultaneously assessing cell viability, genotoxicity, cell death, autophagy, and secretory activity. This integrated approach enables the construction of a broader model describing the relationship between intracellular stress signalling and extracellular communication, rather than merely documenting individual phenotypic changes.

Particularly noteworthy is the experimental design, which allows discrimination between the individual effects of the applied agents and their potential synergistic interactions. This represents an important contribution to understanding the complex mechanisms underlying cellular responses to therapeutic interventions.

Overall, the dissertation belongs to one of the most dynamic areas of modern molecular medicine: the study of mechanisms by which cells communicate and adapt to stress. From a translational perspective, the work is particularly relevant given the growing interest in extracellular vesicles and their molecular cargo as potential biomarkers for disease diagnosis, monitoring, and therapeutic response. The dissertation demonstrates an excellent

understanding of the scientific problem, successful application of contemporary experimental methodologies, and the level of independence and scientific maturity expected from a doctoral candidate in molecular biology.

Scientific Contributions

The dissertation lacks a separate section explicitly outlining its scientific contributions. Based on the presented experimental results and the conclusions drawn by the author, the following principal contributions can be identified:

1. **Novel experimental evidence has been obtained regarding the interplay between autophagy regulation, DNA damage-induced stress, and the secretion of extracellular vesicles and extracellular nucleic acids.**
2. **The role of combined cellular stress in modulating tumour cell secretory profiles** has been demonstrated, contributing to a better understanding of cellular adaptation and intercellular communication under stress conditions.
3. **A comprehensive experimental evaluation of the relationships among cell viability, genotoxicity, autophagy, cell death, and extracellular vesicle release** has been conducted, expanding current knowledge of cellular responses to genotoxic stress.

In my opinion, these contributions are original and scientifically significant, extending current knowledge of extracellular communication under conditions of cellular stress and improving our understanding of the complex interactions between autophagy, DNA damage, and tumour cell adaptation.

Critical Remarks, Recommendations and Questions

The dissertation is scientifically sound and demonstrates high-quality experimental work. The following comments are intended to be constructive and to outline possible directions for future research.

- The literature review could benefit from a more detailed discussion of the relationship between programmed cell death and extracellular vesicle secretion, particularly the distinction between active vesicle secretion and the passive release of cellular components following cell damage.
- Future studies could further strengthen the work by using more comprehensive molecular characterization of extracellular vesicle cargo via modern omics approaches, enabling the identification of stress-specific molecular signatures.
- It would also be valuable to explore the clinical relevance of the findings, particularly regarding the potential application of extracellular vesicles and their nucleic acid cargo as biomarkers or predictors of therapeutic response.

These recommendations do not diminish the scientific value of the dissertation but rather emphasize the strong future potential of this line of research.

Questions to the Doctoral Candidate

1. How do you interpret the increased secretion of extracellular vesicles and nucleic acids following combined autophagy inhibition and DNA damage as an adaptive survival mechanism or as a driver of tumour progression?
2. Which additional experimental approaches would you propose for a more comprehensive characterization of extracellular vesicles and the identification of clinically relevant biomarkers?

Conclusion

The submitted documents demonstrate that Aleksander Aleksandrov is a young scientist with consistent academic development, a clearly defined research focus in the molecular biology of cellular stress and extracellular communication, and proven scientific productivity through peer-reviewed publications and participation in nationally funded research projects.

The candidate has demonstrated excellent theoretical knowledge, proficiency in modern experimental methodologies, and the ability to analyse and interpret complex biological processes. The scientific results contribute to a better understanding of the mechanisms regulating cellular responses to stress, the role of autophagy, and the significance of extracellular vesicles as mediators of intercellular communication and potential biomarkers.

The dissertation fully satisfies the requirements for the award of the educational and scientific degree of Doctor (PhD) in Molecular Biology. It provides sufficient evidence of the candidate's scientific competence and his potential for future independent research.

In conclusion, I consider the dissertation entitled **"Synergistic Induction of Extracellular Vesicle and DNA Secretion by Autophagy Inhibitors and DNA-Damaging Agents"** to be an original, comprehensive, and scientifically significant contribution to the field of molecular biology.

Based on my overall evaluation of the scientific results, the originality of the research, the publication record, and the personal contribution of the doctoral candidate, I give my unequivocal positive assessment and vote in favour of awarding Aleksander Aleksandrov the educational and scientific degree of Doctor (PhD).

Sofia

Prof. Milena Georgieva, PhD:

10.07.2026
