OPINION

by Prof. Stefan Dimitrov, DSc
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regarding the dissertation of Prof. Dr. Iva Ugrinova Zlatkova, titled "Common Target, Diverse Agents: The Fundamental Vulnerabilities of the Tumor Cell as a Basis for Selective Antitumor Action" for the acquisition of the scientific degree "Doctor of Sciences" in professional field 4.3. Biological Sciences, scientific specialty "Molecular Biology"

I.Short Professional Biography

An alumnus of Sofia University, Prof. Ugrinova graduated in 1992 with a degree in Biotechnology, specializing in Gene and Cell Engineering. She went on to earn her PhD from IMB-BAS in 2002 with a dissertation focused on the *post-synthetic acetylation of the non-histone protein HMGB1*. Her academic foundations were further strengthened by prestigious research fellowships and guest professorships at the École Normale Supérieure de Lyon in France, supported by NATO and the Région Rhône-Alpes.

Professor Iva Ugrinova currently serves as Deputy Director of the Institute of Molecular Biology at the Bulgarian Academy of Sciences (IMB-BAS). She also leads the institute's "Chromatin Structure and Function" section, a role she has held since 2013, alongside a prior tenure as the Institute's Director from 2014 to 2022.

Prof. Ugrinova's past research centers on chromatin organization and the role of non-histone HMGB proteins in cell nucleus processes. Her recent work bridges the gap between ontogenesis mechanisms and experimental oncology, directly driving the development of innovative antitumor therapies. Her research team actively tests novel compounds—such as co-amorphous drug structures, metal complexes, and nanomaterials—to evaluate their targeted cytotoxic effects against cancer cells. Funded by the National Science Fund, she currently leads advanced projects into precision medicine and newly synthesized DNA-intercalating naphthalimides.

II. Strategic importance of the thematics

The main challenge of modern oncology is to discover drugs that specifically target the cancer cells and kill them, with having the smallest possible off targeting effect. The thesis is focused on this crucial problem and uses a highly commendable methodology, employing a comprehensive suite of contemporary cell and molecular biology techniques. The experimental design targets three pivotal vulnerabilities in cancer cells using therapeutic preparations of highly diverse chemical compositions. This combination of structural diversity

in the compounds and state-of-the-art methodology ensures that the functional data gathered is both high-resolution and translationally relevant.

The underlying framework of the three tasks of the thesis is exceptionally well-conceived. By utilising a cell model that contrasts the presence versus the absence of functional p53, the project establishes a robust platform to evaluate distinct cellular responses. This enables a precise assessment of how cells react when their core machinery is disrupted—specifically focusing on the vital networks that control genomic stability, redox homeostasis, and cell survival programmes.

III. Characteristics and Evaluation of the Dissertation and Its Contributions

The presented dissertation summarizes a significant volume of research work carried out over nearly ten years, dedicated to the development and biological characterization of novel synthetic and natural compounds with antitumor activity, the elucidation of their mechanisms of action, and the development of approaches to improve their pharmaceutical applicability. The first point I would like to note as a significant merit of the dissertation is the systematic way in which this substantial and seemingly diverse experimental material is organized. The work does not represent a mechanical collection of studies on individual compounds. The author strives to unify the results around a common biological concept—that structurally diverse antitumor agents can exert their effects by targeting a limited number of fundamental vulnerabilities of the tumor cell. Among these, central roles are occupied by disruptions in redox homeostasis, replication stress, DNA damage, cell cycle checkpoints, and the activation of various cell death programs. In my opinion, this is precisely what transforms the presented work from a series of pharmacological observations into a coherent biological study.

From the perspective of my own scientific interests, the results related to replication stress and the maintenance of genomic stability deserve special attention. DNA replication occurs within the context of chromatin, and any significant disruption of this process poses a dual necessity for the cell: to preserve genomic integrity while maintaining proper chromatin organization. In this sense, tracking the effect of the studied compounds on DNA synthesis, the accumulation of γH2AX, changes in the cell cycle, and the subsequent loss of proliferative potential represents a well-founded experimental approach.

The results obtained with the novel naphthalimide derivatives are particularly convincing. The reduction in EdU incorporation, accompanied by the accumulation of γH2AX, clearly demonstrates that the antitumor effect is not merely non-specific cytotoxicity, but is instead linked to replication disruptions and the subsequent activation of the DNA damage response. Additional value is brought to these experiments by the comparison of cell models with different p53 statuses. The distinct changes in the cell cycle in the presence or absence of

functional p53 allow for tracking how the state of this regulatory system influences the cell's response to the exact same replication stress.

This also leads to one of the interesting conceptual conclusions of the dissertation—that p53 plays a central role in integrating signals triggered by various types of cellular stress, but does not mediate the sole possible pathway to cell death. The presence of an antitumor effect in p53-deficient cells shows that if cellular homeostasis is sufficiently disrupted, alternative mechanisms can be triggered. This result is also important from a potential therapeutic standpoint, as disruptions in p53 function are characteristic of a large proportion of human tumors.

I also positively evaluate the manner in which complementary experimental approaches have been utilized. Cell lines of different origins and different p53 statuses were studied, non-tumor controls were included, and various aspects of the cellular response were analyzed—including oxidative and replication stress, DNA damage, the p53 and NF-κB signaling pathways, cell cycle changes, autophagy, and apoptosis. In such complex biological processes, the persuasiveness of conclusions can rarely rely on changes in a single parameter. It stems from the consistency of several independent experimental observations, and I consider this to be one of the core strengths of the presented work.

A good example of this approach is the research on the ferrocene-containing derivative DK-164. Its antitumor activity is examined in relation to oxidative stress, the p53 and NF-κB signaling pathways, cell cycle changes, and cell death. The comparison with the structurally related compound CC-78 is particularly informative. The loss of tumor selectivity resulting from a change in chemical structure is an important outcome, because it demonstrates that high cytotoxicity alone is not sufficient justification to consider a compound a promising antitumor agent. In this sense, I believe that tumor selectivity is correctly placed as one of the central criteria in evaluating the investigated compounds. The results support the idea that certain molecules can exploit pre-existing vulnerabilities of tumor cells—such as increased replicative load, impaired redox homeostasis, and limited capacity to cope with additional genomic damage. This is conceptually more interesting and promising than solely pursuing maximum cellular toxicity.

As another merit of the work, I would point out the link between fundamental and applied research. For a portion of the leading compounds, biological characterization is followed by the development of polymeric nanosystems aimed at overcoming unfavorable physicochemical properties. In this way, the study follows a logical progression—from the identification of a biologically active compound, through the elucidation of its mechanism of action, to the first steps toward improving its potential pharmaceutical applicability.

The results included in the dissertation have been published in 14 scientific papers in international peer-reviewed journals indexed in Web of Science and Scopus. This represents a solid scientific output and shows that a significant portion of the presented results has already undergone independent international scientific evaluation.

The scientific contributions are clearly formulated and can be grouped as fundamental, experimental, and applied. Among them, I would highlight as most significant: the establishment of common cellular mechanisms targeted by structurally diverse antitumor agents; the determination of the role of p53 as a central regulator of the response to various types of cellular stress; the establishment of p53-independent mechanisms of cell death; the characterization of replication stress as an important part of the mechanism of action of the novel naphthalimide derivatives; as well as the establishment of a link between the mechanism of action and selectivity toward tumor cells.

Naturally, the wide scope of the presented topic means that not all directions have been developed to the same mechanistic depth. In the future, it would be interesting to study some of the most promising compounds in greater depth regarding their immediate molecular targets, the dynamics of replication structures, and the mechanisms linking replication stress to chromatin changes and cell fate. However, this does not diminish the value of the current work, but rather outlines the logical next questions arising from the obtained results.

In conclusion, the presented dissertation contains a significant volume of original experimental material and demonstrates the author's ability to unify results from different research directions into a coherent and scientifically argued concept. I consider the systematic approach and the striving to interpret antitumor activity through fundamental mechanisms of cell biology—such as replication and oxidative stress, disruptions in genomic stability, cell cycle control, and programmed cell death—to be of particular merit.

The presented dissertation meets the requirements of the Development of Academic Staff in the Republic of Bulgaria Act, the Regulations for its implementation, and the corresponding regulatory requirements for acquiring the scientific degree. On this basis, I give my positive evaluation of the dissertation and confidently recommend to the esteemed members of the Scientific Jury to award Prof. Dr. Iva Ugrinova Zlatkova the scientific degree of "Doctor of Biological Sciences" in professional field 4.3., scientific specialty "Molecular Biology".

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Review prepared by: ……
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