TO WHOM MAY IT CONCERN:

Evaluation of the doctoral Thesis of Ms. Sylwia Lewoniewska, entitle "The role of estrogen receptor status in proline dehydrogenase/proline oxidase-dependent apoptosis in breast cancer cells".

As requested by the Medical University of Bialystok, I have evaluated the Doctoral Dissertation of Ms. Sylwia Lewoniewska. The studies presented in the dissertation by Ms. Sylwia Lewoniewska reports evidence for the important role of estrogens in the mechanism of PRODH/POX-dependent apoptosis in breast cancer. The role of PRODH/POX in the ATP production and ROS production is described and the consequences of both actions in the survival or apoptosis of the cell, and how the proline availability may play a key role in the switch of both ways. Estrogens are involved in collagen metabolism as stimulators of its biosynthesis. These processes need a large amount of proline, limiting substrate availability for PRODH/POX-dependent functions.

The dissertation is 120 pages long including the title page, table of contents with source of research funding, articles included in the dissertation, abbreviation list, the materials and methods included in the two papers related, the Scientific Achievement, and the author-coauthorship statements, that help the reader to reach a better idea of her value as a scientist.

It is very remarkable that this thesis includes 2 publications, a review, and an original paper, in which Ms. Sylwia Lewoniewska is the first author, in 2021 in peer-reviewed journals featuring in the Journal Citation Reports and recognized in the international scientific areas. It is worth pointing out that academic achievements of Ms. Sylwia Lewoniewska are not limited to the works related to her dissertation, as is shown in le list of other scientific publications and monographs, a total of 14, plus 13 conference abstracts. Noteworthy is also the fact that her studies were funding from National Center of Science (Poland), from European Union Funds and by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowske-Curie grant.

The Doctoral Dissertation includes an introductory general section that allow the reader to follow all the dissertation and the two papers derived. This introduction is clear and informative, being critical to understanding the work, and is absolutely supported by the first review paper.

The study has well-defined goals that correlate with the paper derived directly from the Thesis. The achievement of this goal required a detail planning and use of laboratory methods included in the Material and Method section. These includes cell culture, DNA, collagen biosynthesis, determination of enzymatic activities, Western Blot, LS-MS, ROS generation quantification. All of them performed by Ms. Sylwia Lewoniewska except the silencing of the cells, that have been developed in the Department of Medicinal Chemistry at University of Bialystok.

Ms. Sylwia Lewoniewska has generated many results, as is demonstrated on the original paper derived from her work. She was able to present her results in a very accessible and simple way, a clear indication of an in-depth understanding of the field. The results section is very clear and

concise allowing the reader to easily understand the detail results extended in the original paper.

The conclusions are relevant and clear: First she developed a PRODH/POX silenced MCF-7 and MDA-MB-231 breast cancer cells generated by shRNA technology, in collaboration with the Department of Medicinal Chemistry, that enable an analysis of the functional significance of PRODH/POX in apoptosis/survival in breast cancer cells. Second, they were able to inhibit collagen biosynthesis in breast cancer cells, inducing PRODH/POX, with a PPAR-γ agonist (TGZ), inducing apoptosis through a ROS mechanism. Finally, it is suggested a possible novel breast cancer therapy combining PPAR-γ agonist and anti-estrogen treatment.

I would like to point out the fact that this dissertation lacks a General Discussion section in which main conclusions drawn from the experimental studies are positioned and discussed against the data from other authors describes in the literature. Of course, this discussion can be extracted from the original paper, but if it would be included in the dissertation would provide Ms. Sylwia Lewoniewska the opportunity to formulate novel ideas that would be too speculative to write down in a manuscript for publication, allowing the candidate to formulate her thoughts on the role of this combined treatments in breast cancer.

And at last, but not least, I strongly recommend Ms. Sylwia Lewoniewska, not describe the aims and in general all the work as a personal aim (*I have hypothesized…, My scientific interest…, I have hypothesized ….)*, I am sure that during the period he have been working on the research she has been work as a team, with the supervisors and other research group members, as she described in the co-authorship statement. Research is a Teamwork.

In conclusion based on this evaluation, I strongly recommended The Senate of The Medical University of Bialystok, with this positive report, that this dissertation move to further stages of the doctorate process and may be subjected to a public defense. Furthermore, I also propose the dissertation to be distinguished with the maximum honors.

If you need any further information do not hesitate to contact me.

Sincerely

Firmado por VIANA ARRIBAS MARTA MARIA - ***9657**
el día 19/09/2022 con un certificado emitido por
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