

## Scientific review

of the doctoral thesis entitled „The role of tumor associated proteins in DNA damage and Unfolded Protein Responses and their use as target for the development of novel canine cancer therapy”

by PhD student Beatriz Hernández Suárez MSc,

supervisor Aleksandra Pawlak DVM, PhD, associate professor

second supervisor David A. Gillespie, PhD, FRSE

### 1. Presentation of basic information about the candidate:

The scientific background of the candidate, Mrs. PhD student Beatriz Hernández Suárez MSc is very solid, comprising of two important internships, in cancer research and in molecular biology, the first one being a one-year internship at Centre des Recherches en Cancérologie in Toulouse, France (10.01.2022 – 09.01.2023), studying the „RNAreg : RNA binding proteins and genotoxic stress” in the team of Stefania Millevoi. The second one was a three months Erasmus + internship (06.09.2021 – 05.12.2021), in the team of Andres Aguilera, supervised by Sonia Baroso, regarding „DNA combing assay” at Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER) in Seville (Spain).

PhD student has a very good practical training, both regarding cancer therapy and she masters the state-of-the art protocols and techniques, due to the fact that she attended important and relevant training in these fields, such as: Genetic modified cell lines (GOM) as a model, during 17-18.11.2022, organized by CNRS Formation, France; a training course on „Image J” software organized by Inserm DR Toulouse – Formation (France) during 28-30.03.2022, an introductory training regarding omics sciences entitled „Intro to Omics course, a 5 day course, during 24-28.10.2022, organized by University of Glasgow, certificate issued by Glasgow Polyomics (online); two one day online trainings, organized by Harvard Medical School, in 25.06.2021, with two important topics, one regarding „HMX Pro Immunology – Immuno – oncology” regarding the immune response to cancer cells and the second one „HMX Pro Pharmacology –essentials” regarding the interactions between the body and drugs and two trainings organized by Abcam, the first one regarding „Antibody basics training” (08.2020) and the second one comprising a „Western Blot training” during October 2020. She was also interested in enhancing her knowledge regarding grant application so she attended a practical workshop „European Grant Writing and Management Workshop” organized online, by Lund University, during 09.11.2021 and 14.12.2021.

Since 2019 till present she is following a PhD student scholarship, an interdisciplinary programme in the frame of the University of Life Sciences in Wroclaw, Poland. As a reward of her doctoral outstanding activity she was rewarded twice, during 02.2020-09.2020 and 10.2022-06.2023 with PhD scholarships for academic excellence „STER – Doctoral Scholarships for Foreigners no. PPI/STE/2018/1/0002/U/001.

Mrs. PhD student Beatriz Hernández Suárez MSc succeeded to disseminate very well the results of her research, by attending important congresses and conferences in the field. Among them, the most important are the ones organized by the EACR (European Association for Cancer Research), starting in 2020 as participant, and continuing after that with a poster presentation in 2021 regarding „CHOP overexpression in canine lymphoma cells” and two posters in 2023, the first one regarding „Canine cancer: a perfect model to study the role of DDR pathways in tumorigenesis and therapy” and the second one about „The use of PhenDC3 to induce cell death in canine lymphoma and leukemia cells by stabilizing G-quadruplexes”. It is enough to follow the titles of these works to be able to see the progress in the field she did. The congress New Trends in Scientific Research was

attended twice, in 2020 with two oral presentations, one regarding the „Influence of proper lysis buffer selection in western blot technique” and the other one regarding „Testing UPR system in canine cancer cells” and a poster entitled „Dog as a model for DDR study” and in 2022 with a poster „The importance of antibody selection – analysis of C/EBP homologous proteins (CHOP) in canine cancer cells”.

The complete scientific achievement was reached by the PhD. student by her publications, starting with the first one, back to 2018 with a published article in Scientific Reports as a co-author, continuing in 2020 and 2021 co-authoring some articles published in scientific journals like Molecules, Catalysts, Journal of Veterinary Internal Medicine and the last one in Veterinary Compared Oncology, as first author, being a scientific review regarding „DNA damage response proteins in canine cancer as potential research targets in comparative oncology”. In 2023 she published her own research results, as first- author in journals like Frontiers in Veterinary Sciences „Studying the DNA Damage Response pathways in hematopoietic canine cancer cell lines – a necessary step for finding targets to generate new therapies to treat cancer in dogs” and the second one, regarding the second objective of her research, the Unfolded Protein Response pathways in hematopoietic canine cancer cells, the first step for the introduction of new therapies in dog’s cancer, published in Journal of Veterinary Research, which are the main important pillars of her scientific achievements.

The entire scientific activity led to a H-index of 3 in Web of Science and a H-index of 4 in Google Scholar, which is a significant scientific result obtained within only a few years of research.

## 2. Presentation of information about the evaluated doctoral thesis:

The present doctoral thesis is entitled: „The role of tumor associated proteins in DNA damage and Unfolded Protein Responses and their use as a target for the development of novel canine cancer therapy” and was written by Mrs. PhD student Beatriz Hernández Suárez MSc, under the supervision of Mrs. Associate Professor Aleksandra Pawlak and David A. Gillespie, PhD, FRSE, as a second supervisor.

The structure of the present thesis follows the pattern of a scientific thesis, which is based on the published papers of the author. In the introductory part the author presents the current scientific detailed knowledge regarding the two most important mechanisms which are present in human and dog cells in order to protect themselves from carcinogenesis, DNA damage response (DDR) and Unfolded Protein Response (UPR) pathways. The aim of the thesis is very well established and specified as being the discovery of new target molecules which can facilitate new anticancer therapy in canine cancer. In order to achieve the purpose, the changes in the two selected pathways – the DNA damage response (DDR) and the Unfolded Protein Response (UPR) pathways are analysed at the molecular level. The methodology used in the scientific research is presented, followed by the introduction to the first publication which is a state-of-the art literature review regarding the use of DNA damage response proteins in canine cancer as potential targets for research in comparative oncology. The next steps are the presentation of the two research publications: the first one regarding the study of the DDR response pathways and the elucidation of their molecular mechanisms, and the second one, representing the initial characterization of the UPR pathway in hematopoietic canine cancer cells, the first step for introducing new therapies in dog oncological treatment. Finally, the conclusions of the research are very concisely presented and well synthesized, directly linked to the purposes of the paper.

The PhD student prepared a very well documented and exhaustive list of scientific literature. The theoretical approach of the thesis is provided by a comprehensive literature research of the state-of-

the art discoveries and reviews in the oncology field (156 titles). The first research chapter which presents an initial characterization of the expression and activity of the key components of the DDR in a panel of hematopoietic canine cancer cell lines, with the use of available antibody reagents, was previously documented from 102 references. The second research regarding the characterisation of UPR pathway in canine cancer cell lines was based on 38 scientific literature titles. Furthermore, for the entire thesis content 87 references were consulted and cited.

The aim and hypothesis for the entire study is presented at the beginning of the thesis and the aim and objectives of the research are very well presented and synthesized by the Ph. D student. The author found that there is missing information in the scientific literature regarding the most important route in canine cancer: ATR-Chk1 pathway, which may function similar in humans, one of the goals of the research being the validation of methodologies and reagents to allow the investigation of molecular features of DDR in canine cancer. The second research aims to facilitate the molecular research of UPR, which can lead to the initiation of apoptosis process, both apoptosis and alterations of UPR pathways being previously described in cancer cells. The two directions of research and the working hypothesis are very well synthesized and these objectives, once established in the beginning are followed during the entire research.

In order to achieve the goal of the research, Mrs. PhD student Beatriz Hernández Suárez MSc implemented in the present study the most modern methods of molecular biology, such as: cultivation of a panel of canine lymphoma and leukemia cell lines, RNA sequencing of two selected cell lines in order to check the expression of the DDR mRNA levels, Western blot technique to analyse, both DDR and UPR protein levels, qPCR for analysing mRNA levels, DNA combing assay to study the dynamics of replication at the replication fork in the two selected cell lines and flow cytometry with annexin V FITC/PI or caspase 3/7 in order to study apoptosis. All the results were statistically analysed, using Mann-Whitney test for the combing assay analysis and TIBCO software Inc. Statistica. The multitude of very modern techniques used in this thesis proves the comprehensive practical knowledge of the PhD student.

The discussions presented in thesis are very detailed, a step-by-step approach explaining each stage of the results of the research. The first article is, in fact, a discussion regarding the present information in the field regarding DNA damage response and its role in cancer, selected DDR proteins and their potential role in canine research, clinical aspects of DNA damage research in canine cancer. The second, being a research article presents a detailed discussions for each obtained result, comprising the fact that the RNA-sequencing analysis revealed the presence of principal components of the DDR pathways in canine cell lines, such as CLBL- 1 and GL – 1 which were selected as representative cells of common hematopoietic cancers like lymphoma (CLBL- 1) and leukemia (GL – 1). The discussions regarding the expression and activation of the DDR pathway components in canine cancer cells emphasize the screening of the basal expression of DDR proteins, using BLAST analysis for ATR proteins which prove to have a large percentage of similarities with humans proteins, such as: ATR (94.75%), Claspin (84.47%), Chk 1 (96.7%), Rad51 (99.12%). These proteins are recommended to be used as promising targets for future analysis. The discussion of the second research study provides the validation of the techniques and reagents which can be used for future studies regarding the molecular basis of UPR in dogs and their high similarity between UPR components and mechanisms of dogs and humans and the first evidence that canine lymphoma and leukemia cell lines may be a suitable model to study UPR in cancer.

The current research has an important practical approach due to the fact that it is for the first time where the DDR and UPR pathways in canine cells were characterized, together with finding the tools and techniques that can be used to study these pathways in dogs. It is also for the first time

when the validation in dogs of some reagents, which were originally used for humans or mice, will facilitate the research on the role of DNA damage and endoplasmic reticulum stress in carcinogenesis in dogs. The new discovered research tools will allow the future development of new therapies in veterinary oncology.

I want to ask the PhD candidate to answer me the following questions regarding the research:

- On what criteria do you selected and used this panel of lymphoma/leukemia cell lines, (CLBL-1, CLB-70, GI-1 and CNK-89) and why did you used this panel of cells for research?
- In order to induce the DNA damage you used etoposide treatment (20  $\mu$ M for 2 h). Why did you choose this concentration and why you did not repeat the treatment, knowing that chemotherapeutical treatment is applied repetitively? Why you didn't use comparative groups of cells with different concentrations applied different amount of time?
- Do you have any possible explanation why higher expression of target genes was found in the CLBL-1 cell line compared to GL-1 (except for EIF2a). Why do you consider that this exception occurred?
- Which do you consider that will be the first steps forward, after concluding this research, regarding targeted-anticancer therapies in canine and human cancer?

As it was previously presented, the present doctoral thesis presents many original solutions for canine cancer research and future therapy. The changes in the expression levels of principal proteins of DDR and UPR pathways which were found during this research in canine cells may indicate the potential use of elements of these pathways as new therapeutic targets.

The general scientific background of Mrs. PhD student Beatriz Hernández Suárez MSc is at a very high level, both regarding the current theoretical knowledge in the field of cancer research and also in mastering some modern techniques of molecular biology, such as: RNA sequencing, Western blot technique, qPCR for analysing mRNA levels, and the novel technique of DNA combing assay.

4. If the reviewer concludes that the doctoral thesis deserves distinction, the justification for this conclusion should be provided.

I conclude that the doctoral thesis deserves distinction, based on all the above mentioned features. I want to underline again, the high originality level of the research presented in the paper, the comprehensive documentation in the oncology field, the characteristic of the interdisciplinary research (molecular biology – oncology – veterinary medicine – human medicine), the potential practical application in cancer therapy, the state-of-the art techniques applied for the research, the presentation of the results and the discussions and the great ability of synthesis and scientific writing of Mrs. Beatriz Hernández Suárez MSc .

**Based on the above, I conclude that the doctoral dissertation meets the requirements specified in article 187, paragraphs 1-4 of the Act of July 20, 2018, the Law on Higher Education and Science (consolidated text - Journal of Laws of 2023, item 742, as amended), in polish: art. 187 ustawy z dnia 20 lipca 2018 r. prawo o szkolnictwie wyższym i nauce (Dz.U.2023.742 t.j ze zm.). Therefore, I propose to the Discipline Council of Veterinary Medicine at the University of Environmental and Life Sciences in Wrocław to admit the doctoral candidate to the next stages of the doctoral procedure.**

Cluj-Napoca

Prof. Dana Pusta, DVM, MSc, PhD

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