

Review

the PhD dissertation of Beatriz Hernandez Suarez MSc with the title.:
„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”

*The present review was composed pursuant to the directive issued by the Veterinary
Discipline Council at Wrocław University of Environmental and Life Sciences
dated 19-09-2023.*

In the dissertation, Ms Beatriz Hernandez Suarez, MSc, undertakes an extensive exploration into the intricacies of the DNA Damage Response (DDR) and the Unfolded Protein Response (UPR). DDR is a sophisticated cellular process designed to identify and mend DNA lesions, whereas the UPR is a cellular apparatus ensuring protein homeostasis. DDR encompasses myriad mechanisms ranging from cell cycle checkpoints to DNA repair pathways and apoptosis. Notably, the interplay between DDR and UPR has been discerned under hypoxic conditions and interfaces significantly with immunological pathways. The implications of DDR in prognosticating the outcomes of immunotherapy hold paramount importance for oncological therapeutics. Progress in this domain promises potential avenues for devising the most efficacious combination treatments and illuminating novel biomarkers for optimizing immunotherapeutic strategies.

It is noteworthy that the scientific community's engagement with DDR has amplified; evidenced by an approximately 100-fold surge in scholarly publications over the past quarter-century. The research themes broached encompass pivotal elements both from an academic perspective and the practical aspects of the preliminary phases of drug innovation. Topics

span from oncogenic transformations to cell mortality under genetic instability, cellular death due to oncogenic stress, enhancement of repair mechanisms, and survival pathway dynamics. A significant portion of these aspects intricately intertwine with DDR and UPR. One of the formidable challenges in enhancing preclinical paradigms pertains to the faithful replication and translation of the tumor microenvironment (TME) in animal models. The foundation of the doctoral work rests upon a trilogy of interconnected scientific papers, each of which has been recognized in esteemed academic journals.

Given the foregoing considerations, the objective and ambit of the doctoral endeavor, which is centred on the identification of novel molecular targets for advancing canine oncological treatments, are both pertinent and timely. The research work is set against the backdrop of investigating the DDR and UPR mechanisms and epitomizes cutting-edge methodologies congruent with contemporary trends in oncology drug development.

Spanning 101 pages, the dissertation incorporates the full text of the aforementioned trio of publications. Distinct sections include the title page, acknowledgement, funding sources, list of foundational publications, abstracts in both English and Polish and a compendium of abbreviations. Moreover, there are sections dedicated to the introduction, objectives, methodologies, and the individual papers. Preceding each article is a succinct preface. The dissertation culminates with the summary, conclusions, bibliography, documented scientific achievements, and attestations of authorship, which are duly signed by Ms. Hernandez, highlighting her seminal role in each manuscript. The table of contents facilitates effortless navigation through the doctoral text. Although the abbreviation listing is comprehensive, the incorporation of protein name synonyms corresponding to the acronyms would augment the utility. A few acronym choices, potentially those not aligned for example with Uniprot recommendations, might necessitate revision.

Within the dissertation, each abbreviation or symbol introduced for the first time is meticulously elucidated, ensuring clarity for readers. Both Polish and English summations encapsulate the essence of the thesis, with the English rendition being an accurate representation of its Polish counterpart.

The basis of the dissertation encompasses three scholarly articles that delineate a coherent thematic trajectory. Information regarding the respective impact factors (IF) of the journals, as of the pertinent year, and the scores attributed by the Ministry of Science and Higher Education is provided. It's imperative to underscore that solely from this trio of publications, the Author accrued an impressive 410 points and amassed a cumulative IF exceeding 7.9. Ms. Beatriz Hernandez Suarez, MSc, holds the distinction of being both the primary and corresponding author for all three articles. The precise extent of her involvement in these publications has been demarcated descriptively in the attached statements, covering all facets of the research process. Given the breadth of her contributions, it is unequivocal that Ms. Hernandez played a pivotal role in the fruition of these published works.

The Introduction adeptly sets the stage, elucidating the intricate research methodologies that are further detailed in the individual publications. The research objectives are articulated with precision, offering no ambiguities. Subsequent sections delve into the methodologies, with comprehensive descriptions encapsulated within the accompanying articles. The dissertation is crafted with clarity, factual accuracy, and professional eloquence. A meticulous attention to stylistic nuances and detail is evident. The trilogy of attached articles, supported by judiciously selected references, mirrors the core theme of the dissertation and its declared objectives, underscoring Ms. Hernandez's profound academic prowess. Her scholarly acumen is further highlighted by the mature narrative style she employs. Each article is prefaced with a succinct segment spotlighting the salient findings.

The first article, foundational to the doctoral endeavor, provides a comprehensive examination of the current understanding of DDR in canines. This exhaustive review is anchored on an analysis of 156 publications. Notably, the authors deftly interlink extant knowledge, attempting to bridge canine findings to human medicine and its implications. This methodology is invaluable, often serving as the cornerstone for refining preclinical models and validating prospective clinical markers in human medicine. The authors frequently emphasize structural similarities (expressed in percentages), yet in a clinical context, pathophysiological relevancies related to specific structural components may hold more significance. In concluding Table 1 of the first paper with a summary of similarities and differences in DDR proteins between humans and dogs, it should be underlined that some of those proteins do not exist in the human clinical trials world. Based on [clinicaltrials.gov TopBP1](https://clinicaltrials.gov/ct2/show/study/TopBP1), [Chk2](https://clinicaltrials.gov/ct2/show/study/Chk2), p21, p27,

cyclin A, in practice does not exist in current clinical studies. Of course another yes indeed, like Chk1, Rad51 (>30 studies); PTEN, PCNA (>300); BRCA (>400) and p53 (>500 clinical studies). But even p53 is used only in 0.5% of clinical studies indexed by this specific database (“cancer” as a keyword > 100,000 studies). Biomarkers' efficacies hinge on several attributes, including sensitivity, dynamic range, specificity, and translatability, and these ought to be weighed judiciously. It would therefore be advantageous if, for instance, Table 1, titled “Percentage of protein homology between human and dog species,” incorporated insights into the real-world clinical applicability of the enumerated proteins. Additionally employing resources like proteinatlas.org for discerning the distribution of proposed markers across cancer cell lines would be a prudent addition in future deliberations. Nonetheless, these observations do not diminish the merit of the thesis; the trajectory of the research and the envisioned applications of these markers in clinical contexts are commendable.

The second manuscript offers a notable revelation regarding the shared DDR gene expression in canine lymphoma and leukemia cells, elucidating how specific key components manifest differently across tumor types. The manuscript pioneers the confirmation of ATR at the protein level in canine lymphoma/leukemia cells. Drawing parallels with human clinical applications, the document elucidates how variations in ATR expression correlate with sensitivity and resistance to oncological drugs. Another biomarker, claspin, was validated across three distinct canine cell lines. What is remarkable only one study has analyzed claspin in canine cells. The Author mentions claspin as a marker highly expressed in prostate cancer cells. Based on a comparison of 8 different human prostate cancer cell lines with adrenocortical cancer cell lines looks like adrenocortical cancer will be a better example. However, data from only one line was made available (see proteinatlas.org). It was very interesting that in the case of Rad51 the antibody employed recognized Rad51 protein in all the canine cell lines tested. The highest was confirmed in the CLBL-1 line, both at the protein and gene level. In this place is important to explain the applicability of these findings by current progress in human clinical practice. Now Rad51 is used as a biomarker in 61 clinical studies around the world (clinicaltrials.gov). The current work is a great example of the applicability of “pure science” in the discovery of tools for real drug development processes both in human and veterinary medicine. Proof of the concept and key conclusion from the second paper explain this statement very clearly “(...) canine cancer cells represent a tractable

model to study cancer (...)" . As can be seen from the example of this study, the author blazes a new trail and sets future trends.

The third manuscript navigates the intricacies of the unfolded protein response (UPR) pathway, illuminating its pivotal role in oncogenesis. Cancer cells' ability to thrive amidst prolonged ER stress by manipulating the UPR system is well-acknowledged. The Author astutely posits the proteins within this pathway as potential therapeutic targets. With rigorous analysis, it's established that a mere 1% of genes expressed in canine lymphoma and leukemia were instrumental in the UPR system. The manuscript's commendable validation of several antibodies positions them as valuable tools in UPR system evaluation. The manuscript's avant-garde approach propounds that canine lymphoma/leukemia cells can serve as model systems for understanding the UPR pathway in cancer. Its trailblazing nature and applicability align seamlessly with the anticipations of the academic fraternity.

The attached publications contain all the elements necessary for original papers. The publications have been prepared state of the art and are appropriately illustrated, which makes it much easier to understand and verify the scope of the research. The scope of the reported works required the PhD candidate to master advanced methods. It confirms that Beatriz Hernandez Suarez MSc has mastered the difficult research workshop and also confirms her great commitment to structuring it and finalizing the research.

The summary and Conclusion sections end the dissertation in a compact and synthetic way. In this part of the thesis, the PhD candidate referred to the detailed objectives mentioned earlier and summarized the obtained results. The results obtained and the hypotheses are very valuable both from the point of view of veterinary medicine and from the point of view of clinical practice in human medicine. The prepared work perfectly communicates all aspects of the work carried out, and the text shows the great commitment and enthusiasm of the Author in creating individual elements of the dissertation. The research described represents a logical sequence of works. Both the scope and conclusions clearly reflect the expectations of the scientific community and correspond to the high dynamics of growing needs in this area.

In the Literature section, 87 references have been judiciously cited, illuminating the research topic's expanse. These references, consistently formatted, are meticulously

analyzed, enhancing the dissertation's depth. The manuscript not only resonates with inspiration but also provokes pertinent queries:

Question 1: *Your research touches on the important problem of validating the dog model in testing drugs intended for humans. Please summarize the current state of knowledge regarding spontaneous cancer models in dogs that can be used or are used in research on drug candidates intended for humans.*

Question 2: *Based on the example of bortezomib and based on your experience related to analyzed markers. What other drugs currently used in human pharmacotherapy but not present in veterinary medicine (even at the experiment stage) would you propose to study in canine cancer and why?*

Question 3: *Why do differences in the tumor microenvironment (animal models versus human clinical studies) influence the translation of the pharmacodynamic effect?*

The list of scientific achievements of the Author of the PhD dissertation is long and covers 8 publications showing her deep expertise and experience in the field. In summary, I confirm that the PhD dissertation by Beatriz Hernandez Suarez MSc meets the requirements set for PhD theses. The results of the presented research have been published in reputable journals and represent a summary of the doctoral candidate's innovative achievements and analysis. The research objectives set by the candidate have been achieved, and the results of the conducted research have been subjected to detailed verification. The scope of the research carried out by the candidate has significant practical and cognitive importance, as well as high potential for non-clinical and clinical practice applications both in veterinary and human medicines development. The manuscript of the thesis has been prepared with attention to detail. The evaluated work is written clearly and uses professional terminology. The doctoral candidate has demonstrated the ability to use an extended group of references and master complex laboratory methods.

The comments mentioned above, do not detract from the substantive value of the reviewed PhD thesis. Therefore, in my opinion, the **PhD dissertation of Beatriz Hernandez Suarez MSc with the title "The role of tumor-associated proteins in DNA damage and unfolded protein responses and their use as targets for the development of novel canine cancer therapy"** fully 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.)

Taking the above into consideration, I hereby submit to the Scientific Council of Veterinary Sciences at the University of Life Sciences in Wrocław a request for the admission of Beatriz Hernandez Suarez MSc to further stages of awarding the doctoral degree process.

I also submit to the Scientific Council of Veterinary Sciences at the University of Life Sciences in Wrocław a request for the distinction of the aforementioned dissertation.

prof. dr hab. Tomasz Grabowski