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Reviewers Dissertation Thesis Report

Autor of the Dissertation: Youssif Budagaga, M.Sc.

Doctoral Study field: Pharmacology and Toxicology

Authors workplace: Faculty of Pharmacy in Hradec Králové, Department of Pharmacology and Toxicology

Dissettation thesis title: Study of interactions of new anticancer drugs and their utilization for modulation of multidrug resistance in solid tumors

Supervisor: Assoc. Prof. RNDr. Jakub Hofman, Ph.D.

Reviewer Jan Juřica, Assoc. Prof, PharmD., Ph.D..
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The main focus of the dissertation is pharmacokinetic drug resistance mediated by transporters and enzymes in lung cancer and the possibility of its modulation by pharmacokinetic interactions of targeted anticancer drugs. The thesis is conceived as a collection of annotated papers. The studies performed focused on elucidating the interaction between targeted drugs

used in the treatment of NSCLC and ATP-binding cassette (ABC) transporters and cytochrome P450 (CYP) enzymes, the key players in MDR development. Through in vitro, ex vivo, and in vivo models, the research demonstrates the potential of these novel drugs as dual activity modulators, effectively combating pharmacokinetic resistance. The integration of three-dimensional primary NSCLC organoids and patient-derived xenograft (PDX) models highlights the translational relevance of the findings. The dissertation emphasizes the importance of innovative research approaches in oncology and advocates for personalized treatment strategies tailored to individual patient needs, improving chemotherapy efficacy and patient outcomes.

The main objectives of the dissertation were:

- Investigation of the inhibitory interactions of targeted therapeutics toward ABC efflux transporters and CYP450 biotransformation enzymes
- Assessment of the chemotherapeutic MDR-reversal efficacy of interacting novel targeted therapeutics using *in vitro* and *ex vivo* models
- Evaluation of the mRNA expression levels of MDR-related transporters/enzymes following the exposure to novel targeted therapeutics.
- Establishment of NSCLC PDX *in vivo* mouse model to validate the outcomes of *in vitro*/*ex vivo* MDR-reversal combinations.

In the dissertation, there were used *in vitro*, *ex vivo* and *in vivo* standard (WB, metabolic activity assessment), novel and even highly innovative approaches (3D NSCLC organoids and in-house developed PDX model) to study interactions of anticancer drugs with respect to MDR.

The results of the dissertation indicate variable levels of inhibition of MDR-associated ABC transporters, potentially overcoming resistance in NSCLC. Also, different patterns of CYP inhibition by the tested compounds were revealed. The tested compounds were found to inhibit the activity of the ABC transporters and CYP enzymes rather than influence their mRNA levels. Thus, the author concludes that the investigated drugs have little potential to strengthen the MDR phenotype or induce drug-drug interactions based on enzyme induction.

The conclusions are clearly stated, the author's publications annotated in the dissertation are original and bring new important scientific findings. I appreciate the commentary on the authors' percentual contribution for each annotated paper. The papers have been published in high-quality journals (Q1/Q2), certainly, all of them have undergone a rigorous refereeing process and their scientific contribution is credible.

Comments:

The formal preparation of the dissertation is at a good level (language, style, formatting), only figures 3,5 and 8 are of poor quality (in the printed version).

In the introduction, the section on the clinical use of monoclonal antibodies in the treatment of NSCLC is completely missing.

Questions:

- On page 14, the author mentions that CYP enzymes "play a significant role in influencing pharmacodynamic activities of drugs.". **Could you mention some examples of CYP playing a significant role in drug PD?**
- **Which model or combination of models (not only according to your experience from the dissertation) do you find most useful and best correlated with clinical effects?**
- Beta-actin is often used as a loading control in the Western blot analysis. However, sometimes, it may be the source of bias. **Did you check beta-actin variability in the blots? Are there some other loading controls available?**
- ABCB1 is not only an originator of the MDR, but also the essential protective mechanism of the human body. As we can see in the case of Paxlovid, inhibitors of multiple transporters/enzymes are very often avoided due to their significant DDI potential. **Could you suggest the future direction of the research on how the MDR could be surpassed selectively?**

Conclusion: The author's concentrated research focus on the topic of his dissertation is evident throughout the thesis. The author has brought new original findings in the field of modulating MDR in anticancer chemotherapy. It is clear from the thesis itself and from publications, that

the author has mastered the research methods used and has made a major contribution to knowledge in the field of personalized therapy in oncology.

The objectives of the thesis were met, the submitted dissertation fulfilled the usual standards and criteria of a dissertation thesis, and I recommend the thesis for defence.

Dr. Budagaga has demonstrated the ability of independent creative scientific activity. Based on the successful defence, I recommend the award of the academic degree of Ph.D. according to § 47 of the Higher Education Act No. 111/98 Coll.

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