Assessment of doctoral thesis

Study on the role of pharmacokinetic mechanisms of drug resistance in new anticancer drugs with a focus on solid tumors

by Mgr. Dimitrios Vagiannis

This doctoral thesis presents the study of multidrug resistance mechanisms with primary attention to ABC transporters active in drug efflux and main metabolizing enzymes, cytochromes P450. Interactions between small targeted molecules and these players in multidrug resistance (MDR) to anticancer drugs were studied using state-of-the-art technologies. *In vitro* and *in silico* experiments revealed significant modulatory effects potentially limiting the risk of MDR. The main aims of the doctoral thesis are original and both scientifically and clinically relevant. MDR may cause the failure of oncological therapy and increase the risk of patients' death. Even incremental progress in controlling this phenomenon in clinical setting would have a significant socio-economic impact.

The thesis is written in well understandable English and divided into Abstract, Introduction, Theoretical Background, Aims, Results, Summary, Conclusions and Perspectives, and References sections. All relevant publications are attached, and section Results also includes a detailed description of the candidate's contributions to each paper. He is the first author of two out of five published and two out of three submitted works.

Considering formal matters, I do not understand the meaning of separate sections, Introduction, starting with general information about cancer, and Theoretical Background, again starting with additional information about cancer. These parts could perhaps be in one section. In general, the introductory part is sometimes simplistic and a bit flat, with moderate narrative value. I feel the author missed the opportunity to survey the MDR area on top-level that would be in harmony with exciting results presented further. The problem of drug inactivation concerns mainly the liver and not tumor cells, where we frequently observe the downregulation of majority of drug metabolizing CYPs (The Human Protein Atlas). The role of the second phase of biotransformation, e.g., GSTs, SULTs, or UGTs in MDR is just depicted in Fig. 8 without further explanation in the text. The same applies to ABCs, whose physiological roles (A, D, and G families) remain untouched together with the lack of transporting activity in E and F, although there are connections to cancer recently revisited. Other ABCs suspected to work in MDR as ABCC10/11 are not mentioned either. Role of susceptibility, e.g., genetic polymorphisms, somatic variability, or epigenetics is left unattended. One or two sentences with proper references would be enough to broaden the insight. Some terms are somewhat clumsy, e.g., "an increase in detoxification" (page 17), i. e. inactivation, or uselessly unspecific, e.g., "the risk of febrile neutropenia increased many-fold" (page 18) – how many fold? Furthermore, why original papers of Hanahan & Weinberg introducing cancer hallmarks in 2000 and updated in 2011 were not cited? Although it's perhaps my one-sided opinion, I missed at least one reference from Fred Guengerich who published many seminal works, including great surveys, about CYPs roles in drug metabolism with connection to cancer, e.g., metabolic activation of chemicals, including drugs (e.g., doi: 10.1007/s00204-020-02971-4 and preceding papers). Nevertheless, introductory parts do not harm the understanding of the matter and interpretation of results provided further.

Study aims are well-formulated and attract a reader to continuing the reading. Indeed, I read all relevant papers authored or co-authored by Mgr. Vagiannis with great interest. Most works had a similar design, i. e. thorough profiling of molecular interactions and cytotoxicity of small molecules using well established *in vitro* models with an engineered expression of major MDR-relevant ABCs

(B1, C1, and G2) followed in some instances by *in silico* molecular docking and studies of induction/inhibition of major ABCs and CYPs in alternative models. I highly appreciate the establishment of *ex vivo* primocultures of human non-small cell lung cancer cells from patients. Their incorporation into the experimental pipeline brings all efforts of the authoring team closer to the clinics. I believe that some of the original results published in high rank pharmacological or oncological journals will contribute to precision oncology in the future. Among others, I would like to mention a brilliant idea, now supported with experimental data, about dual (MDR-limiting through ABC inhibition and simultaneously anticancer) effects of small molecules as alectinib, ensartinib, tepotinib, and dabrafenib. On the other hand, the interesting observation of reversal of CYP3A4-mediated resistance to docetaxel by ensartinib and dabrafenib will be hard to prove *in vivo* due to the relatively high effective concentration (15 and 25 microM).

Taken together, I would like to state that the thesis submitted by Mgr. Vagiannis reaches the highest standard compared to other works I had the chance to review. Five papers in Q1 journals document outstanding achievements of the candidate and supervisor's team. Results represent a significant advancement in scientific knowledge and, after confirmation *in vivo,* may become clinically highly important.

All published papers were reviewed by experts in the MDR area. Thus the following questions shall contribute to general discussion and do not challenge the candidate's work.

1/ I wonder about protein expression in the used models. Do you have data about the expression of ABCs in MDCKII models? How much do parent and engineered subclones differ? Have you searched for protein expression of major CYPs in experiments where you used transcript levels only, is there a correlation between transcript and protein?

2/ On fig. 9 of paper P1 it looks like some of the models do not constitutively express ABC levels (G2 in LS174T and B1 in A549). Is there a known reason for the lack of expression, e.g., genetic defect or epigenetic deregulation, or are these ABCs induced after drug administration?

3/ Samples from patients presented in SP1 seem to differ by histology (Supplementary table 1), but to my surprise, there is quite small interindividual variability in protein expression of studied ABCs, mainly B1. Do you think that such variability may be a reason for different individual susceptibility to MDR? Were immunoblots of NSCLC explants taken after or before sample processing to primocultures? Did you try to compare expression from bulk tissues with passages at the end of experiments?

According to my best knowledge, this doctoral thesis fulfills the criteria for this type of work, and I strongly recommend continuing in the process of a Ph.D. title defense.

In Prague 21st May 2021

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