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Review
of the doctoral dissertation of Dr. Alexander Cortez
done at the Silesian University of Technology

The Discipline Council "Biomedical Engineering" of the Silesian University of Technology at Gliwice employing me as a reviewer in doctoral procedure of Dr. Alexander Cortez. The decision was followed by providind Doctoral Dissertation of the candidate titled: "Molecular mechanisms of tumor cell resistance to the FGFR kinase inhibitor".

According to current regulations I have not received any data concerning personal data, scientific background and appropriate activity. So, all of it including nationality that would be of interest, remains secret. Fortunately, two strains of information were to be found in dissertation book.

The first one is a list of nine research projects with own participation of the candidate. One of them is (was?) supervised by Dr Cortez. An involvement in other projects was indicated mostly by a term project contractor. The projects were granted by the Agency for Medical Investigations (4), specialized committees of the Polish Ministry of Science and Education (3) and other sponsors. The list of projects reveals quite busy research activity in many areas of tumor biology. Two of the listed projects seem to be directly associated with the research topic of dissertation. Nevertheless, acknowledgement at page 114 informs additionally about European Social Grant AIDA supporting the studies presented in dissertation.

Another important information is coming from the list of scientific achievements including 19 publications in 4 Polish and 15 in international journals. A majority (17) appeared in indexed journals having impact factor ranging from 1,58 to 8,71. A number and quality of publications are impressive. There are researchers who decide to apply for

habilitation with such (occasionally lower!) achievement. On the other site I have to admit that only twice Dr. Cortez' name has appeared at position of the first (leading) author. To put these two things together I assume that Dr. Cortez is having a position of wanted team-worker providing results worth to be published but not successfully competing with contribution of other members of a team. In any case team-workers could deserve a respectful posit in science as well.

There is also information about very frequent presentations (oral and posters) on scientific meetings. I understand such activity as a need for discussing and confrontation of own results on open way. This is appreciated.

Dissertation is written in English except bilingual abstract (English and Polish) and acknowledgements expressed in Polish.

Concerning its structure, the dissertation is not holding a typical partition divided in principle into introduction, material and methods, results and discussion. Such structure is not obligatory but helpful for a reader (including reviewer). Anyway, some elements of typical structure remain.

Rather short sections including: Abstract, Introduction, hypothesis and aim open together the text and present goals of the undertaken study. The main target was to identify biomarkers helpful in targeted therapy because of an ability to establish resistance/susceptibility to FGFR kinase activity. Further goal was to establish mechanism of biomarkers activity. Until now there is one gap of information. The novel biomarker of activity of FGFR kinase established within CELONKO project and substantial for further studies is not identified as the phrase is written in a style of patent language where everything is coded and secret. The same is repeated at page 52. Fortunately looking at reference [170] I was able to decipher the studied compound as pyrazole-benzimidazole derivative. Such information should be given openly and straight. Secondly, the author considers mainly cancer biomarkers applied in cancer diagnosis to jump suddenly to the biomarkers of interest.

The next section describes fibroblast growth factor receptors (FGFRs) in physiology and pathology. Besides short textual information there is also a well-done drawing showing different aspects of FGFR role in cellular and molecular pathways in various organs.

A short characteristics of lung, stomach and bladder cancer that are the subjects of undertaken studies make the body of the next section.

Then we have considerations on FGFR receptors focused on lung, stomach and bladder cancers that are becoming study subjects. The choice of cancer types to further studied is connected with frequency of FGFR alterations. It would be almost convincible except omitting head and neck cancer meeting the criteria of inclusion into study (look at Fig. 2). In any case it refers to my own research involvement. Nevertheless, you comment at this point is expected.

Further, the author is presenting tyrosine kinase inhibitors (TKIs) together with short section on TKIs resistance. A valuable part of it are Tables 1 and 2 although taken from literature they were modified. I appreciate also Fig. 4 combining together various pathways of resistance to FGFR inhibitors. It really helps in understanding the matter. Talking about FGFR resistance author mentioned "acquired resistance". The latter term exists only in opposition to constitutive resistance that was not mentioned. Comment expected. No further remarks to this section.

Following 11 pages turn an attention to biomarkers. Taking together biomarkers of early phase, markers of tumor progression with biomarkers of resistance/susceptibility is not proper. Concerning biomarkers of early phase I would like to remind that there are also cancers easy to observe and diagnose by autopsy as skin, oral cavity or larynx tumors, usually no requiring biomarkers. I would be glad to find in here more general considerations pointing at desired situation. I mean, real biomarker should work along black or white or 1 v. 0 system not leaving any doubts. Otherwise we have to talk about good, reliable or useful marker but still unfortunately living space for incorrect information followed by possible error-prone therapeutic decision.

As a minor remark I have to pstop at page 33. Symbol abbreviation of KRAS gene is written in block straight fonts. Look also at page 47. To compare, at page 7 there are protein symbols abbreviations written in block italics. I remember letters of prof. Chorąży addressed to journals and research institutions reminding the rule: Human gene symbols are written in block italics and human proteins symbols in straight blocks. I am sorry to admit that prof. Chorąży heritage is going to decline.

Pages 38- 51 present a concept of pipeline application to biomarker discovery and identification that is a crucial point of the dissertation. Fig. 6 that must serve as an image of the idea is so banal that should be omitted. The process of biomarker identification is presented as a multiphase procedure with increasing number of patients to be studied.

Dependently on a goal (single or multiple cancer types) alternative versions as umbrella or basket trial could be adopted.

It was also necessary to show obstacles one can meet on research pathway. The main is associated with diversity on any steps. Diversity includes inter-tumor, inter-patients, intra-tumor variations and it is well digested and discussed in the dissertation. Discussion in this point introduced driver and passenger mutations. I would put here more attention on the role of type and gene location of mutations. This is also applicable to intra-tumor diversity explained by Nowell in terms pf clonal evolution. Carcinogenic agent generate a spectrum of alterations in genetic material that are subjected to clonal evolution. The process proceeds in many cells, in various microenvironment and prolonged time. Aspiration of genetic material is taking place at a given time and diverse alterations could be present there. For these reason our old studied on chromosome aberration by classical cytogenetics established some frequent and some rare aberrations in laryngeal tumor biopsies. Study repeated a couple of years later by comparative genomic hybridization has shown different profiles of *CDKN2A* gene homozygous deletions. So, intra-tumor diversity evidently exists.

In this section I cannot accept the statement on tumor heterogeneity as the major obstacle to achieve personalized cancer therapy. This is not an obstacle but a reason to establish personalized therapy for an individual case taking into account the recognized mutation profile.

The problem of diversity is known for years. Clinicians perfectly know how diversity complicates diagnosis, treatment and prediction. Having two patients with the same diagnosis concerning tumor location and type, the same TNM characteristics we could expect quite different outcome and that was an essence of dissertation.

At this point I am asking a question how it was possible to talk about diversity not using the term genetic polymorphism.

Experimental design starts good. As a notorious reviewer of manuscripts submitted to journal I am getting bad mood when authors use different tumor cell lines do not providing information about differences. When two cell lines represent the same tumor they should be derived from tumors with different TNM indices, primary v. secondary tumor, responding or not to treatment etc. Dr. Cortez perfectly explained differences of the material used to cell line establishing. The only question at this point concerns bladder cancer. Is renal pelvis carcinoma a good material for comparative studies?

Differential analysis methods to establish gene expression profile (pages 59 -61) are on the edge of my competence andt herefore I not going to comment it.

Looking for genes involved in establishing resistance towards FGR-TKIs the PREDICTpipeline analysis was performed separately for all three types of cancer. Each step of analysis gradually reduce a number of potential gene candidates. Then the found genes were clustered along main cellular and molecular processes. Finally, the reduced number of potential gene-candidates equalled to 53 for stomach, 50 for bladder and 13 for lung. Four genes overlapped between stomach and bladder. On this was the author assumed that the latter four, namely *SSRP1*, *CCNB2*, *CDT1* and *CENPO* could be further tested for applicability in determination resistance to FGFRK1-TK1. Discussion is somehow hidden in between results mostly given by references with analogic results.

Goig to the end I have to ask the question did the study reach a final goal. Concerning title aiming for mechanisms of tumor cell resistance to FGFR kinase inhibitors the studies on RNA expression and sequencing with the use of PREDICT pipeline have pointed at several pathways involved in acquiring resistance. So, this goal has been reached. Concerning another aim that was an identification of biomarkers, dissertation has shown four potential biomarkers that is still an approximation requiring further studies. However, I am having in mind an information that dissertation is framed by funding resources, a typical time-schedule for doctorate and as well as a patience of candidate and tutors. Hence, my answer is YES. The aim has been achieved. The dissertation provided a real novum in the field of tumor biology and opens an opportunity to establish more efficient therapy.

Altogether, the dissertation fulfils the formal criteria (Obwieszczenie Marszałka Sejmu Rzeczypospolitej Polskiej z dnia 10 marca 2023 r. w sprawie ogłoszenia jednolitego tekstu ustawy - Prawo o szkolnictwie wyższym i nauce) and my suggestion to the Scientific Council is to proceed further the doctorate procedure.

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Podpisał Piotr Szyfter (podpis odręczny)