

Posudek na dizertační práci „High-throughput screening for the discovery of small molecules modulating cell fate“

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The presented thesis focuses on the development and implementation of high-throughput screening (HTS) procedures that could be used for the discovery of novel chemical compounds targeting important biological pathways.

The thesis is written in English in the format “Introduction followed by the collection of papers”, and is based on total 4 peer-reviewed publications and one European patent application. In the two of these publications the applicant shares the first authorship with a researcher from a collaborating group. Given the multilab collaborations that are nowadays standard in the field the first-authorship sharing is quite common, though, in my opinion, the exclusive first authorship would be somewhat preferred to clearly stress the contribution of the application towards the two manuscripts. At the same time, I truly appreciate the practical outcome of the applicant’s work in the form of the EPA that really documents the translational potential of the research performed at the National Infrastructure for Chemical Biology.

The general introduction (literature review) is very well written with minimum typos and errors (with the exception of discrepancy between figure numbers in the text and actual figures starting from the Fig. 40). The thesis (and resulting publications) clearly documents a variety of HTS techniques mastered by the applicant during the PhD training and these include various viability, cytotoxicity and differentiation assays, flow-cytometry, qPCR, immunoassays as well as a statistical analysis that is indispensable to evaluate data generated by the HTS campaigns. The fact that four publications presented here underwent a successful peer-review process in internationally recognized journals confirms the high quality and originality of defendant’s research.

I would like the candidate to address the following issues/questions:

1. Although the general introduction provides an overview of biological pathways targeted by the HTS campaigns, I am missing details on the actual HTS method development and implementation that should be the major strength of the applicant. Could you in a couple of minutes (e.g. in a form of a “typical example”) introduce me into the process of the HTS assay development, including

pitfalls, problems and their solutions? Additionally, could you give an overview of instrumentation/techniques available at the screening facility?

2. In a publication aimed at the Wnt signaling there are several experimental models (human, mice, *D. rerio* and *X. laevis*) used and monensin, the novel drug discovered by the HTS, is effective in all of these models. This fact alludes on high structural/functional conservation of a target molecule(s). Could you comment on the overall conservation of the Wnt signaling pathway in different species including the conservation of a putative monensin target (if known)?

3. Could you comment on your contribution to the Publication No. II (Artificial Organs, 2014)?

4. Why CCD841 cells were used for toxicity studies instead of for example macrophages/blood cells that might be more relevant to the *Leishmania* treatment?

5. The co-administration of bromosulphophthalein with DPI abolishes the killing effect of the latter, suggesting that the glutathione metabolism might be targeted. Could you comment on differences in the glutathione metabolism between humans and *Leishmania* that could be exploited for the *Leishmaniasis* treatment?

6. Are there any additional findings (since the thesis was submitted) concerning the identification of putative cellular targets on the newly discovered compounds?

In conclusion, the PhD thesis by António José Ribeiro Pombinho fulfills all criteria required for the successful defense of this work. Therefore, it is my pleasure to fully recommend the applicant to be awarded the PhD degree.

Závěrem můžu konstatovat, že předkládaná dizertační práce splňuje všechny požadavky Oborové rady biochemie a proto ji doporučuji k obhajobě.

V Praze, 26. září, 2014

RNDr. Cyril Bařinka, PhD

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