

Jury Member Report – Doctor of Philosophy thesis.


Name of Candidate: Dominique Leboeuf

PhD Program: Life Sciences

Title of Thesis: UBR-ubiquitin ligases of the ARG/N-degron pathway as new targets for therapy: implications in cancer and inflammation

Supervisor: Associate Prof. Timofei Zatsepin

Name of the Reviewer:

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Signature:</p>  <p>Date: 04-08-2020</p>
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The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

The summary of issues to be addressed before/during the thesis defense

- Brief evaluation of the thesis quality and overall structure of the dissertation.

It was my pleasure to assess the PhD thesis of Dominique Leboeuf. I followed the research as it was reported regularly on our internal meetings and now was presented as a completed piece of elegant scientific endeavor. The thesis is written in a canonical way on 184 pages and split in literature review, materials and methods, results and separate discussion paper. It contains 48 figures, of which 32 are representing experimental results in a clear and accurate way, satisfying the stringent criteria for publication in specialized journals. The review is written in a good style and covers N-degron pathways and also RNAi methodology starting from discovery of biological processes and ending up with its clinical applications. The literature review can be published as a perfect up to date compendium for broad biomedical audience

- The relevance of the topic of dissertation work to its actual content

The topic of the dissertation was suggested by Professor Konstantin Piatkov, a student and a collaborator for several years of Alex Varshavsky, Caltech Professor and a classic scientist who coined the name for N-end rule and now replaced it with N-degron pathway. Despite being distinguished with a Nobel prize almost a decade ago, the field continues to be very hot, and at the same time reaching maturity and providing background for translational research. The thesis represents a successful attempt to combine investigation of the Arg-N-degron pathway using RNAi instrument with applied studies aiming at validation of combinatorial approach to hepatocarcinoma therapy. The content of the dissertation follows to the point the topic and the established goals.

- The relevance of the methods used in the dissertation

The thesis capitalizes on the rare for Academic lab opportunity to apply state of the art LNP-siRNA knock down approach supported by the experimental platform of professor Zatssepina Laboratory in Skoltech. RNAi is the best, and most likely the only, method that allowed simultaneous knockdown of four E3 mammalian Ubr genes in vivo. The method was immaculately executed with rational design of multiple sets of effective siRNAs for Ubrs 1,2,4 and 5. In vivo models of HCC were used accurately, with right balance of necessary amount of animals involved in moderate to severe procedures for achieving the statistically significant results.

- The scientific significance of the results obtained and their compliance with the international level and current state of the art

The strong generalized pro inflammatory effect of simultaneous knockdown of Ubrs 1,2,4 and 5 in the liver of healthy animals was unexpected, and at the early stage of the work might endanger the further investigation. However, Dominique managed to turn it into her strength. She argued for the rationale of this effect. She also investigated potential targets of proteasome degradation, which may affect pro inflammatory phenotype and obtained some support to it in NGS transcriptomic profiling. She also managed to harness the proinflammatory response by optimizing the dose of siRNA and combining low dose of siRNA with pro apoptotic drugs, Doxorubicin and staurosporine.

- The relevance of the obtained results to applications (if applicable)

This in fact allowed Dominique Leboeuf to come with the rational combinatorial treatment of HCC in mice, the patentable IP which can be explored in the future for translational application

- The quality of publications is of high international standard, the papers with DL first author are in prestigious journals, *Biomolecules* (IF 4,5) and *Molecular therapy* (IF 8,4), and together with the third bioinformatics paper cover the whole scope of the thesis.

Obviously as in every serious study there are open questions still to answer. The proinflammatory effects of Ubr knockdown require further explanation, particularly the generalized effect described in the spleen and pancreas. Mechanistic investigation targeting candidate proinflammatory pathways, cytokines, transcription factors affected by N-Arg-degron warrants further investigation. In this respect some mechanisms evaluated in the discussion require experimental support.

Provisional Recommendation

I recommend that the candidate should defend the thesis by means of a formal thesis defense

I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report

The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense