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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: Petr Sergiev

I confirm the absence of any conflict of interest	Signature:
	[signature]
	Date: 15-07-2020

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 ore asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers ore asked to bring a copy of the completed report to the thesis defense ond to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries obout the thesis which they wish to roise 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

In the PhD thesis entitled "UBR-ubiquitin ligases of the ARG/N-degron pathway as new targets for therapy: implications in cancer and inflammation" Dominique Leboeuf addressed several fundamental as well as practical issues. She advanced our understanding of the functional role for N-terminal aminoacid dependent degradation system. In the literature review, she described general mechanism of the ubiquitin mediated protein decay system as well as the action of the particular kind of this machinery dealing with the degradation of proteins dependent on the identity of their N-terminal aminoacid. Next, Dominique described the main method applied in her study, namely, RNA interference. Started from the natural function of this system, she continued with applications of RNAi for gene silencing with particular emphasis on the methods of targeted siRNA delivery. The review is well written and is very useful as an introduction to the following experimental part.

The main instrument of the presented research was siRNA-mediated silencing of UBR1, UBR2, UBR4 and UBR5 components of the ARG/N-degron pathway. Post-transcriptional inhibition of these genes was achieved both in vitro on several cell line models, as well as in vivo via nanoparticle-mediated siRNA delivery into mouse hepatocytes. The study followed a practical goal, namely, to develop a treatment for the hepatocellular carcinoma (HCC) based on the N-rule degradation system. Contrary to the expectation, straightforward inhibition of this system on the transgene-induced HCC model lead to the acceleration of tumor growth. The likely explanation of this phenomenon is an induction of inflammatory response. However, a combination of ARG/N-degron pathway inhibition with DNA-damaging chemotherapy by doxorubicin resulted in the anticipated reduction of tumor size. Thus, the practical goal of the study was successfully achieved.

Additionally, the study addressed a fundamental question of a regulatory role for ARG/N-degron pathway. Dominique found that inhibition of this system lead to the inflammatory response in a context of the organism as well as on the level of immune cell culture. She demonstrated that the stabilization of pro-inflammatory fragments and excessive secretion of proinflammatory cytokines, such as IL-1b, was observed, especially upon co treatment of the macrophage cell culture with anti-UBR siRNA and LPS.

As a result, I'm glad to state, that the thesis is very well written and the research is an interesting piece of work. However, as any substantial research project, this thesis has few minor points which might be addressed for the sake of its improvement as detailed below.

1.page 14 – "Figure x" should be replaced with (most likely) "Figure 4"

2. page 17 - (Ling) incomplete citation

3. page 62 - "BRCA1 containing the Asparagine residue" – likely "BRCA1 containing the Aspartate residue", if Asp is meant

4. Figure 22b. Downregulation of UBR4 by the control siRNA treatment is puzzling. While a possible explanation is provided, this point might require further clarification.

5. Figure 24b. Panel labels are missed, likely to be PBS, LNP Ctrl, LNP UBRs.

6. page 76 on. The study of pro-inflammatory protein fragments accumulation would benefit from the clarification of several points. The approach taken is hypothesis driven, i.e. the predicted fragments are searched for and studied. It might be worthwhile to use unbiased approach to compare proteomes of control and UBR knockdown samples.
7. Figure 31 and its discussion. To monitor pro-inflammatory protein fragments accumulation upon UBRs inhibition an artificial approach was used, namely, a construct coding for a fusion protein, whose deubiquitination results in the formation of those fragments. Are these effects physiologically relevant?

What share of endogenous proteins are indeed cleaved and whether an increase in fragments stability would have biologically meaningful consequences? Would an increase in the amount of naturally formed fragments would be sufficient to trigger any response?

8. Figure 33 and its discussion. What is the identity of moderately shorter IL1b fragment? Where (in what compartment) the cleavage and degradation happen? Whether IL1b is located in ER/Golgi immediately after synthesis and whether ARG/N-degron pathway function in these compartments?
Is the cleaved fragment functional? No decrease in full-length protein is obvious. Whether ectopic expression of cleaved form of IL1b have any phenotype?
9. page 107 on. It is obvious to say that ARG/N-degron pathway function post-translationally and hence also post transcriptionally. Wouldn't it make sense to analyze a difference between the intact and UBR5 downregulated liver samples on a PROTEOME level?

Few above listed suggestions on the improvement of the thesis are only of a recommendatory nature. It's my pleasure to repeat that Dominique Leboeuf made an excellent study of both applied and fundamental value. Her thesis, in my opinion, is undoubtedly acceptable for the defense of PhD degree.

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*