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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:

I confirm the absence of any conflict of interest	**Signature:**  **Date: 02-08-2020**

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 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

Dissertation by Dominique Leboeuf proposes and elaborates a new strategy of targeted anticancer therapy based on suppression of Arg/N-degron protein degradation pathway by RNA interference. It is well known that in animal models siRNAs are mainly accumulated in liver. This strongly restricts applicability of RNA interference *in vivo*, but at the same time makes this approach very useful for treatment of liver due to decreased off-target toxic effects for other organs. So, a mouse model of liver cancer was used in this work.

siRNAs against UBR1, UBR2, UBR4 and UBR5 ubiquitin ligases were developed and used in different models *in vitro* and *in vivo*. Experiments on cell lines showed strong downregulation of target mRNAs and proteins by siRNAs. Through characterization of siRNA effects on cell *in vitro* demonstrated that knockdown of the Arg/N-degron pathway results in significant decrease of cell proliferation and migration, as well as increase of apoptosis.

Encouraged by these results, author applied the best siRNA species to *in vivo* models: healthy mouse liver and oncogene driven hepatocellular carcinoma (HCC). A strong target mRNA and protein downregulation in the liver (with no significant overall toxicity) was observed. Notably, siRNA action was targeted only to liver, as no significant downregulation of URPs was detected in other organs except adipose tissues.

Contrary to the expectations of the author, siRNA treatment of HCC in mice did not suppress but favored cancer growth. I can imagine that it was a very difficult moment, but the author did not give up and suggested not only explanation of these unexpected results but also successfully overcame the problem. It was found that downregulation of Arg/N-degron pathway leads to upregulation of inflammatory proteins; the resulted chronic inflammation supported liver cancer progression. So, a new role of the Arg/N-degron pathway in the regulation of inflammation was discovered. Moreover, author suggested a new combinatorial treatment based on siRNAs against UBRs applied together with apoptosis-inducing drugs (e.g., doxorubicin). This combination showed synergetic antitumor effect in the HCC mouse model.

To conclude, dissertation by Dominique Leboeuf represents an extensive and intelligent research. The author used many diverse methods, from bioinformatics to cells *in vitro* to complex animal models. Importantly, the suggested siRNA-based knockdown of UBRs is not just a research strategy, but has clear potential to be translated to medicine in the future. The dissertation is written in good language, beautifully framed including fonts and spacing; it is clear that the author put a lot of work into its preparation.

The main results of dissertation work were published in reputable scientific journals: one article in Molecular Therapy (IF=8.986; the first author) and two articles in Biomolecules (IF=4.082; the first author or joint first authorship).

In my opinion, the thesis is almost ready for the formal thesis defense; I have only a few minor remarks mainly about wording:

1. Abstract: Please explain “HCC” abbreviation here (in addition to the List of abbreviations).
2. Page 13: “The study by Tasaki and colleagues revealed seven E3 ligases bearing the UBR box (Figure x)…”.
3. Fig.19: Please describe the scale bar in the legend.
4. Page 59: “…we designed 10 19-mer siRNA sequences per gene and ranked them based on their possible off target recognition and known miRNA and immune stimulatory sequence motifs … The

10 best scored siRNA against each UBR ubiquitin ligase (Table 2) were screened …". From this description, it follows that you selected the best 10 out of 10 siRNAs; so probably there is some misprint in numbers.

5. Page 62: "This reporter system is comprised of a ubiquitin molecule fused to the N-terminus of a reference FLAG-tagged derivative of the mouse dihydrofolate reductase (fDHFR-UbR48) coupled to FLAG-tagged BRCA1, a known target of the Arg/N-degron pathway (see Figure 29 for illustration)". This description seems to be different from what is depicted in Fig. 29 (also, it is inconvenient to go far away through the dissertation to look at the Figure). Please add a scheme of the reporter as a panel in Fig. 17.
6. Page 62: "Cotranslational cleavage deubiquitylases produces …". Preposition "by" is missed.
7. Page 62-63: "we observed a decrease of both cell proliferation and migration after transfection with si-UBRs compared to si-Ctrl (Figure 18)". In contrast, Fig. 18b shows "non-significant" differences in migration rates (although values for si-Ubrs(1) and si-Ubrs(2) were clearly lower than in control samples). Please comment.
8. Page 63: "… confirming the on-target effect of siRNA mediated downregulation on cell proliferation, migration and apoptosis". As the experiments showed upregulation of apoptosis (Fig. 19), it should be "… confirming the on-target effect of siRNA mediated downregulation on cell proliferation and migration and upregulation of apoptosis.".
9. Page 67: "Maximal mRNA and protein downregulation in the liver occurred 3 days after injection, followed by a slow recovery (Figure 21c)". In fact, data are available starting only from the 3rd day for mRNAs and the 5th day for proteins. Potentially, maximal downregulation can occur earlier, the authors just have no data on this. In addition, there are no detectable "slow recovery" of UBR1 protein in the graph. Please rephrase this sentence to describe these results more accurately.
10. Page 76: A line break within the sentence "… Leu^{680}-Matrin-3 (Table 7)."
11. Table 11: "Espnl – Actin component". I would suggest changing to "Actin cytoskeleton component".
12. Ideally, "Conclusion" section should additionally contain a concise list of the main findings – proven experimental facts demonstrated in this work for the first time.

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*