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: Friedrich Felix Hoyer

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	Signature: *Hoyer* **Date: 23-07-2020**

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 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

The work conducted for the presented thesis significantly refines our understanding of the Arg/N-Degron pathway's role and function in the context of hepatocellular carcinoma and inflammation. The delivery of lipid nanoparticles for RNAi are a novel, scalable and specific approach to modulate the ARG/N-Degron pathway's activity. The thesis is overall structured well. The introduction is written in a clear way and provides not only a general overview but also all required details and background information. The introduction covers in-depth the ubiquitin/proteasome system and various types of RNAi delivery methods. The literature research is solid and comprehensive, and covers the current state of knowledge.

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

The relevance of the thesis' content to the topic is high. Hepatocellular carcinoma is frequent, yet, treatment options are limited. There is a high need for novel therapeutic approaches to treat this aggressive form of cancer. Dominique Leboeuf's work does not only provide novel insights into how E3 ligases' activity interferes with apoptosis, inflammation, and cancer progression but also outlines how targeted RNAi may be useful in potentiating concomitant chemotherapy.

- The relevance of the methods used in the dissertation

The methods used for this work are very diverse, relevant, and of high-quality. Cutting-edge technology, such as lipid nanoparticle based RNAi are paired with elaborate molecular biology methods. A large gamut of methods has been applied for this work including cell culture experiments, protein analyses, functional cell assays, fluorescence-assisted cell sorting, and bulk-RNA sequencing. The experiments were conducted in a meticulous manner. The author chose an oncogene-driven mouse model to induce hepatocellular carcinoma, which is a relevant and a state-of-the art mouse model to mimic human disease.

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

The study's findings are of high scientific significance as demonstrated by the publications that resulted from this work. The results obtained in this study draw a complex picture of E3-ligases' role regarding the development of hepatocellular carcinoma, inflammation, and apoptosis. Whereas alterations in dosage impact inflammatory signaling, silencing of only select E3 ligases may likewise affect adversely the progression of hepatocellular carcinoma. Dominique Leboeuf did a great job to decipher these complex processes, and finally harnessed E3 ligases' beneficial effects to potentiate doxorubicin chemotherapy while reducing adverse effects that come with the suppression of the ARG/N-Degron pathway's activity. Beyond analyzing the multitude of processes, the candidate identified 30 previously unknown targets that may be involved in apoptosis and cancer development. These targets may present an interesting route for future and follow-up studies aiming to constrain cancer development. Overall, the presentation of results is at a level of internationally renowned journals publishing high-impact findings.

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

The obtained results are highly relevant for clinical applications. The findings suggest an additional benefit to standard-of-care for the treatment of hepatocellular carcinoma. Considering that similar approaches of RNAi have been successfully applied in other hepatic diseases in humans, the presented findings have the potential to be greatly beneficial. Yet, the complexity of the thesis' findings indicates that proper dosing and the selection of silencing-targets may remain a difficult hurdle. In light of the promising *in-vivo* data, this task, however, should be tackled in future studies. Furthermore, the unravelled regulatory functions of the ARG/N-Degron pathway in apoptosis and inflammation may be relevant for various other diseases. Finally, the identification of previously unknown molecular targets that may interfere with apoptosis and cancer warrant functional follow-up studies.

- The quality of publications

The overall quality of publications is high. The CellPress journal Molecular Therapy has a respectable Impact factor of ~ 9. In addition, Dominique Leboeuf is a first and second-first author on two papers published in Biomolecules, which has an Impact Factor of ~4.6. The PhD candidate therefore has a successful publication record, which also reflects the high-quality of the thesis submitted for defense.

- Summary of issues to be addressed before/during the thesis defense

The candidate should address the following issues:

1. Flow cytometry in Figure 23C and following figures. The candidate should consider to choose a more descriptive way to describe the macrophage population in the middle panel (such as CD11B-positive, Ly6C-positive myeloid cells instead of macrophages). Kupffer cells, which are the resident macrophages in the liver, do not by default express CD11B. Infiltrating, monocyte-derived macrophages may, however, transiently express CD11B. The population at hand is likely a mix of infiltrating monocytes (Ly6C-high) that differentiate into macrophages. It is unclear whether the candidate included macrophage markers such as CD64 or F4/80. I therefore recommend to omit the term "macrophages" and instead replace it by CD11B^{high} Ly6C^{high} myeloid cells. Further, there are two gates in the middle panel, however, the author only refers to macrophages. Please remove the lower "intermediate" gate.

2. The chosen lipid nanoparticles are taken up by hepatocytes, as the candidate clearly describes. Since there is significant accumulation in adipose tissue, the author speculates that peripheral macrophages take up the nanoparticle as well. The author should add a sentence on macrophage phagocytosis and lipid nanoparticles with subsequent implications for macrophage targeting. The chapter on RNAi and delivery methods is already fairly comprehensive. Yet, few information on lipid nanoparticles and macrophages would complete the picture.

3. The PhD candidate often uses the term "influences on" throughout the thesis. Please check every instance where you use this term and erase the word "on" where necessary.

☒ *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