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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Evgeniia Alekseeva

PhD Program: Life Sciences

Title of Thesis: Evolutionary analysis of intrahost interaction between pathogens and adaptive immunity

Supervisor: Professor Georgii Bazykin

Name of the Reviewer: Richard Neher

I confirm the absence of any conflict of interest	**Date: 09-06-2023**

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

The thesis presented by Evgeniia Alekseeva describes two detailed investigations of two rapidly evolving and interacting systems: (i) the adaptive human immune system, and (ii) the SARS-CoV-2 viruses during a chronic infection. The thesis is well structured and provides the necessary background on adaptive immunity, B-cell receptor maturation and evolution, and RNA virus evolution in Chapter 2. This chapter

contains an extensive literature review of adaptive immunity, B-cell receptor sequencing and evolution, as well as viral evolution under immune selection. This introduction covers existing knowledge well and extends to the most recent publications, which is remarkable given the rapid pace at which research in viral evolution and immunology has progressed during the SARS-CoV-2 pandemic. The results by the candidate are discussed in Chapters 3 and 4, followed by a discussion in Chapter 5. The exposition of the results in Chapters 3 and 4 is closely aligned with the corresponding (co)-first author publications by the candidate.

Chapter 3 describes the work on persistence and differentiation of human B-cells and evolution of B-cell receptors in healthy individuals. This work is a collaboration between clinical/experimental colleagues with the candidate and her advisor, who are responsible for the computational and evolutionary analysis of the data. The latter part is crucial and earned the candidate co-first authorship on the paper which is published in a very visible international journal. The study focusses on Ig heavy chains from different B-cell in peripheral blood. The candidate devised a computational scores to track the persistence of different lineages and methods to quantify signatures of molecular evolution of these lineages. This analysis identifies distinct groups of B-memory cells with different distribution among Ig classes and different evolutionary properties. HBmem lineages are close to the germ line and are persistent. LBmem clusters seem to be reactivate older lineages. The latter undergo additional de novo rounds of affinity maturation. These results shed light on important questions of how immunity is reactivated and further shaped by repeated exposures. The computational analysis by the candidate was crucial for this insight.

Chapter 4 describes an analysis of a chronic SARS-CoV-2 infection of a patient without B-cells and in absence of plasma or COVID-19 nAB therapy. Over 10 months, the viral population accumulated many mutations several of which mediate escape from T-cell immunity. The rate of SARS-CoV-2 evolution within this patient is much faster than then background rate and comparable to rapid evolution that gave rise to variants of concern. As with the first project, this is a collaboration between experimental and computational groups, where the candidate did the bulk of the computational analysis. T-cell escape is rarely studied in acute viral infections and the observations presented in this study are an important contribution to our understanding of SARS-CoV-2 biology and evolution. The study investigates the effects on T-Cell responses within the chronically infected patient and explores the potential effects of this kind of evolution in the larger population given the HLA distribution. This well executed study sheds light on important question and involves a variety of computational analysis that the candidate contributed.

In both projects, the candidate showed that she can apply advanced concepts of population genetics and evolutionary biology to large next generation sequencing data sets to answer important open questions in immunology or viral evolution. Through this thesis and the high quality papers, she has made substantial internationally recognized contributions to these fields. The topics are original and important and addressed with creative approaches using state-of-the art methodology. I therefore wholeheartedly feel that this thesis is acceptable and the candidate should be allowed to proceed and defend her results.

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*