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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:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 12-06-2023**

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

In the presented PhD thesis "Evolutionary analysis of intrahost interaction between pathogens and adaptive immunity" Evgeniia Alekseeva looked at the intricate interaction of the pathogen and the host adaptive immune system from two complementary sides by studying the evolution of B cell clonal lineages over time and by analyzing the intrahost evolution of the viral pathogen and its evasion from T cell-mediated response.

The thesis is structured as a compilation of two independent studies on longitudinal B cell repertoire analysis in individuals with seasonal/food allergies and intrahost evolution of SARS-CoV-2 over the course of long COVID in immunocompromised patients comprising chapters 3 and 4 respectively. The main part is preceded by a well-written introduction and followed by a concise conclusion. The topic of the thesis and publications is highly relevant and the methodology (multicolor flow cytometry with cell sorting, intracellular cytokine staining,

BCR repertoire NGS sequencing, whole viral genome sequencing, and subsequent bioinformatic analysis) are state of the art. The presented work has been published in renowned journals.

Two of the papers, of which Evgeniia is co-first author, are dedicated to 1) the study of persistence of B cell memory and differentiation into antibody-secreting cells over the course of one year; 2) the study of SARS-CoV-2 escape from recognition by cytotoxic T cells over the course of long COVID in immunocompromised individuals.

Alekseeva and co-authors demonstrated that B cell clonal lineages fall into two separate clusters representing a) persistent memory with predominantly non-class-switched clonotypes and few antigen-secreting cells and b) predominantly IgA or IgG switched clonal lineages with many antigen-secreting cells representing the activated population. In addition, two lineages had significant differences in germ line to MRCA distance. In one case, an observed transition between clusters indicated reactivated persistent memory.

In the second study, by analyzing viral genomes obtained from an immunosuppressed lymphoma patient on rituximab therapy with COVID-19 infection running over the curse of more than 10 months, Alekseeva and co-authors showed accumulation of mutations falling into the epitopes presented by the patient's HLA class I alleles. Using prediction algorithms, they estimated that the mutations reduced or abolished binding of mutant epitopes to HLA class I alleles compared to wild-type counterparts, and using ex vivo T-cell stimulation assays, they confirmed that patient T cells recognized wild-type but not the mutant epitopes. This suggests that in the absence of a human response, viral evolution was driven primarily by escape from the T cell response.

The main criticism relates to the central part of the study (Chapter 4). The limitation of the study is that the authors did not investigate the response to other SARS-CoV-2 epitopes that are known to be present in the patient's HLA class I alleles and thus did not show what fraction of SARS-CoV-2 specific CD8+ cells was affected by the accumulation of the studied mutations. Another limitation of the paper is that the authors limit their pipeline by studying mutations that affect predicted HLA binding, whereas it is known that there are SARS-CoV-2 mutations in CD8+ epitopes that do not affect epitope presentation but prevent epitope recognition by T cells (see Dolton et al. Cell 2023 for an example).

Minor comment: some abbreviations are missing from the list (MRCA, HSCT, PHBR, BR)

Despite the limitations of the current study, the results presented are significant and in line with international standards and the current state of the art.

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*