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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Artem Mikelov

PhD Program: Life Sciences

Title of Thesis: Dynamics of immunoglobulin repertoires in memory and antibody-secreting B cell subsets in health and disease

Supervisor: Associate Professor Dmitriy Chudakov

Name of the Reviewer:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 10/04/2023**

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

B cell memory plays an important role in the defence against diseases caused by previously encountered pathogens.
The elements of B-cell memory, i.e. memory B cells (Bmem), plasmablasts (PBL) and plasmacells (PL), are generated by the adaptive immune response to infection or vaccination. Antigenic-experience shapes the repertoire of B-cell memory. First VH selection takes place, as only B cells that bind antigens can be engaged in the immune response and are involved in the germinal center (GC) reaction where the generation of memory occurs. In the GC, two mechanisms modify the B cell receptor (BCR) with the aim of increasing its affinity for the antigen. Somatic mutations (SM) are induced in the immunoglobulin genes and then mutated B cells are selected based on their ability to bind the antigen (affinity maturation). Thus, the antigen, by modifying the BCR, leaves a permanent imprint in the B cells, that can be identified by the sequence of the expressed immunoglobulins.
The Candidate used the NGS technology to study human B cell memory. He sorted and sequenced Bmem, PBL and PL from 6 individuals at various time points (0, 1 month later, 11 months later) and often had duplicate samples at each time point. As comparison for the expressed VH genes at the naive B cell stage he used published sequences obtained from 100 donors (Gidoni, 2022).
He was able to demonstrate that:

1. All elements of B cell memory, Bmem, PBL and PL from the same subject express selected VH reflecting the individual antigen experience.
2. Bmem clonotypes are stable in time, persisting up to one year after the first evaluation.
3. PBL and PC show more variability, as they represent a terminally differentiated effector type destined to antibody production. This function is required and induced only in case of stimulation by antigen and is a transient phenomenon.
4. A small number of clonotypes is shared between pairs of unrelated donors. Shared clonotypes are found among the most abundant Bmem clonotypes, suggesting a certain degree of similarity in the response of different individuals to common and frequently encountered pathogens.

The Candidate also develop a new algorithm, MiStrainer, a tool designed for V- and J-gene allele variants inference and genotyping. To assess the performance of MiStrainer, publicly available dataset were used. MiStrainer was able to detect a larger number of alleles than the available tool and showed a higher sensitivity, in that most alleles were still detectable using dataset with shallow sequencing.
The thesis is very good. The subject is most interesting, especially at this time, when the SARS-CoV-2 pandemic has shown what happens when we have no memory of a pathogen. On the other hand, vaccines have demonstrated the power of adaptive memory in the prevention of severe disease. Because of vaccines which induce immune memory we came out of the emergency.
The thesis is well structured, clearly written and gives answers to several questions.
The methods used are of high quality, based on the experience and previosly developed technologies of the Chudakov lab, where Artem Mikelov works since he was a student. Based on his experience and acquired knowledge, the Candidate also developed an additional method for inferring new allelic variants, thus demonstrating the high level of expertise reached during the PhD.
The quality of the publications is good for a PhD student and I am sure that new paper will be published soon.

Few issues to be address at the discussion concern the difference between Bmem and PB:

1. Why are PB more mutated than Bmem?

2. Why is the CD3 length longer in PB than in Bmem and shorter again in PC? We think that PB and PL derive from the same GCs than Bmem. How can we reconcile the hypothesis with this findings? Are there different mechanisms selecting Bmem and PBL?
3. Repertoire stability : the studies performed during the COVID-19 pandemic have shown a continuous evolution of Spike-specific Bmem? Can this evolution be evaluated by the technology described in the thesis?

Provisional Recommendation

☐ X*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*