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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Sofya Kasatskaya

PhD Program: Life Sciences

Title of Thesis: Origin of T cell subsets studied through the lens of TCR repertoires

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)	**Signature:**  **Date: 15 May 2021**

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

A PhD thesis by Sofya Kasatskaya is a well-written, comprehensive and very impressive doctoral work examining origins of classical and non-classical T cell subsets utilising TCR analyses. The findings presented in this thesis have furthered our knowledge and understanding of the molecular immune profiling of specific T cell subsets across different anatomical sites and during the human lifespan. It was a real pleasure to examine Sofya's PhD thesis.

Introduction provides an adequate and comprehensive review on human T cells, especially helper T cell subsets and gamma delta T cells. A substantial body of work presented in Introduction are devoted to a comprehensive overview of TCR repertoire and TCR signatures. Objectives and aims set up the scene for this beautifully crafted PhD thesis.

Chapter 1 researches the peripheral selection and survival of human naïve CD4+ and CD8+ T cells. The effects of aging are also investigated by comparing naïve TCR repertoires in young and elderly individuals. The main conclusion was that with age, shorter and less diverse CDR3 regions are found, especially in the recent thymic emigrants (RTE) subset. This work was published in *Frontiers Immunology*.

Chapter 2 further assessed analyses of survival of naive T cells. This PhD research demonstrated novel findings of immunological memory in helper T cells acquired in embryonic intestinal tissue. Distinct of CD4+ Treg and non-Treg memory T cell subsets were found in the fetal gut through mass cytometry, RNA-seq, and TCR-seq. This cutting-edge work was published in *Nature Immunology*.

Chapter 3 presents the work on the plasticity of Th1/Th2 and Th17/Treg subsets, the subset stability and heterogeneity of TCR repertoire within specific subsets. The candidate demonstrated that the functional T cell subsets share the same patterns of prominent differences in TCR features, even amongst unrelated blood donors. High plasticity within Th subsets was shown, clonal exchange within some but not all Th subsets. This Chapter provides an in-depth and comprehensive analysis of distinct repertoire features and plasticity within helper CD4+ T cell subsets. This work was published in *eLife.*

Chapters 4 to 6 are centred around the role of TCRs in human gamma delta T cells in PBMCs and tissues. The PhD candidate found new innate-like and adaptive-like subsets of blood gamma delta T cells and a tissue-resident populations in the liver. Public TCRs in cancer settings were also studied. The work was published in *Nature Communications, Journal of Hepatology, Cancer Immunology Research.*

Conclusions provide a comprehensive and thought-through general discussion and overall conclusions on TCR repertoires in human T cell subsets across anatomical sites and human lifespan.

Overall, Sofya's PhD work represents advances in the molecular understanding of T cell immunity and their TCR signatures across different anatomical sites and human lifespan, key to the rational development of new vaccines and immunotherapies.

Congratulations Sofya on a very successful PhD!

Minor comments:

- Abstract: "..multivariate subsets pecific differences in physicochemical TCR features. ." one full stop too many
- Publications: Journal of hepatology: Hepatology should start with a capital letter
- Page 29, "conventional ab T cells" ab should be $\alpha\beta$

Specific questions for the PhD candidate:

Please explain differences observed between your PhD data using the broad TCR analyses of non-antigen specific T cells, especially with respect to shortening of CDR3 length in the elderly and published reports on CDR3 elongation within antigen-specific T cells in the older individuals?

Please briefly outline different TCR sequencing approaches and discuss their benefits and disadvantages.

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*