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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	**Signature:**  **Date: 18-05-2021**

Reviewer's Report
In the presented PhD thesis "ORIGIN OF T CELL SUBSETS STUDIED THROUGH THE LENS OF TCR REPERTOIRES" Sofya Kasatskaya studied the intricate mechanisms of the human adaptive immune system with a particular focus on the T-cell receptor (TCR) repertoire. The thesis is structured as a compilation of 6 published articles contributing to the field of research of human alpha/beta and gamma/delta T cell receptor repertoires. It is preceded by a well-written introduction and followed by a concise conclusion. The topic of the papers and the thesis is current and the methodology (multicolor flow cytometry with cell sorting, TCR repertoire NGS sequencing and the subsequent bioinformatic analysis) is the state of the art. The research group to which Sofya belongs is one of the leaders in the field of TCR sequencing. The presented papers were published in the prestigious journals. Two of the papers in particular, of which Sofya is a shared co-first author, are dedicated to 1) the investigation of the mechanisms of selection and survival of naive T cell clones in different functional subsets and donors of the different age; 2) study of the physicochemical features of TCR of the different helper T-cell subsets. In the most important study (Kasatskaya et al. eLife 2020) Sofya and co-authors have demonstrated that TCR sequence and hence its structure may predispose T cell to a specific functional and phenotypical T cell subset. Besides studding the TCR repertoires of conventional alpha/beta T cells Sofya delved in the biology of human gamma/delta T cells and the role of their TCR structure. In the collaborative papers Sofya and her

co-authors have described new innate-like and adaptive-like subsets of human gamma/delta T cells in the peripheral blood and proposed a new approach to gamma/delta TCR sequencing and data analysis.

The presented results are by all means significant and comply with the international level and the current state of art.

The main criticism is addressed to the central part of the thesis (Chapter III). In the paper Kasatskaya et al. eLife 2020 the authors analyzed the alpha and beta TCR sequences of various subsets of helper (CD4+) lymphocytes. Several features in the composition of the central part of the beta chain CDR3 were substantially different between various T-cell subsets, suggesting that the rearrangement of the particular TCRB sequence may dictate the phenotypical fate of the naïve helper T cell. Besides that, the authors showed that some TCRBs were present in two distinct T-cell subsets. From this observation they draw a conclusion of the certain plasticity of helper T cell population. This term is used multiple times in the paper and in the abstract of the thesis to describe the underlying reason for the observed overlap in repertoires. Albeit T cell plasticity is one of the possible explanations, in their work the authors did not strictly show that T cells can and do transit from one phenotype to another. Neither it was feasible to demonstrate by the used methodology. Another possible interpretation is that some clones just do exist in more than one subpopulation (in this explanation the fate of the cell progeny diverges rather than phenotype of individual changes over time). This notion is in my opinion is no less significant than the plasticity.

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*