Skoltech
Skolkovo Institute of Science and Technology

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: 16th 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

This thesis provides a wide-ranging discussion of many aspects of the T cell receptor (TCR) repertoire in humans. The thesis begins with a clear and succinct overview of the basic biology of the TCR repertoire and the basic techniques used to study it. This covers T cell recombination, as well as an overview of the biological functions of CD4 cell subsets. The main body of the thesis is made up of 6 peer reviewed published papers, which cover the influence of age on the T cell repertoire, the generation of CD4+ T cells in human fetal intestine, detailed analysis of the chemico-phisico properties of TCRs from different T cell populations, an analysis of Vdelta 2 subpopulation of gamma/delta T cells, an analysis of gamma delta cells in the liver, and a final paper on gamma delta T cells in breast cancer. Each chapter corresponds to a published paper, and in each case the contribution of the candidate was focused on an analysis of TCR sequences. The studies are all published in high impact international journals.

This thesis constitutes a considerable body of work, including a number of interesting and novel observations. My discussion will focus mostly on the study of TCRs in aging, and the study of the physical properties of TCRs from different functional sub-populations of T cells, both studies where the candidate was joint first author on the resulting publication. A brief discussion of the gamma/delta studies is also included.

The analysis of changes in TCR repertoire with aging in humans analyses global properties of the repertoire in naïve and effector T cells from young and old repertoires. The major finding is that significant decrease of CDR3 length, NDN insert, and number of non-template added N nucleotides within TCR beta CDR3 with aging, and a decrease in "strongly binding" aromatic-driven residues in the CDR3s of elderly people. Interestingly these changes are seen in the bulk naïve population, but also in new thymic emigrants. The study also provides some further support for the notion that embryonic T cell repertoire persists long term in the adults. Another interesting point for discussion is the gradual increase in "public" TCRs in older CD4 repertoires. The study is challenging and technically convincing. Of course an intrinsic weakness of these sort of studies is they are largely observational. Hence the discussion of the possible meaning of these changes is of necessity speculative. Nevertheless, the results are intriguing and form a basis for future work aimed at teasing out what is the functional impact of these changes on immune function.

The study of the physical properties and the sharing of the TCR in different subsets of T cells is perhaps the most original and novel aspect of this thesis. In this study, the candidate analyses repertories from numerous human T cell functional subsets. The principal findings is that the physical properties of distinct functional T cell types vary, thus suggesting differences in the underlying selection process leading to the differentiation of the different functional subsets. In addition, more extensive TCR sharing is observed between TH22/TH2/TH17 and TH1/TH17 – the authors interpret this as increased plasticity of these subsets, although it would be interesting to discuss the alternative hypothesis that these types share precursors. Surprisingly follicular Th cells were found to have short TCRs, with very few strongly binding aromatic amino acids. The candidate makes some interesting hypotheses about this, although the caveats mentions above about observational rather than interventional studies remains true.

The last three chapters of the thesis can be considered together, since they all focus on probing the gamma delta TCR repertoire, albeit in different contexts. The candidate makes some significant methodological contributions, in terms of developing the well-established techniques for alpha/beta TCRs to the Gamma/delta context. This techniques seem to work robustly and will be of interest and value to all those working in this field. The candidate is also responsible for all the TCR repertoire analysis, in blood, liver and tumor. It was interesting to see that multiple copies of the D region were commonly observed in the delta gene, allowing rather long sequences. Identity of public TCRs in cancer. The extent of

heterogeneity within the gamma/delta compartment was striking, as was the convincing evidence of clonal expansion. The candidate makes a convincing case that gamma/delta T cells comprise both a more innate-like and a more adaptive-like component. This is an interesting conceptual point that would be interesting to discuss further during the thesis defense.

Overall this is a strong thesis, which includes a great deal of interesting material, well presented and clearly discussed. While the contribution of candidate to the writing of the publications which form the bulk of the thesis is difficult to assess, the parts of the thesis written by the candidate (introduction, and the brief introductions to each chapter) are well written, with minimal typographical errors, and easy to read and to follow.

Issues which could be discussed during the thesis defense. 1. The difficulty of comparing and harmonizing different TCRrep data sets. 2. The biological significance of TCR publicity, and why it should change with age. 3. The biological importance of the physical changes in TCR observed with aging, and in Tregs. 4. The evidence for T cell subset plasticity and how this might be maintained. 5. The balance between innate and adaptive responses in gamma/delta T cells. 6. The perspectives for gamma/delta cells in immunotherapy of cancer. More general discussion could focus on the candidate's views about the current challenges to TCR repertoire studies; and the impact of single cell RNAseq on the study of the repertoire.

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*