

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: Professor Dmitriy Chudakov

Name of the Reviewer: Peter Chumakov

I confirm the absence of any conflict of interest	Signature:  Date: 15-05-2021
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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

Sofya Kasatskaya's thesis is devoted to studying T-cell populations in the prism of the T-cell receptors expressed by them and their repertoires. The dissertation is structured as a collection of articles published in leading international journals, to which the author herself has made a significant contribution. In addition, the dissertation contains a review of the literature, remarkable in form and content, highlighting the essential information in the field of T-cell biology, their subtypes, functional differences, and structural heterogeneity of T-cell receptors (TCR). According to the focus of the study, the main focus of the review is on information related to helper T cells. The review focuses on the structure of TCR subtypes, the mechanisms of gaining diversity, and repertoire replenishment due to V (D) J and VJ recombinations. Technological approaches to sequencing TCR repertoires are explained and described in detail, indicating the specific contribution of the author to work performed. The importance of studying the TCR repertoire as a promising direction in immunology, which, among other things, allows tracking the immunological history of an individual, is explained. In addition to the bioinformatic processing of sequencing results, the author also performed "wet" tasks in selecting donors, sampling, selecting antibody panels for flow cytometry, optimizing sequencing protocols, and preparing NGS libraries. Much attention is paid to the role of age-related changes in the repertoires of T-cells and T-cell receptors, from the embryonic stage to the aging condition, and differences in repertoires in T-cells obtained from different tissues.

Moving on to describing the results, the author followed the sections defined by the content of the published articles. Thus, the first section of the products is devoted to studying naive T cells in different age groups. Recent thymic emigrants (RTEs) and more mature naive T cells have been found to exhibit a similar TCR repertoire. However, RTEs are more prone to senescence when TCRs contain truncated and less diverse CDR3 regions. The results of this work are published in the journal *Frontiers in Immunology*. The second chapter focuses on naive fetal T cells. In subpopulations of T cells from the intestine of the fetus, it has been shown that the development of immune memory occurs in the fetus's tissues. CD4 + T cells can acquire an immune memory phenotype and exhibit the properties of resident memory T cells in the intestine. The expansion of T-cells of subsets of T-helpers and regulatory T-cells with different characteristics of TCR in naive subsets and subsets of memory has also been described. That indicates a very early formation of immune memory in both helper and regulatory T cells. The results of this work are published in the journal *Nature Immunology*. The third section is the central part of the dissertation work, to which the author made the most significant contribution and became the first author of an article published in the journal *eLife*. It is devoted to the uniqueness of the structure of T-cell receptors in human T-regulatory cells. Deep profiling of TCR subpopulations of T-cells from five donors was carried out, and eight subpopulations of effector CD4 + T-lymphocytes were analyzed, revealing subpopulation-specific differences reproduced in unrelated donors. For the first time, a detailed picture of the repertoire features, plasticity, and publicity of subsets of helper CD4 T-lymphocytes is presented.

The fourth and the following sections examined the $\gamma\delta$ TCR repertoire from nontraditional T cells. E Using TCR sequencing, novel innate and adaptive-like subsets have been described in nontraditional human $\gamma\delta$ T cells.

The fifth section describes the study of the functional state of $\gamma\delta$ T-lymphocytes in the liver tissue. Samples of chronic liver diseases and healthy donors were studied. V δ 2- $\gamma\delta$ T cells predominated everywhere. However, resident $\gamma\delta$ T cells exhibited increased clonal expansion

compared to circulating $\gamma\delta$ T cells. Naive T cells were practically absent in the liver. The last sixth section explores the public portion of the $\gamma\delta$ TCR repertoire that is present in unrelated donors and has been observed in independent $\gamma\delta$ TCR datasets. A significant proportion of generally available TCR clonotypes was found in samples of tumor-infiltrating lymphocytes from breast cancer patients.

Sofia Kasatskaya's dissertation study was carried out at the highest methodological, technical, and scientific level, in close cooperation with several prominent international research teams. The results and conclusions of the work have been published in leading international journals, and their high quality is beyond doubt. The work is framed exceptionally carefully. I have not noticed any typos or inaccuracies in the description of both the research subject and the experiments performed. The results obtained represent a significant contribution to the study of the repertoire of T-cell receptors and understanding of the diversity of functional T-cell subtypes. The quality of the work performed is supported by the level of publications, which guarantees their reliability through the high assessment of peer reviewers in this highly competitive field of immunology. The author's profound, sometimes decisive, contribution to various aspects of the study is beyond doubt. I believe that Sofya certainly deserves the desired Ph.D. degree.

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