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Review of the PhD thesis: *Immunophenotypic and functional characteristics of lymphocytes of patients with primary immunodeficiency*

The PhD thesis submitted by Marina Bakardjieva summarizes the experimental data from five manuscripts, two of which were first-authored by the candidate, including one pre-print.

The thesis, along with the **literature review**, is very well-written, practically without any mistakes (p. 26 row 4 – trimmed sentence). The overview of current knowledge on immune cell development and differentiation is well-structured, easy to follow, and linked to a comprehensive summary of currently described primary immunodeficiencies (PIDs). It also addresses current diagnostic and treatment approaches.

The thesis **objectives** are well-defined, and the **methods** chosen to achieve them are appropriately selected. In addition to utilizing highly standardized multi-parametric flow cytometry and mass cytometry, advanced data analysis, a novel trajectory inference tool has been developed.

The **results** were published in reputable journals including *Frontiers in Immunology*, *Journal of Allergy and Clinical Immunology*, *Science Immunology*, *American Journal of Hematology*, reflecting their exceptional quality. Additionally, results of one of the critical projects were submitted to the *European Journal of Immunology*.

The first part of the thesis concentrates on developing diagnostic and immuno-phenotyping tools for severe PIDs detection within the EuroFlow framework. This includes a SCID-RTE cytometric test and an assay for evaluating B cell subset alterations in patients with predominantly antibody deficiencies (PADs). To further explore B cell developmental pathways, the candidate developed a mass cytometry protocol and a novel computational framework for deep immunophenotyping of human B cells.

The second part focuses on understanding non-apoptotic Fas signaling in B cell differentiation, utilizing autoimmune lymphoproliferative syndrome as a model. It also describes a novel mutation in the TLR8 gene causing autoimmune and autoinflammatory disease.

The **discussion** section provides detailed interpretations of the results and places them in context. The thesis's summary is clear and concise. The thesis contains a statement on the author's contribution to each publication and cites 193 publications. While the review articles represent a minority of them, I would recommend (when appropriate) distinguishing them at least graphically from original research articles.

Understanding PIDs is critical for clinical diagnosis and research, utilizing them to study the immune system. Developing innovative diagnostics and immunophenotyping tools is crucial for enhancing patient care.



As the main focus of the thesis are the cytometric tools enabling deep characterization of lymphocyte populations in healthy individuals and PID patients, my questions are related only to the articles where the candidate is the first author.

Q1: Has the SCID-RTE assay been already used on unknown samples? Is it now routinely used for diagnostics?

Q2: Could you give us an update on the revision process of the manuscript submitted to EJI?

Q3: Could you reveal how the manual de-barcoding was performed in the EJI manuscript? Were some antibody clones superior over the others (CD45 clones x HLA-I clone)?

Q4: How heterogeneous is the stem cell population defined in the EJI manuscript – does it also contain MPPs, CLPs, MEPs and GMPs?

Overall, the thesis showcases **outstanding quality**, demonstrating Marina Bakardjieva's expertise in various advanced techniques. These include high-end standardized multiparametric flow cytometry as well as mass cytometry and advanced computational analysis of data generated by these techniques. Her exceptional results and research papers validate her as a mature scientist capable of independent work, meeting the requirements for a PhD degree in Immunology.

Jana Balounova, Ph.D.