Wroclaw 24.01.2025

Prof. dr hab. Eng. Julita Kulbacka
Department of Molecular and Cellular Biology,
Faculty of Pharmacy, Wroclaw Medical University
Borowska 211a, 50-556 Wrocław

Review of the PhD Dissertation of M. Sc., Dominika Miroszewska

Title: Characterization of the function and heterogeneity of infiltrating T cells, especially Th17/Treg cells in colorectal cancer and inflammation

The doctoral dissertation of M.Sc. Dominika Miroszewska was prepared at the Laboratory of Experimental and Translational Immunology Intercollegiate Faculty of Biotechnology University of Gdańsk & Medical University of Gdańsk under the supervision of Dr. Zhi Chen. The presented dissertation addresses innovative aspects of molecular and cellular biology, with a focus on gut microbiota, immune response regulation, and their implications in colitis and colorectal cancer. The dissertation is based on a series of scientific publications authored or co-authored by the candidate.

General evaluation of the dissertation

The dissertation adheres to the format of a cumulative dissertation and consists of seven scientific papers authored or co-authored by the candidate. These publications are thematically consistent and collectively address the stated research aims. The work includes a graphical abstract illustrating the scope of the conducted research. The PhD candidate provides a

comprehensive background, introducing the reader to the topics of T cell-mediated colitis, gut microbiota, and immune regulation in the tumor microenvironment (TME). The discussion of results is thorough and well-referenced, reflecting the candidate's ability to relate findings to the current state of knowledge in the field.

In my evaluation, I would like to focus on the specific aims set by the doctoral candidate as part of the dissertation and whether they were achieved.

Assessment of specific aims:

Aim 1: To investigate the effect of animal care facilities' conditions on gut composition and their impact on colitis development mediated by T-cells in mice models.

Evaluation: The candidate successfully demonstrated how housing conditions influence the gut microbiota and, in turn, the progression of colitis. The research highlights specific bacterial strains that modulate colitis severity. This aim was achieved through a detailed analysis of how housing conditions influence gut microbiota composition and colitis progression. The research highlights specific microbial strains correlating specific bacterial strains with colitis severity and highlighting *Akkermansia muciniphila* as protective, and their role in disease modulation.

Aim 2: Identify the role of USP28 in T cell activation and function, especially Th17 and Tregs, and its role in intestinal inflammation in DSS-induced colitis.

Evaluation: The PhD candidate elucidated USP28's regulatory role through knockout models and detailed immunological analyses, revealing its impact on Treg suppression and IL-22/STAT5 signaling pathways. This contributes valuable knowledge to the field of immunology and inflammation. Results show USP28's involvement in modulating inflammation.

Aim 3: Investigate the TME and participating immune-related interactions in a spatial context using spatial transcriptomics in FFPE tissue.

Evaluation: PhD candidate applied advanced spatial transcriptomics to identify immune cell interactions within the TME. The obtained results add valuable insights into immune modulation in colorectal cancer, analyzing the tumor microenvironment, identifying gene expression gradients, and novel immune interactions, such as Tregs' roles in TME.

Aim 4: Identify protein expression changes linked to tumor progression and immune response in CD4+ enriched CRC tissue sections.
Evaluation: This aim was met by identifying key protein expression changes in CD4+ T cells, revealing their association with tumor progression and immune responses in colorectal cancer. The doctoral candidate used advanced analysis methods such as deep proteomics to demonstrate complex protein expression patterns in CD4+ T cells, including immunosuppressive and pro-inflammatory markers. Specific proteins like MCEMP1 were highlighted for their roles in CRC. The obtained results revealed significant protein expression patterns, contributing to understanding tumor progression mechanisms and immune responses in colorectal cancer.

Aim 5: Identify plasma protein changes and their association with immune response and tumorigenesis using proteomics strategies.
Evaluation: There were presented data from proteomics studies that identified plasma proteins linked to CRC and immune response, including potential biomarkers like CSF3 and SAA4. Validation was performed in independent cohorts. The aim was achieved, as proteomic analysis of plasma proteins provided critical data on biomarkers associated with immune responses and cancer progression, with potential translational applications.

Based on the above, I can conclude that all of the aims were achieved, and I would like to underline the scientific contribution of the presented research. The dissertation provides novel insights into gut microbiota, USP28, and TME, significantly advancing the understanding of immune regulation in colitis and cancer. The dissertation includes methodological innovation, i.e., advanced techniques, including spatial transcriptomics and proteomics, underscores the candidate's strong technical skills. Moreover, there can be noted translational potential, as there were identified biomarkers and immune modulators, that have direct implications for the development of therapeutic strategies.

Concerns regarding cumulative dissertation:

The dissertation is based on a collection of scientific papers, and the attached statements of the candidate and co-authors are too general. It is important to emphasize that a cumulative dissertation should distinctly outline the individual contributions of the PhD candidate to each publication. While the attached statements of the candidate and co-authors provide a general overview of contributions, they lack the specificity required to clearly evaluate the candidate's independent input in conceptualizing, conducting, and analyzing the research. For each

publication, detailed contributions, which part was exactly performed by PhD candidate, should be provided. This will better establish the candidate's role in the research and demonstrate her ability to conduct independent scientific work, for example, clarifying what aspects of the conceptual framework, experimental design, and data analysis the candidate led. Was the PhD candidate responsible for writing the manuscript or performing key experiments? A more precise delineation of the candidate's role in each study will validate the claim of independence in conducting scientific research, as required for the doctoral degree.

Additionally, I would like to refer to paper no. 4, which is also included as a part of this dissertation. The paper "*Deep proteomics characterization of colorectal cancer tumor microenvironment enriched in CD4+ T cells*" is also a part PhD thesis of Vurbiola Salvador's from 2024. While such overlaps are not uncommon in collaborative projects, it is crucial that the PhD candidate's independent contributions should be clearly outlined. In this dissertation, the attached statements of contributions remain too general and do not provide sufficient detail to assess the candidate's role relative to that of the co-author. To ensure compliance with the requirement of "original solutions to scientific problems," the candidate should (i) demonstrate how their contributions to the shared publication advance the thesis's unique objectives and (ii) show that the thesis does not entirely rely on shared results but also includes other independent work and publications. It would be good to include some information related to e.g., which proteomics analyses were performed exclusively by the candidate? Was the candidate primarily responsible for experimental design, data processing, or interpretation in the shared study? Did the PhD candidate contribute distinct insights that are independent of the co-author's thesis? Honestly, taking into account the number of papers included in this dissertation, this paper did not even need to be included in the list due to the number of results in previous works.

The PhD candidate demonstrates a solid knowledge and understanding of immune and cancer biology, particularly in Th17/Treg dynamics, spatial transcriptomics, and proteomics. This knowledge is reflected in the thorough background sections and interpretation of findings. The research provides novel insights into gut microbiota's role in colitis, the function of USP28, and CRC TME interactions using advanced techniques like spatial transcriptomics and proteomics. The identification of novel biomarkers and mechanisms supports the originality criterion.

The thesis includes two first-author publications and equal contributions in a high-impact study, reflecting the candidate's significant involvement in conceptualization, execution, and analysis. PhD dissertations should be completed with scientific papers, where recommended achievements are made and where the PhD candidate has first authorship. However, the experimental design, the scope of the conducted experiments, and the research expertise were very broad in all papers, which is a sufficient contribution.

There can be noted practical applications, and the results have potential translational relevance, including biomarkers for CRC diagnosis and insights into immunotherapy targets.
From the point of view of the Reviewer, I indicate some points for clarification and questions for PhD candidate:

- address the limitations of the studies, including sample sizes, reliance on animal models, and generalizability of biomarker findings to clinical practice.
- strengthen the conclusion section by proposing future studies, particularly those that validate biomarkers and elucidate USP28's role in human diseases.

Final recommendations

The dissertation of PhD candidate Dominika Miroszewska, entitled "*Characterization of the function and heterogeneity of infiltrating T cells, especially Th17/Treg cells in colorectal cancer and inflammation*" meets the conditions specified in Article 187 of the Act of July 20, 2018, Law on Higher Education and Science (i.e., Journal of Laws of 2022, item 574, as amended). The presented research demonstrates the candidate's theoretical knowledge, independence in conducting research, and ability to address complex scientific questions. The results contribute to the field of molecular and cellular biology and offer significant potential for future therapeutic developments.

According to the above, I recommend that PhD candidate Dominika Miroszewska to the Biotechnology Discipline Council at the University of Gdańsk be awarded a doctoral degree in the field of natural sciences in the scientific discipline of biotechnology. Furthermore, given the quality and innovative nature of the research, I also propose **that the dissertation be considered for distinction.**

Uniwersytet Medyczny we Wrocławiu
KATEDRA I ZAKŁAD BIOLOGII
MOLEKULARNEJ I KOMÓRKOWEJ
kierownik
Julita Kulbacka
prof. dr hab. inż. Julita Kulbacka