To the the Biotechnology Discipline Council at the University of Gdańsk

As the appointed evaluator of doctoral dissertation entitled '*Characterization of the function and heterogeneity of infiltrating T cells, especially Th17/Treg cells in colorectal cancer and inflammation*' by a Master of Science **Dominika Miroszewska** I state the following.

1. An overview of the dissertation and theoretical knowledge about the subject

This dissertation is presented to obtain a Ph. D. Degree in the field of natural sciences in the scientific discipline of biotechnology. It has been carried out at the Laboratory of Experimental and Translational Immunology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk. It has been supervised by Dr. **Zhi Chen**. The thesis consists of a review of the literature, altogether seven scientific original publications followed by succinct conclusions of the presented work. The doctoral dissertation includes an abstract both in English and in Polish.

Thesis is based on seven (7) original publications. Five of the papers are already published whereas two are presented as unpublished manuscripts. M.Sc. Dominika Miroszewska is the first author in one of the publications (unpublished), shared first author in one published article (*Experimental Biology and Medicine*), second author in one published article (*Molecular Cell Research*) and in one unpublished article as well as third or fourth author in three additional published papers (*Frontiers in Oncology*, *Biomark Insights* and *Frontiers in Immunology*).

In her dissertation, M.Sc. Miroszewska has investigated molecular mechanisms how the immune response is regulated in the gut. The immune signalling needs to be carefully tuned to avoid destructive inflammation and/or autoimmunity, and to protect from malignancies. Collectively, these thesis present a series of studies aiming at deciphering the heterogeneity of immune responses linked to the T-cells functions, especially Th17 and Tregs, and associated changes in protein and gene expression in tumor settings and inflammation.

I consider all the subprojects to be well-connected, and they form a balanced entity.

The doctoral dissertation demonstrates sufficiently the candidate's general theoretical knowledge about the subject.

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2. Topic, research questions and published articles

In her thesis project, M.Sc. Miroszewska has used mouse models of colitis, humans blood samples and tissue samples. First, M.Sc. Miroszewska and her co-workers noticed that different housing conditions impacts gut microbiota and the subsequent development of colitis in the T cell adoptive transfer colitis mice model (**Publication 1**). Differences in the mikrobiota was associated with the risk of development of colitis. Mice which underwent adoptive T-cells transfer developed colitis differently depending on the microbiota composition present in their environment. There were increased circulating inflammatory cytokines as well as Th1 and Th17 cells in mice housed in units having micobes compared to mice maintained in a specific-pathogen free units. These results provide insight about the environmental factors, such as chenges in the microbiota, that are involved in the development of inflammatory bowel diseases. In another mouse study utilizing Dextran Sulfate Sodium(DSS)-induced model of colitis, the role of USP28 ws studied in the development of different types of T-cells (**Publication 2)**. Genetic deletion of USP28 in mouse led to protection against intestinal inflammation. In the acute DSS treatment, USP28 deficient mice lost more weight compared to control mice. Activated T cells lacking USP28 had increased STAT5 phosphorylation and elevated IL22 cytokine levels. Thus, USP28 plays a role in regulating T cell functions and intestinal health.

In addition to mouse studies, sophisticated gene **(Publication 3)** and protein expression (**Publication 4**) analysis of human tissue samples were carried out. Formalin-fixed and parafin embedded tissue sections from tumour samples were analyzed to detect changes in immune cells. For example, spatial transcriptomics analysis was done to study changes in gene expression and in immune cells distribution in tumor microenvironment. These studies provide novel understanding about the dynamic interplay between the immune cells and of cancer cells.

The last two original publications were based on proteomics analysis of serum samples from patients having colorectal cancer (**Publication 5 and 6**). These studies identified potential biomarkers for colorectal cancers, and for example, a complement component C5 was identifed as one such potential biomarker.

The last publication (**Publication 7)** is a review that summarizes how the proteomics studies can be used to study immune responses in cancer and tumor immune microenvironment.

I consider this research entity important as besides the great health impact of colorectal cancer, also autoimmune and inflammatory diseases are of great clinical importance. Inflammatory diseases are many in numbers (there are more than 100 different autoimmune diseases that manifests either systematically or at practically all possible tissues). Incidences of many of these diseases are constantly increasing (for example the inflammatory bowel disease (IBD)) and many of the diseases are still challenging to treat. Thus, better understanding the mechanisms how microbes in the gut as well as different immune cells affect development of colitis is of great interest.

As a summary, I consider that the topic is well chosen, methodology is adequate, modern and diverse, and M.Sc. Miroszewska has apparently had an important role using these techniques. Based on the published (and unpublished) articles it is obvious that the **candidate has the ability to conduct research both independently and as a member of an (international) team.** Furthermore, **the doctoral dissertation constitutes an original solution to a set scientific problem**.

3. Conclusion

As a summary, the dissertation has studied important research question, the molecular basis of inflammatory diseases and cancer. The contribution of the author is clearly sufficient for Ph.D. thesis. Thus

the doctoral dissertation meets the requirements set for doctoral dissertations by The Higher Education and Science Act dated 20 July 2018 (Polish Journal od Laws od 2018 item 1668, as amended). Therefore, I am applying to the Council of the Biotechnology Discipline for admission M.Sc. Miroszewska to further stages of the doctoral procedure.

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