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RE: Questions and comments on the PHD THESIS of Mr. Víctor Urbiola-Salvador.

Dear Committee,

The presented thesis of Mr Urbiola-Salvador, is well written that focuses on use of proteomics to characterize immune response in inflammation and cancer with an eye toward identification of immune regulators and biomarkers. I find it more than adequate as a written representation of scientific efforts conducted as part of study toward a doctor of philosophy degree.

A variety of methods were used to generate proteomic data. The student appears to excel in the analysis of such disparate, clinical proteomic data. The thesis begins with orthogonal proteomics approaches, mass spectrometry and proximity extension assay used to analyze plasma samples from COVID-19 patients with and without pre-existing comorbidities and controls. The goal was to determine plasma protein changes due to SARS-CoV-2 infections. Next the previously optimized proteomics approaches were applied to plasma samples from a multi-center colon cancer cohort and matched healthy controls to understand better colon cancer development, progression, and inflammation related to cancer. Finally, proteomics is used to understand changes in proteins of immune cells from the colon cancer tumor microenvironment. Following are questions that I have for the candidate.

CHAPTER 1. Introduction. Very well written and thorough review. I would consider submitting some version of this as a review article perhaps targeting the immunology community where the technologies may be useful but where the practitioners may not be up to date on the latest methods used herein.

CHAPTER 2. Aims of the thesis. This chapter clearly lays out the scope of the thesis. However, while the first sentence is commonly accepted "*Inflammation is the most relevant contributor to several diseases. Chronic inflammatory diseases, and cancer, are considered the most significant causes of death and their prevalence is increasing.*" is there a reference you can point to that supports this claim? It is a really broad statement and probably most of us believe it to be true but students should reference such broad claims. For example, what are the inflammatory biochemical markers that are causative in development of disease and cancer? We know there are PAMPS in microbial diseases, but what about cancer and inflammation?

CHAPTER 3. Plasma Proteomics Elucidated a Protein Signature in COVID-19 Patients with Comorbidities and Early-Diagnosis Biomarkers. Page 22. "*untargeted LC-MS/MS proteomics analysis was applied to plasma samples from SARS-CoV-2 infected patients with and without pre-existing comorbidities together with their age-and-sex matched HCs and disease controls to characterize the protein changes caused by SARS-CoV-2 infection.*" This sounds pretty standard as an approach. Generally one might expect to identify ~250 proteins from such analysis of plasma. You found ~235 proteins overall and the Venn diagram in Figure 3.1 shows 208 of those are in common to all three conditions. Does this suggest that being able to access further down in the detectable dynamic range or protein expression would help further segregate the patients and controls?

Page 30. "*In this study, changes in plasma levels of proteins involved in tissue damage and remodeling (K1C10, K22E, and ECM1),… were associated with SARS-CoV-2 infection". Doesn't this suggests that fibrosis is ongoing in these patients? Either acute or chronic?*

CHAPTER 4. Plasma proteomics unveil novel immune signatures and biomarkers upon SARS-CoV-2 infection. Nice work. While most clinical analysis is done using a cohort of patients versus controls, as you have done, humans are inherently unique. This uniqueness contributes to noise in analytical data. How would use intra-patient longitudinal analysis instead to better define proteins resulting from the disease condition you studied? Why aren't longitudinal human studies common?

CHAPTER 5. Mass spectrometry proteomics characterization of plasma biomarkers for colorectal cancer associated with Inflammation. Page 44 *It's not clear what you mean by "filtered" in this statement: "Proteins with missing values in over 50% of patients and 50% of healthy controls were filtered." Were the filtered values removed? Please clarify.*

CHAPTER 6. Plasma protein changes reflect colorectal cancer development and associated inflammation. Nice work! Please compare and contrast the relative merits and demerits of the targeted PEA approach to the discovery LC-MS/MS based proteomics methods used in other chapters?

CHAPTER 7. Deep proteomics characterization of colorectal cancer tumor microenvironment enriched in CD4+ T cells. Considering Figures of Venn diagrams, I see there is Fig 3.1, Fig 5.1, Fig 6.3 and Fig 7.1. The first two are based on LC-MS/MS data-dependent acquisition based discovery experiments, while 7.1 is created from data-independent method. Why do you think there is such disparity between all of these other than 3.1 and 5.1 which show the same trend that most proteins are the same between conditions/patients? Continuing 6.3 shows the least similarity between conditions/patients. Please develop some reasoning for these disparate outcomes and explain how this might affect your interpretations.

CHAPTER 8. CONCLUSIONS AND FUTURE DIRECTIONS. Sorry if I missed this but considering all diseases examined and their cognate controls, what biochemical functions (e.g. functional pathways) – if any - were in common across all studies???

In conclusion, this thesis is more than adequate to fulfill the written requirement for a Ph.D. Therefore, I recommend that Mr. Victor Urbiola Salvador be allowed to proceed to publicly defend his thesis for a doctoral degree in the discipline of biotechnology at the University of Gdańsk.

Sincerely,

David R. Goodlett, PhD
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