Gdańsk, 18.05.2023

PhD thesis review by Georges Bedram, M.Sc. "Exploring alternative sources of tumor antigens using large-scale immunopeptidomics", Supervisor of the thesis Prof. Dr. Theodore R. Hupp from the International Center for Cancer Vaccine Science. Auxiliary supervisor: Dr. Javier A. Alfaro.

General and Editorial Notes

Georges Bedram's doctoral dissertation entitled "Exploring alternative sources of tumor antigens using large-scale immunopeptidomics" is described in a way typical for a doctoral dissertation.

It contains 132 pages of computer printout divided into a section on general introductions in the field of MHC, cancer and immune system, selection of neaontigen candidates, promising sources of antigens and on the end thesis outline. In the outline 2 main aims were described separately technical and biological ones.

The main points of the PhD thesis are described in chapters 2 and 3 where 2 publications are presented and discussed separately. The first publication is accepted in Cancer Immunology Research and the second publication under revision.

Chapter 4 summarizes, milestones and future directions.

Moreover, an appendix 1 is separated form other parts to describe technical guide for COD-dipp pipline.

At the end 3 supporting documents are attached for contribution statements of the co-authors as well as acceptance letter from publication nr 2. While the acceptance letter for

the Nature Communications publication has not been provided, anecdotally the article has been accepted with minor revisions.

Substantive evaluation of work:

In the introduction in chapter 1, description of current status of knowledge about cancer antigens in the new age of vaccines and antigen specific T-cell therapies is given. First major histocompatibility complex (MHC) is described. Next cancer and the role of immune system are presented. Next parts described selection of neoantigen candidates and promising sources of antigens. Particular focus in the introduction concerns strange antigens presented to the immune-system from aberrant splicing events, noncoding translation and post-translational modifications.

The aim of the study

The first aim of the study was a characterization of alternative sources of tumor antigens derived from non-coding intronic regions or post-translational modifications in MHC Class I molecules. The chapter presents the development of a sophisticated pipeline for the detection of non-cannonical peptides making use of three fundamental concepts of proteomics searching. In a first for the whole field of proteomics he combined open, closed and denovo searching into a single pipeline. This means that in the results of his search he was able to gauge the spectrum of detectible canonical, non-cannonical and post translationally modified proteins and adjust FDR for each search respectively. In an analysis of 29 previously published papers, comprising the full spectrum of high-quality datasets as of December 2020 he explored the full landscape of non-cannonical antigen presentation. As far as I know, this is the largest landscape study of these strange peptides that have previously been reported in a handful of papers in the literature. Significant efforts were made to optimize the mass spectrometry pipeline for the characterization of these events.

The second aim of the study was presented in the second publication in chapter 3. This was a fascinating study. Viruses and cancers are known to alter the glycosylation patterns. An event often associated with immune-escape from antibody immunity. But, in this chapter Georges developed a pipeline to detect glycosylation events in MHC Class I and II antigen presentation. Another compendium of data was searched using the new tool and a new landscape of glycosylated antigens presented to the immune system was revealed. This landscape is full of new biology ready to be mined regarding the immune-visibility of glycosylation – though the thesis is focused mainly the detection of this new type of peptides. The challenges in analyzing glycosylated MHC associated peptides were described and the bottlenecks in their large-scale assessment. Here also online available databases were used and characteristics of disclosed glycosylated peptides. It's important to recognize that the novelty arises through the development of a computational methodology to detect these glycosylated peptides, which allows the re-examination of old datasets using a fresh eyes.

In both publications the candidate divided aims of the study into technical and biological. In technical aims 4 separate aims are described. Interestingly with description of technical aims these results led to better understanding of 3 separate gaps on the field of biological knowledge.

The publication entiteled "**The immunopeptidome from a genomic perspective: Establishing the non- canonical landscape of MHC class I–associated peptides.**" Was accepted for publication in Cancer Immunology Research – impact factor 12.02 (letter of acceptance attached in supplementary files). Here data from 26 MHC class I immunopeptidomic studies from 11 different cancer types were presented. The atlas showed 8601 ncMAPs with a suggestion of 17 cancer-selective ncMAPs that might be a good candidate for therapeutic targets. Here a combination of open-source pipeline and an atlas of ncMAPs found to be good source of new targets for T-cell therapies or vaccine development.

The second publication (chapter 3) entitled "**HLA-Glyco: A large-scale interrogation of the glycosylated immunopeptidome**" is available in bioRxiv repository since December

8 2022 and here Mr Bedran shares the first authorship together with Mr Polasky. Anecdotally this manuscript has now been accepted to for publication after minor revision at the prestigious journal Nature Communications.

Here a new method of computational glyco-immunopeptidomics workflow that integrates the ultrafast glycopeptide search of MSFragger with glycopeptide-focused false discovery rate control. Here also a open available database were used (8 studies). A HLA-Glyco was created that contains over 3400 HLA II N-glycopeptides form 1049 distinct protein glycosylation sited. This new field of glyco-immunopeptidomics the optimized workflow was provided as a free web source.

In conclusion, I consider the doctoral dissertation presented to me for review, consisting of the three chapters with two publications described above, to be very valuable both in terms of science and possible future applications of the obtained research results in clinical procedures, not only in the field of oncology but also other basic sciences. A very important scientific contribution of the doctoral student is basing his doctoral dissertation on a new method of computational glyco-immunopeptidomics workflow that integrates the ultrafast glycopeptide search of MSFragger with glycopeptide-focused false discovery rate control. Additionally, we can see from the first publication that a 17 cancer-selective ncMAPs might be a good candidate for therapeutic targets. However, the number of detected ncMAPs is much broader, and the author actually presents a comprehensive landscape of thousands of potential antigen presentation in cancer. Here a combination of open-source pipeline and an atlas of ncMAPs found to be good source of new targets for T-cell therapies or vaccine development. Even as the second publication is still under revision this hybrid type of doctoral thesis presented by Georges Bedran meets all the conditions for doctoral theses set out in Article 13 sec. 1 of March 14, 2003 on academic degrees and titles and degrees and titles in the field of art (Journal of Laws No. 65, item 595, as amended) and further criteria, including those regarding the new mode of defending doctoral theses. Therefore, I am applying for admission of the Candidate to further stages of the doctoral procedure.

I rate the substantive value of the research carried out very highly. The doctoral student's study is very insightful, cross-sectional and innovative.

In addition, due to the very valuable contribution to the study of the presented material and the presentation of innovative methods not previously described by other authors, as well as due to the professional and above-average preparation of the dissertation, I would like to ask the Honorable Council to award the doctoral dissertation.

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