L-Università ta' Malta

Faculty of Health Sciences
Dept. of Applied Biomedical Science
Rm 417, Biomedical Sciences Building
University of Malta,
Msida MDS 2080 Malta
T +356 99945592 david.saliba@um.edu.mt

Report for Doctoral Thesis entitled: "Dissecting the mechanism and functional landscape of cancer PD1 signalling in osteosarcoma" by Ms. Katarzyna Dziubek (Mpharm).

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)

In this dissertation the candidate presents important knowledge advancement of the role of Programmed cell death-1 (PD1) expression in U2OS - an osteosarcoma cell line. Despite the low incidence (3.4 cases per million per year) of osteosarcoma, research in this relatively rare tumour of the bone is warranted since osteosarcoma is often considered an "immunologically cold tumour" due to minimal PD1 and PD-L1 expression. This can, in part, explain the resistance to immune checkpoint inhibitors targeting the PD1/PDL-1 axis in osteosarcoma. However, as rightly pointed out by the candidate, this is only one reason for poor therapeutic efficacy observed during ICB in osteosarcoma. Often overlooked, and the subject of this PhD dissertation, is a growing emphasis on understanding the role of cancer-intrinsic PD1 and its influence on ICB.

The candidate presents a framework of key concepts including 1) immune checkpoint blockade with a focus on PD1/PDL-1 and CTLA4/CD80/86 axes; 2) hyperprogressive syndrome following ICB; 3) T cell signalling costimulatory/coinhibitory signalling and 4) PD1 signalling in T cells in a detailed introduction. As rightly pointed out by the candidate, there is a gap in knowledge on the role of cancer-intrinsic PD1 and its potential role in hyperprogressive expansion of cancer cells in osteosarcoma. Research is now shifting towards reassessing the safety of current treatments for patients with tumour-expressed PD1, while exploring the signalling pathways and molecular interactions of cancer-intrinsic PD1 to address the limited response to ICIs in specific cancers. The above underscores the candidate's profound theoretical knowledge in the discipline of ICB with an emphasis on osteosarcoma.
The overarching aim of this thesis is clear and the objective have been formulated to address the research questions outlined in the introduction.
Notably, prior to this research, PD1 expression had not been documented in osteosarcoma. The findings strongly indicate that PD1 exerts intrinsic effects on human osteosarcoma cells and may function as a tumor suppressor. This finding warrants further expansion in future studies as pointed out by the candidate especially in light of some conflicting results obtained through knockdown using different siRNA oligos. Coupling the siRNA knockdown to mass spectrometric analysis is certainly a step in the right direction considering this analysis has the obvious readout of dissecting out the effect of this perturbation on the cellular proteomic profile rather than the RNA profile through RNA-seq. Flow cytometric analysis highlighted that PD1 is expressed predominantly in the intracellular compartment rather than on the cell surface. This warrants further investigation

and could be a point of discussion. Is there an endoplasmic pool of PD1 which is translocated to the cell surface upon some as yet unknown trigger? In this context it is interesting that over expression of PD1 results in a dramatic increase of PD1 expression on the cell surface (Chapter 5 Fig. 28).

It is my opinion that the strongest data of this dissertation was the protein:protein interactome of osteosarcoma expressing PD1. By employing a twin-streptag-V5 pulldown followed by MS profiling, the candidate identified a novel interaction between PD1 and AXL. This interaction was confirmed by Proximity Ligation Assay that highlighted the interaction to occur on the plasma membrane. Remarkably, this interaction appears to be independent of PD1 phosphorylation status and molecular modelling and *in silico* docking analysis suggest that both the ECD and the ICD of both proteins participate in this interaction. I highly recommend submitting this section for publication in a peer-reviewed journal as it represents original knowledge generation crucial for understanding the challenges of ICB in osteosarcoma. This research not only sheds light on the reasons behind poor treatment outcomes but also provides valuable insights into potential novel therapeutic targets that could enhance treatment efficacy.

The thesis exhibited a remarkably clear writing style, presenting key points in a logical progression that seamlessly transitioned between sections, enhancing readability and comprehension. The candidate's ability to interpret research findings within the current field of knowledge not only demonstrates their capability but also propels the boundaries for future investigations. Collectively, this dissertation highlights the candidate's capacity to autonomously conduct scientific research, showcasing a remarkable skill set and expertise in the field.

Therefore, I am applying to the Council of the Biotechnology Discipline for admission of *Ms Katarzyna Dziubek* to further stages of the doctoral procedure.

Yours Sincerely,

Prof. David G. Saliba (PhD Edin.)