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Dean Prof. Zbigniew Madeja
Department of Biophysics
Jagiellonian University in Krakow

Dear Professor Madeja

I have reviewed with great interest the Thesis of Mr. Krzysztof Zak on the structural biophysics of PD1/PDL1 and its inhibitors. The thesis compiles an amazing amount of work that most certainly would qualify his/her author to earn a PhD degree at my academic institution.

The thesis is remarkable in many respects. First, it deals with an important question: the molecular mechanism of PD1 and PDL1 interactions, an important cancer target and one that has eluded detailed understanding for the human proteins; Second, using the above insights to discern the mechanism of action of the first-in-class small molecule inhibitors of PD1 and PDL1, opening a novel route for disrupting this checkpoint; Third, technically, the candidate laid out an outstanding introduction, discussing the biology and the broad scope and previous work of the problem. I also want to note the excellent command of the English (written) language displayed by the candidate, making the Thesis very easy to read; and, Fourth, the candidate has contributed to 10 publications, including four as first author.

The challenges on the crystallization of PD1 start by noting that this protein has been impossible to crystalize, existing only NMR structures of the apo protein. Mastering a broad set of experimental techniques (NMR, chromatography, fluorimetry, SDS, etc,), the candidate developed a rational search for the optimal protocol for protein expression, purification and crystallization, resulting in the first crystal and co-crystal of the human PDL1, and PDL1/PD1 complex. These studies rationalized important changes between bound and unbound structures.

No small molecules have been designed or discovered for this target. This thesis describes the NMR based screening and structural validation of PD1/PDL1 inhibitors. Most interestingly, the candidate solved the mechanism of action of this class of inhibitors by showing that compounds help dimerize PDL1,

preventing PD1 interactions. Moreover, he checked by differential scanning fluorimetry (DSF) that the compounds were specific to PDL1 and do not bind to PD-L2.

In summary, I am more than convinced that Mr. Zak has acquired the right skills to solve difficult problems in structural biology, and I have no doubt to his potential to develop an independent career.

Please feel free to contact me if I can provide any more supporting material towards or details regarding the great work that Mr. Zak performed in his Thesis.

Best Regards,

Carlos J. Camacho, Ph.D.
Associate Professor