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HR EXCELLENCE IN RESEARCH

Review of the PhD Thesis
of M.Sci. Martyna Plens-Gałąska

Targeted inhibition of STATs in chronic inflammation and cardiovascular disease

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Scope, significance and assessment of the thesis

In a current paradigm, atherosclerosis is a chronic inflammatory process, where the innate and adaptive immune systems play central roles in initiation and subsequent progression of disease. Given the relationship between inflammation and atherosclerosis, treatment of atherosclerosis from an inflammatory perspective appears to be a good and more effective anti-atherosclerotic modality. The signaling pathways mediated by inflammatory mediators are implicated within the atherosclerotic lesion. Understanding signal transduction helps to create a new treatment modalities. Because of the pivotal role of STATs (signal transducers and activators of transcription) in the development of atherosclerosis, the pharmacological inhibitors targeting STATs may have beneficial effects in protecting from inflammatory damage in atherosclerosis.

The Author – Ms. Martyna Plens-Gałąska in carrying out the series of studies undertakes to verify the hypothesis of the effectiveness of

inhibitors of the STAT family of proteins (STAT 1-3) in terms of modulating both the induction of inflammatory processes itself and the crosstalking with other molecular pathways in the environment of atherosclerotic lesion of the vessel.

The layout of the thesis is original, unusual for standard doctoral theses, but it contains the important and required chapters: introduction, (chapter 1), scope of research (1.11) (described in chapters 2 and 3, respectively). The whole is closed with chapter 4, the purpose of which, as the doctoral student points out, is to summarize the presented research results. The conclusions from the analysis of the results were presented mainly at the summary of the individual chapters of the theses. The bibliography contains as many as 202 items, correctly cited, arranged alphabetically, sporadically inhomogeneously cited (abbreviated names of journals were used, e.g.: Bäck et all. 2019; Cybulsky, M. I., & Gimbrone, M. A. 1982; Gupta et all. 2011 e.t.c). On page no. 42 of the dissertation, the following entry appears in the description of the methodology: 38, 39, probably in this place appropriate references to the bibliography should be found.

The dissertation under evaluation is very complex, both in terms of the research workshop and the stages of research. The PhD student, taking into account the inflammatory background accompanying vascular diseases (especially atherosclerosis), considers blockade of STAT proteins as a potential and promising treatment strategy.

In Chapter 1, information relating to the pathogenesis, symptoms and consequences of atherosclerosis is collected (p. 10 - 13). According to the assumptions about the pro-inflammatory basis of atherosclerosis, information on the inflammatory mediators of atherosclerosis was collected (p. 10-13) and, logically with the assumptions of the research, the family of STAT, IRF, NFkB proteins was described, with precise information on the association of these factors with pro-inflammatory signal transduction and the corresponding implications in relation to atherosclerosis (p. 19-24).

Presenting the strategy of inhibiting STAT proteins, the Author describes the strategies applied using peptides, peptidomimetics, small

molecule drugs, natural compounds or ASOs (p.25-29). The presented range of information is very synthetic, but it gives sufficient insight into the assumptions of the strategy of inhibiting STAT proteins.

The subject of inhibition of the signal transduction pathway involving STAT proteins is discussed by the doctoral student in the following chapters of the dissertation, where, among other things, she points out the possibility of using JAK kinase inhibitors.

Analyzing the entire dissertation, it is clearly visible how, as she conducts research and analyzes the obtained results, the doctoral student not only draws appropriate conclusions from her own observations, but formulates further research hypotheses, expanding the area of research. This proves the scientific maturity of the doctoral student and her perspective approach to the research.

The Author conducts *in silico* research, in which she searches for and analyzes the most promising potential STAT inhibitors based on available databases and literature data. In her research, she obtains results, on the basis of which she determines STATTIC and STX-0110 inhibitors for further research, showing similar binding properties/similar affinity to the SH2 domain, STAT1 and STAT2 proteins. Importantly, the PhD student identifies a new inhibitor: C01L-F03. The Author confirming cross-binding mechanism of action - the similar strength of binding of the listed inhibitors to the SH2 domain, calls them pan-STAT.

Thus, she adopts a hypothesis to verify the possibility of STAT protein inhibition with an indication of genome-wide effects of their action.

Chapter 2 describes in detail the methodology of the research, its results and analysis. HMEC cells were cultured under normal conditions and an *in vitro* model for activation of the cytokine inflammatory reaction was developed using IFNa, IFNg and LPS in the tests. The response to these factors was modulated by selected STAT inhibitors.

The mere determination of the range of concentrations, duration of stimulation, and appropriate combinations of factors constitutes a very

large scope of research and indicates their complexity and time-consuming nature.

In the next stage, the Candidate allocates the cellular material for the isolation of RNA and proteins to verify the effect of the inhibitors used in relation to changes in the level of transcription and translation, changes in the amount and activity of the proteins tested.

The molecular studies to verify the effectiveness and direction of modulation of the inhibitors used are detailed and logically linked. The Author not only confirms changes in the expression of key genes for modification of STAT protein activation caused by the presence of the tested inhibitors. She shows the reduction of "pro-inflammatory" genes transcription, as well as changes in the expression of genes encoding adhesion molecules.

A very interesting approach in the assessment of gene expression changes is the adoption of the wide-genomic strategy. The use of microarray and gene ontology (GO) analysis in the research revealed a whole range of connections between the changing expression of genes under the influence of the inhibitors used. The Candidate identified groups of genes not only related to the immune response, but also related to the regulation of cell proliferation, apoptosis, migration and cell adhesion. The microarray studies also reflected the strength of inhibitor activity and their multi-effect.

Conducting tests using the technique of chromatin immunoprecipitation independently confirmed the blocking of STAT proteins binding to DNA in the presence of the tested inhibitors. At the same time, it was a valuable supplement to the preliminary results of research conducted with the use of the western-blot technique.

The Author does not end her search in terms of the effectiveness of the tested inhibitors only on indicating changes in the level of gene expression. She shows the pool of genes that can be directly linked to the inflammatory background of atherosclerosis, and additionally, *in silico* studies, it indicates the presence of STAT binding sequences within the promoters of genes with changed expression. This is another result

confirming the effectiveness of anti-inflammatory inhibitors and their efficacy in blocking pro-inflammatory background of atherosclerosis.

Due to the pathomechanism of atherosclerosis, functional tests of endothelial cells or fragments of the vascular wall are particularly important, therefore it is worth emphasizing that these analyzes were conducted by a PhD student. These studies were conducted in cooperation with foreign research centers (*Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain, Institute of Health Research INCLIVA, University Clinic Hospital of Valencia, Valencia, Spain, Department of Pharmacology, School of Medicine, Universidad Autónoma de Madrid, Madrid, Spain, Instituto de Investigación Sanitaria Hospital Universitario La Paz (IdiPAZ), Madrid, Spain*). The above-mentioned studies significantly increased the value of molecular research and became an additional confirmation for the verification of the research hypotheses, including the indication of the relevance of using pan-STAT inhibitors for atherosclerosis therapy.

Obtaining so many valuable research results was undoubtedly associated with arduous work, and as I have already mentioned, just fine-tuning the time, concentrations, and/or combinations of using modulators required a lot of effort and numerous analyses. Perhaps this was one of the factors that became the basis for additional research activities of the Author - the creation of the SINDBAD database. An unusual type of research task, differing in terms of technique from other research, but very precisely related and focused on the research topic of the Candidate. The database created not only allows to search and find information on the STAT inhibitors used. It provides insight into data on their physicochemical properties, laboratory procedures used, tests conducted, including pre-clinical and clinical tests, included in the study of disease entities, or even information regarding the manufacturer/source of a given STAT inhibitor. The created database is an accessible, open database, it is not only a source of valuable data, but, as the Author points out, it has certain ethical values. The universality of the created database means that it can be expanded and supplemented with data by the users themselves.

The summary of the entire large research project is to conduct research and present the possibility of using a combined supply of inhibitors, indicating the possibility of verifying the pan-STAT or STAT-specific effect. The Candidate also shows that in *in silico* studies she can extract parameters useful for the verification of potential STAT inhibitors in terms of their further testing and prediction of targeted action as a pan-STAT or STAT-specific inhibitor.

The openness of her research project and the possibility of continuing her research are shown by the doctoral student in the last pages of her thesis. This is another, very important aspect of the dissertation being assessed, which again confirms the scientific maturity of the Author.

The results of the ongoing research described in the dissertation were presented in high-scoring scientific publications. The "author contributions section" of the published papers precisely states the extent of work that Ms. Martyna Plens-Gałąska contributed to the creation of each publication.

During the analysis of the dissertation, the following questions emerged:

- Could the RNA interference phenomenon be an effective therapeutic tool for regulating STAT protein activity?
- For which target group of patients would therapy with the described STAT inhibitors be effective?
- What is the Candidate's opinion on evaluating the balance of the effects of STAT protein blockade on the pro-inflammatory but also on the cytoprotective potential in cardiomyocytes of patients with coronary atherosclerosis?
- Can you propose epigenetic strategies for regulating STAT protein activity?

Final conclusions

Considering the particularly valuable results obtained by Ms. Martyna Plens-Gałąska, good mastery of the research technique and correct interpretation of the research results, herein I conclude that the dissertation of Martyna Plens-Gałąska entitled: " *Targeted inhibition of STATs in chronic inflammation and cardiovascular disease"* meets all conditions specified in Article 187 of Act of July 20, 2019 Law on Higher Education and Science (Journal of Lawa of 2018, item 1668, as amended), and thus I am asking the Scientific Council of Biological Sciences of Adam Mickiewicz University in Poznań for admission Ms. Martyna Plens-Gałąska to the next stages of the doctoral process.
At the same time, due to the high quality and importance of the results of the dissertation, the outstanding scientific achievements of the doctoral student published in leading scientific journals, the reviewed work deserves a distinction.

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