Institute of Microbiology
of the Czech Academy of Sciences

Laboratory of Immunotherapy
(head: Dr. Luca Vannucci, MD, PhD)

Prague, 19.08.2024

To the kind attention of the PhD examination board
Faculty of Science, Charles University
Prague

REPORT

Doctoral thesis:

Non-immunoglobulin binding proteins with immunomodulatory potential in autoimmune diseases

PhD candidate: Mgr. Yaroslava Groza

Study program: Molecular and Cellular Biology, Genetics and Virology

The thesis presented by the PhD candidate **Mgr. Yaroslava Groza** accounts the result of studies addressed to the development of small proteins for possible targeting of IL-6Rα (on tumor and immune cells), to impede the IL-6 binding and consequent cellular effects; of IL-22R1, to downregulate chronic inflammation in IBD; of PD-1, on one hand, to increase targeting and penetration inside tumor sites with more efficiency and sensibility than the currently used monoclonal antibodies, and, on the other hand, to perform more sensible histological staining as well as to be traceable *in vivo* for the identification of PD-1 positive structures (e.g. metastasis).

The methodology and results of these studies are presented in the collected research articles integral part of the doctoral thesis, respectively Cell Communication and Signaling 2022 (DOI: 10.1016/j.cytogfr.2022.04.001, first author, IF: 8.2), Cell Communication and Signaling (under revision) (2024), and Journal of Translational Medicine 2024 (DOI: 10.1186/s12967-024-05210-x, IF:6.1).

Finally, the dissertation integrates also two review papers examining, the first, the role of IL-6 in the renal IgA gammopathy (Cytokine Growth Factor Rev. 2022. DOI: 10.1016/j.cytogfr.2022.04.001, IF: 9.3), and the second a general overview of TREM-2 molecule as a part of immune regulatory factors interacting in the tumor microenvironment (International Immunopharmacology, 2024. DOI: 10.1016/j.intimp.2024.112042, IF: 4.8).

Formally, the thesis accounts 217 pages, of which: 8 introductory pages (including the Table of Contents); 183 pages composing the body of the thesis, classically organized (Introduction, Aims, Results, Discussion and Conclusions), well subdivided in sub-

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chapters and paragraphs; 5 pages of abbreviations and 19 pages of a wide bibliography collecting 330 references, ranging from 1974 to 2024.

The Introduction is clear and, in a sufficiently concise style. It progressively guides the reader through the complexity of the arguments related to the studied molecular systems (IL-6, IL-22, PD-1, TREM-2). I would like to suggest using “intercellular communication” instead of “cell-to-cell communication” in relation to cytokine-mediated communication, leaving “cell-to-cell” for describing the interaction by direct contact between the cellular membranes with their surface-expressed molecules.

The progressive steps to select the final small proteins - starting from combinatorial libraries, protein analysis and affinity tests until the tests *in vitro* and *in vivo* models - are well described, and further specifications are available in the included papers.

The all study, in the line of modern nanomedicine and precision medicine approaches, has clear aims, is innovative for the novel molecules here identified and proved (one patent is under evaluation) and demonstrates a virtuous protocol of research that, repeatedly applied, has permitted to the candidate and the team in which she works, to efficiently select working molecules.

The candidate shows through her papers (either as the first author or as a co-author) the progressive acquisition in knowledge and skills necessary for this kind of research, as also demonstrated by her capacity to make understandable and consequent the complex Introduction and Discussion.

Very relevant are the inhibitory effects that the developed small proteins exert on the IL-6 pathway both in the tumor microenvironment and inflammation, with very interesting and perspective application in conditions of plasma cell deregulation and immune complexes production like in the IgA nephropathy.

Similarly, the very interesting results of the novel small peptides against IL-22R1 in moderating the DSS-induced chronic colitis in mouse open new possibilities of intervention to treat the IBDs. We hope that these studies can further develop and be confirmed to, finally, reach the clinical testing.

Another very promising achievement are the targeting peptides against the PD-1 immune checkpoint molecule. They resulted equivalent and even more efficient in evidencing the PD-1 expression on cells and tissues, *in vitro* and *in vivo*, thanking also their good penetration. To identify the best responders for planning really efficient immunotherapies is still a challenge. The enhanced affinity of these new molecules for PD-1 is really important in the perspective of a more refined selection of patients, and the results of this study put a step forward in this direction.

Beside the experimental papers (one as the first author), the two review papers (one as the first author) help to better understand the importance of the experimental study and its possible implications and outcomes for future clinical applications (especially for the IgA nephropathy). Both reviews are accurate, documented and fluently written.

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The Discussion and Conclusions are adequate. The Bibliography is extensive, covers the last 30 years until today, and shows a not superficial approach in the compilation of the thesis. The language is adequate, but a few little errors can be eliminated by a new, accurate revision.

In conclusion, I approve the dissertation thesis of the candidate Mgr. Yaroslava Groza. She results to have fulfilled her study requirements with excellent results, and therefore she is recommended for achieving the title of PhD (Phylosophiae Doctor).

Questions:

Pleiotropic activity of IL-6 in the microenvironment of tumor and inflamed tissues can allow a sustain to Th17 cells and IL-17 production. Both cytokines appear involved in tissue remodeling promotion and fibrosis. How do you see the action and the use of your small proteins in prevention or even cure of the inflammation-promoted fibrosis (e.g. like in the chronic colitis, liver, lung, pancreatic cancer)?

How many doses can be considered necessary to administer for a stable and effective treatment?

Could be imagined a use of your small proteins even for brain pathologies, i.e. have you any evidence of blood-brain barrier crossing?

Faithfully,

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