Review on dissertation of Mgr. Ondřej Uher

Study of Cancer Immunotherapy Mechanisms in Pancreatic Adenocarcinoma and Pheochromocytoma Murine Models

The presented dissertation deals with a serious cancer problem, namely a possible immunotherapeutic treatment of a mouse model of pancreatic adenocarcinoma (PanC02] and pheochromocytoma (PHEO). It is clear that new treatments are urgently needed, as pancreatic cancer has a five-year survival of 6% to 11%, but always after a feasible surgical intervention. If this is not possible, the patient´s life is in acute danger. The situation is similar with pheochromocytoma, a rare mostly benign adrenals disease. However, if the tumor is malignant, standard treatment is difficult. Tumor occurs most often in young people between the ages of 20 and 50, although it can develop at any age. It causes a number of serious, life-threatening health problems that often affect remote organ systems.

After many years of skepticism, immunotherapy is today one of the four basic pillars of tumor treatment. These are surgery, chemotherapy, radiotherapy and the already mentioned immunotherapy. In the last decade, it has relied mainly on modern and successful treatment with inhibitors of immune response checkpoints. However, even this therapy is not for every tumor patient who needs treatment. Therefore it is necessary to look for other therapeutic options.

The idea of the newly proposed method of immunotherapy, to which dissertation is devoted, came from the assumption that intratumoral application will allow the accumulation of the chosen therapy directly at the site of the desired effect where both arms of the immune response should be activated.

The dissertation builds on previous laboratory results, which effectively eradicated dangerous and poorly treatable B16-F10 mice melanoma using a combination of Toll-like resiquimod receptor (R-848)), poly (I:C), heat-killed *Listeria monocytogenes* and mannan. The latter two components were attached directly to the tumor cells using a suitable anchor (BAM). The original combination has been termed MBT therapy and has been well tested in several mouse tumor models. However, the remarkable results obtained in the melanoma model were not repeated in the aggressive pancreatic adenocarcinoma (PanC02) and pheochromocytoma.

In the new approach, *Listeria monocytogenes* was replaced by another TLR2 agonist, lipoteicholic acid (LTA), which was also anchored to the cell membrane using a biocompatible cell membrane anchor (BAM). The MBT therapy was additionally supplemented by anti-CD40 antibodies that mimic CD40 ligands on Th helper cells. It leads to the activation of antigen-presenting cells and the induction of an effective T cell antitumor response. This new therapy was called MBTA therapy and was used against metastatic PHEO tumor, bilateral PHEO tumor and bilateral PanC02 tumor. Various combinations have been tried, including combinations with immune response checkpoint inhibitors, chemotherapy and radiotherapy. The presence of innate and adaptive immune cells and released cytokines was studied directly in treated as well as untreated distant tumors and in the spleen. It was verified whether and what memory

can be induced by this therapy and also which immune cells perform it. Finally, MBTA therapy in young and old mice was compared.

The results initially demonstrated the successful eradication of an aggressive melanoma in mice, which at the same time acquired anti-tumor resistance as demonstrated by re-transplantation and the ability to suppress the development of metastases. Treatment was further validated in a PanC02 bilateral mouse model of pancreatic tumor. In this case, the therapy was successful only if treatment was applied to both tumors. Immune response checkpoint inhibitors (anti-PD1 and anti-CTLA-4 antibodies) did not show statistically significant results. A similar approach was chosen for the treatment of model pheochromocytoma in mice. The treatment elicited strong innate immunity as evidenced by significant tumor infiltration with cytotoxic neutrophils and an observable shrinkage of the model tumor. The involvement of adaptive immunity was demonstrated as resistance of cured mice to re-transplantation with the same tumor. It is important to emphasize that distant liver metastases was also affected by the treatment. The role of $CD8^+$ T cells has been demonstrated by depletion of this T cell subpopulation. Treatment of mouse subsutaneous pheochromocytoma was also compared in young (6-week-old) and old (71-week-old) mice. In old mice, the incidence of s.c. tumors and their growth was more aggressive, but it is noteworthy that the effectiveness of treatment was comparable.

Dissertation and its results are very nice and original, well and clearly written. The only thing I would complain is the fact that in the publications where Mgr. Ondřej Uher is only one of a large number of co-authors and where Veronika Caisová is typically the first author, his contribution to the publication is not explicitly stated. I have a few questions 1) Intratumoral administration certainly has the advantage of concentrating treatment into the tumor. However, the technique of administration is, apart from skin tumors, practically unusable for human medicine. This is especially true for metastases, which are difficult to locate. Does the author have any idea how to convert i.t. application to human practice or it is just a model approach? 2) What advantage does i.t. administration versus systemic administration? My experience with immunotherapy is that treatment of only one of the bilateral tumors leads to expansion of the other 3) As already mentioned, the main problem of oncology is metastases. But these are not affected by i.t. administration. 4) The tumor is not homogeneous. How to ensure that the i.t. therapy is administered to the outer part of the tumor with abundant immunocompetent cells and will not be applied to the necrotic part? 6) How can it be explained that the treatment of Panc02 tumor was comparable in old and young mice, regardless of the fact that the growth is in old mice more aggressive as during the period of so-called immunosenescence, the performance of both arms of innate and acquired immunity decreases. 7) It´s not disappointing that in a bilateral Panc02 tumor only the one to which the i.t. application was administered is affected? After all, it was proven by re-transplantation that systemic adaptive immunity is activated during the mentioned immunotherapy. Shouldn´t it apply?

The author logically concludes that in order for immunotherapy to be effective, it must be used before chemotherapy and radiotherapy, i.e. at a time when the immune system is not

too depleted by disease and immunosuppressive treatment and can take advantage of all the benefits that immunotherapy provides.

Conclusion: Dissertation Mgr. Ondřej Uher is of exceptional quality and meets all the requirements placed on it. I have no doubt that the candidate is a talented researcher with the ability to independently address and solve scientific issues. I therefore recommend the dissertation for the defense and award of the title Ph.D.

Praha 19. 5. 2022 **Prof. RNDr. Blanka Říhová, DrSc.**