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Statement of the bachelor thesis reviewer

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Title: T Regulatory Lymphocytes During MBTA Immunotherapy of Pancreatic Adenocarcinoma
Supervisor: RNDr. Jan Žeňka, CSc.
Supervisor specialist: Mgr. Ondrej Uher, PhD.
Reviewer: doc. Mgr. Jaroslava Lieskovská, CSc.

The thesis deals with the cancer immunotherapy with particular interest in the MBTA which is the therapy invented by Dr. Žeňka. MBTA therapy is based on the mixture of 4 compounds potentiating anti-tumor immune reactions. The overal aim of this non-experimental thesis was to write review about the role of T regulatory lymphocytes in cancer and summarize the knowledge about MBTA therapy. To assess a potential involvement of T regulatory cells in MBTA therapy, the design of future experiment was outlined.

In first 15 pages, the thesis covers the basic information of tumor biology and overview of immune system followed by description of general interplay between cancer and immunity. This part is well written however contains an excesive textbook informations which could be avoided. Special chapter is devoted to the role of T regulatory lymphocytes in range of 13 pages. Author writes about the classification of regulatory T lymphocytes, their function, cellular markers and differences found between human and murine cells. The importance of regulatory T cells in cancer development and the role in anticancer therapy is added as well. The text is supplemented by numerous figures which illustrate the interaction between particular immune cells and the reciprocal regulation. I do apreciace the ability of author to write about such complex and often complicated interaction it the understandable way. Next, the varioues types of existing immunotherapies and their proposed mechanisms are described. This is very interesting and also well written chapter. Finally, the MBTA therapy is described on 6 pages. It summarizes what is known today about MBTA therapy in various model of tumors, including malanoma, pancreatic adenocarcinoma, pheochromocytom or colon cancer. The conclusion contains the suggestion of experiment which would test the involvement of T regulatory cells in MBTA therapy. The detection of regulatory T cells in tumor and the non-

intratumoral administration of anti-CTLA-4 antibody was proposed. I like this part of the thesis because even it is not experimental work still it shows that the author can plan and think of detail preparation of *in vivo* experiment.

Overal the thesis is well written, with good english and stylistics, complying with the valid terminology. The content of thesis is correct as far as I can say. As concerns to a proportion of individual parts, the thesis is well balanced, with the exception of the excesive textbook information at the beginning which was already mentioned. The references were properly used as well.

Comments

1. In general, the message from the figures should be understood from legend. Therefore, a short description of the figures should be added in two or three sentences into each legend.
2.Unproper titles of chapters are used. i) Chapter 7.3 with heading Reasons for not using intratumoral MBTA. There is not written any reason about it in the chapter. It should be named e.g. MBTA vacciny therapy as that is what all chapter is about.
ii)The title of thesis does not fit well to the content of thesis. First time when we read about T regulatory lymphocytes during MBTA immunotherapy, what is the title of the thesis, is in the chapter Conclusion. And there is just one experiment mentioned potentially addressing the role of T regulatory cells in MBTA.
3.On page 15 is stated that regulatory T cells differentiate in the presence of TGF-β and IL-10. I do not think the IL-10 is a differentiating factor for Treg. This cytokin is effector cytokine with immunosupresive effects produced by the regulatory T cells.

Questions

1. On page 45, you mention that treatment of adenocarcinom with anti-CTLA-4 antibody did not improved the MBTA therapy likely due to lower amount of antibody used in comparison to others. Could you specify what were these difference?
2. The first step in your suggested experiment is to perform the proper detection of Treg in tumor. This seems correct. However, the administration of anti-CTLA-4 antibody may affect all cells which express this molecule. Can you outline what type of cells

can be affected and how? Has ever been examined the MBTA treated or not treated tumor for the presence of Treg?

3. CTLA-4 receptor is expressed on activated T cells and constitutively expressed on the regulatory T lymphocytes. By using anti-CTLA-4 antibody you target both mentioned population of T cells. As CTLA-4 is an inhibitory receptor (and competes with CD28 in binding to B7) do you really expect that its blocade (with anti-CTLA-4 antibody) will lead to a decrease of intratumoral Treg as you wrote on page 45 line 13?
4. Can you point point out i) the common features of MBTA therapy tested on various types of cancers, and ii) differences which are unique to particular types?
5. What do you think about the improvement of the MBTA therapy by depletion of factors needed for the differentiotion of Treg?

In conclusion, the submitted thesis is very interesting and of high quality. The author processed a huge amount of literature, chose the important things, and managed to write it in understandable way. The ability to design an experiment testing the involvement of Treg in tumor biology confirms author‘s deep undertanding of this interesting topic. The thesis fully meets requirements for bachelor thesis. I recommend thesis of Isabel Simmer to the defense and suggest the grade excellent.

In České Budějovice, June 8, 2023. doc. Mgr. Jaroslava Lieskovská, CSc.