Editor-in-chief Rostock December 3rd, 2018

Dear Editor-in-Chief, dear Prof. Tarnawski,

thank you very much for the opportunity to submit our manuscript entitled 'Colorectal Cancer Vaccines: Tumor-associated antigens versus Neoantigens' for potential publication in ***World Journal of Gastroenterology*** to be considered as a mini-review. We are also thankful for the excellent review and modified our manuscript according to the reviewer's suggestions.

In the following, please find our answers on a point-to-point basis. Structurally, we first list the reviewer's comment followed by our answers.

Comments from **Reviewer #1:**

Authors address the topic of vaccination in the setting of colorectal cancer (CRC). This is a very interesting topic, especially in the context of the success that immunotherapy, namely the use of immune checkpoint inhibitors (CPI), is proving in different cancer indications, including the MSI+ CRC patients. Some inaccuracies are present, and need to be corrected. Additional general and specific suggestions are provided below. General comment: Authors are encouraged to critically evaluate the role that vaccination could have in the current era of CPI. In other words, to me the review does not communicate what the authors suggest/feel/imagine as a best setting for vaccination in order to improve immune eradication of CRC. Would it be best in cancers that lack/are deficient in antigen-specific T cells (improve priming and eliciting antitumor immune response)s? Hot/cold tumors, from an immunological point of view? Authors imagine a role of vaccination also in tumors with abundant presence of immunosuppressive cells/molecules, and with what rationale? Any different role imagined in MSI vs MSS tumors?

Specific comments:

1) Abstract, „two general classes of target structures", could read, "….target molecules" or "…..targets."

2) Core tip, "an extremely promising novel tool", should read "…..promising tool".

3) Core tip, „…due to their unspecificity, they frequently trigger severe adverse events. This risk is neglectable …." This needs to be rephrased/toned down: the SAE reported by authors were triggered by the use of adoptively transferred peptide-specific T cells and not by vaccination. Inference is not possible.

4) Core tip, „Intelligent modern CRC vaccines will combine several or", should read "….will likely combine several or".

We changed the phrasing according to the reviewer's suggestions (comments 1) to 4)).

5) Introduction, „peptides alone or loaded onto antigen presenting cells". Why do author focus only on APC?

This sentence was rephrased to clarify that this mini-review is focused on suited targets for tumor-specific vaccination.

6) **Authors should include a table summarizing**: vaccination strategies, peptides used, adjuvants, number of enrolled patients, clinical results, sorted by antigen type (TAA vs tumor-specific), etc.

We included tables similar to these suggestions. We are very thankful for this suggestion because it definitely improves the overall quality of the manuscript.

7) Carcinoembryonic antigen, "the efficiency of CEA peptide vaccines was overall not satisfying[7]." Numbers should be provided.

They have been added.

8) Melanoma associated antigen, "The melanoma associated antigen (MAGE),". Specify that MAGE are representative of a specific class of TAA the CTA.

This information has also been added.

9) Melanoma associated antigen, "A vaccination study with melanoma cell lysate", If melanoma cell lysate is used, it could not be defined as vaccination with MAGE antigens.

10) Melanoma associated antigen, "40 % of MAGE-positive CRC", which of the MAGE antigens?

Comments 9) and 10): We want to thank the reviewer for this watchful comment. This part has been modified.

11) Neoantigens – truly tumor-specific antigens, "neoantigens have only recently been accepted as ideal targets for successful immunotherapy", authors should mention their possible involvement in predicting response to CPI.

We are again thankful for this comment. The potential of neoantigens as critical determinant for CPI responses have been included into the manuscript.

12) TGFβRII and other frameshift mutations, "…coding microsatellites: PTHL3, HT001, TGFβIIR, AC1, ACVR2,….", TGFβIIR is repeated from the section above.

This has been corrected.

13) TGFβRII and other frameshift mutations, "In addition MARCKS-1, MARCKS-2, TAF1B-1, PCNXL2-2, TCF7L2-2, Baxα+1[47] as well as CREBBP, EP300, TTK[48] have been suggested to be taken into consideration for developing cancer vaccines for MSI+ CRCs." Authors should argument on why a specific focus for developing cancer vaccines has been put on these gens or rephrase.

This part of the manuscript has been rephrased and we are confident that the line of reasoning why these genes might be of especially high interest has been strengthened.

14) TGFβRII and other frameshift mutations, "containing peptides of frameshifted AIM2,", AIM2 was not included in the list of genes reported in the previous part of the section as containing frameshift mutations.

This has been corrected, too.

15) Point mutations: KRAS, does CRC have point mutations that could provide neoepitopes only in KRAS?

Here, we were not absolutely sure if we understood the comment of this reviewer correctly. We included one abstract about KRAS as this gene is one (if not the single most) prominent example for point mutations. But neoantigens can result from mutations in every coding region of the genome. Therefore, KRAS was only chosen as a well characterized example for point mutations. This point has also been emphasized in the text of the manuscript.

16) Genetic Configuration and Target Selection, "This lowers the risk of SAEs by only enhancing the existing antitumoral immune response instead of creating new targets." So pursuing new targets is discouraged by the authors? For some scientists the higher the number of neoepitopes targeted, the higher the possibility to evade antigen-driven imune escape.

This sentence was poorly worded and therefore rephrased. Thanks for this helpful hint.

17) Single peptides, peptide-loaded antigen-presenting cells or ex vivo expanded T cells?, ". In addition, the patient's individual set of HLA alleles also influences the efficiency of a peptide vaccine.", authors need to explain.

We added an explanation why patient's individual HLA is as important as it is for anti-tumoral immune responses.

18) Single peptides, peptide-loaded antigen-presenting cells or ex vivo expanded T cells?, "To evade HLA restriction, longer peptides (15-30-mer),.." and "Another way to circumvent HLA restriction as..", authors should better detail what they intend on "circumventing/evading HLA restriction", which effector cells are expected to do the job?

Again, this part has been augmented in order to better explain why HLA restriction should be avoided or at least minimized when designing tumor-antigen vaccines.

19) Adjuvants, authors should comment on pros and cons of the available adjuvants. Is there any one which would be preferable?

This abstract was included to give readers a short overview of the different adjuvants used in the tumor vaccination field. A detailed discussion on how and why choosing the right one for different applications was not in the scope of our mini review.

20) Adverse events, "In a study with engineered anti-CEA T cells, the.." and below, authors need to be careful not to lead the reader to infer SAE from vaccination with TAA stemming from SAE observed using adoptively transferred T cells.

We are again thankful for the careful review of our manuscript. This point has indeed been not correct and we modified it accordingly.

21) Adverse events, "The treatment with autologous anti-MAGE-A3 engineered T cells…", recognized peptide is shared by MAGE-A3/A9/A12.

This has been corrected.

22) Cancer vaccines: The solution to immune evasion? An effort should be made to make clear how cancer vaccines are proposed to takle the immune evasion mechanisms reported, e.g. HLA loss.

Concerning this point, we added some information and ideas how we think it possible that immuno-therapeutic approaches will benefit also patients suffering from tumors with active immune evasion mechanisms. However, we did not try to deepen this aspect since it was again not the focus of the current mini-review.

23) Immune check point inhibitors, „, PD-L1, LAG-3, and IDO", to my knowledge, IDO is not generally considered an immune checkpoint.

We agree with the reviewer – IDO is a candidate to be involved in immune escape, but not an immune checkpoint in a narrower sense. This has been corrected.

24) Immune check point inhibitors, „In clinical trials, almost 80 % of MSI+ CRC patients benefitted from PD-1 blockade whereas microsatellite stable (MSS+) CRC patients rarely did[94,93].". Please provide range and type of responses observed in the different trials. Besides, would authors suggest a different expected impact of vaccination on survival of MSI vs MSS patients?

We here cited a review by Gupta et al. [ref. 98] In this overview, the authors clearly discussed the first mentioned aspect of range and type of responses observed in the different PD-1 blockade trials. Concerning the second point, we definitely think that patients with a hyper-mutated CRC (either MSI or POLE-mutated) are more likely to benefit from checkpoint-blocking therapies. But similar to the first point, we would like to refer to the cited review since also this aspect was not central to our mini-review.

25) Immune check point inhibitors, „, but the correlation between infiltrating lymphocytes and overall survival is only in MSS+ patients significant[95,96]. ", is there conflicting literature evidence on this?

According to the best of our knowledge, this interpretation of the data from literature cannot be considered as a dogma, but the larger studies analyzing lymphocyte infiltration and MSI status concerning overall survival came uniformly to this conclusion.

26) Conclusion, authors should consider providing **a table with current number of trials** evaluating peptide cancer vaccines, as monotherapy or in combination, sorted by TAA and tumor-specific ones to give the feeling of the current interest in the topic.

Similar to comment 6), we added a table according to the reviewer's recommendation.

27) Conclusion, "These genetic alterations can…", not always associated to genetic alterations (e.g. epigenetic, regulatory?).

Thanks for this comment. The focus of the current review are neoantigens (which are for the largest part derived from point or frameshift mutations) and this might have corrupted our thinking a bit. The conclusion has been modified in order to correct this bias.

Comments from **Reviewer #2:**

The authors have done a very decent review on most parts of the cancer vaccines for colorectal cancer, and provides some valid perspective on the future of the field. The authors have tried to cover most, if not all, approaches of cancer vaccines, including the use of immunogenic chemotherapies.

However, it seems that one obvious was missed, which play more and more important roles in cancer therapy and cancer vaccine. Oncolytic viruses have been shown to induce immunogenic cell death and can induce tumor-specific CD8 and CD8+ T cell responses. Indeed, these oncolytic viruses may function as potent therapeutic vaccines. Thus, it may be appropriate to add a paragraph (on page 15?) and make a short discussion on this particular class of cancer vaccines. Articles for references may be, (1). Bartlett DL et al. Oncolytic viruses as therapeutic cancer vaccines. Mol Cancer. 2013; 12:103. (2). Russell SJ, Barber GN. Oncolytic Viruses as Antigen-Agnostic Cancer Vaccines. Cancer Cell, 2018; 33: 599-605.

Minor issues.

1. It needs some minor improvements in English language and use of certain terminology. A few examples are as follows,

(1). In Introduction: "One promising approach to further improve this type…" should be "one approach to further improving this type…"..

(2). "Checkpoint inhibition" should be changed to "immune checkpoint inhibition", as they are a variety of checkpoints these days, such as metabolic checkpoint.

This has been modified according to the reviewer's recommendations (1) and 2)).

2. Page 14, line 11, and other places. "MSI+ CRC…" In literature, the most common way to describe the status of MRI is "MSI-high" and "MSI-low", not MSI+. 3. Page 16, line 8. MSS+ CRC. Is it a typo in "MSS+"?

We changed also MSI+ to MSI^{high} all through the manuscript.

Comments from **Reviewer #3:**

The authors reviewed the literature with regard to the colorectal cancer vaccines. It is a very decent summary of the topic with very clear structure and good evidence. The topic is an important one nowadays with increasing clinical attention. To provide an overview of this topic, I would suggest to use several figures illustrating the mechanism of the vaccines and the targets mentioned in the manuscript.

We first want to thank this reviewer for the positive assessment of our manuscript. More important, we appreciate the very helpful comment and prepared two Figures illustrating the

differences of TAA and TSA on the one hand and the general idea how to prepare an individualized vaccine with a focus on the usage of neoantigens on the other hand.

Comments from **Reviewer #4:**

The authors presented a review of colorectal cancer vaccines, and the manuscript is well-organized and written well with detailed data. Some part like clinical trials is complicated to understand. So, if possible, summary with table of clinical trials or figures on concept of colorectal cancer vaccines could be helpful to improve the readers' understanding.

Similar to our answer to Reviewer #3, we are thankful for the positive evaluation and acted according to the suggestions. Two tables as well as figures have been prepared and added to the manuscript to improve future readers' understanding.