Answering Reviewers

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Title: Immunotherapy in colorectal cancer - available evidence, challenges and novel approaches

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2 Peer-review report

Reviewer #1: I. The author the will be take in consideration included the paper information with have been published, is necessary included more information and references about Immninotherapy in colorectal cancer. II. References are relevant and update, however, the author should add more about of Immnunotherapy in colorectal cancer.

Reply: The authors are not completely sure what the reviewer suggests. We think the topic of clinical immunotherapy of colorectal cancer is covered very well as suggested by the other reviewers. We therefore added clinical to the title as suggested by reviewer 4.

Reviewer #2: The impact of immunotherapy on the survival of patients with colorectal cancer is not obvious compared with other malignancies, such as melanoma and lung cancer. Only a minority of patients with metastatic colorectal cancer, mainly CMS1, respond to immunotherapy. This article presents the status of immunotherapy in colorectal cancer and analyzes the difficulties in improving the efficacy of this modality. And novel approaches in the future are also reviewed in this article. This is a comprehensive review which summarizes important clinical data about immunotherapy of colorectal cancer and points out promising strategies in this field. This article provides useful information about immunotherapy of

colorectal and indicates that a combined strategy may resolve the dilemma in immunotherapy of colorectal.

Reply: We thank the reviewer for this encouraging feedback.

Reviewer #3: Authors extensively reviewed clinical trials related to immunotherapy in colorectal cancer. The review touches on nearly every aspect of clinical trials involving various types of immunotherapy. The manuscript is relatively well written. There are several minor points for a potentially better review article. Ambiguoug meaning: 1) Page 3, paragraph 2: the effect of neoadjuvant nivolumab and ipilimumab seems to be as good as that of metastatic setting according to the authors' description (4/7 complete response and 3/4 partial response). Then, what does "challenging the noted response rates with checkpoint inhibition in dMMR/MSI-H tumors" mean? The term "challenging" in this context usually means that the described observation is different from the known one.

Reply: We want to thank the reviewer for this relevant comment. The authors wanted to state that pathological regression in all patients (7/7) to 2% or less vital tumor cells challenge the noted response rates by imaging in metastatic setting (27/45). To make this statement clearer the authors change the wording accordingly.

Consistency in terms: 1) CTLA 4 or CTLA-4 -> please keep a constant term (I recommend CTLA-4) 2) Just before "Future development" section, "phase l or ll studies..." -> "phase 2 or 3 studies" Several spelling errors: 1) first page of introduction paragraph 2, line 5. therefor -> therefore 2) stage ll, lll -> stage II, III (throughout the manuscript, numeric in stage description should be in capital Roman) 3) some survival data use commas when describing months: 14,1 months (in page 5). Please correct (14,1 -> 14.1) 4) Please use correct greek symbol. For example, INF-y -> IFN-gamma (please use word processor and use "Insert symbol" menu) 5) In "Adoptive cell therapy" section, ex vivo -> please write in italic font;

Reply: Changes were made accordingly.

"tumor draining lymph nodes of 1V MCRC...", what does "1V" mean? Please clarify; More specific, the Rosenberg..." -> "More specifically, the Rosenberg..."

Reply: 1V should mean stage lV but is dispensable since MCRC is written thereafter. Therefore, 1V was erased and the next sentence was changed as suggested.

Reviewer #4: The authors have tried hard to cove the whole field of immunotherapy for CRC for the clinical part. Little of frontier and promising preclinical studies have been discussed or even mentioned. Therefore, it is suggested that authors add one word “clinical” to the title in order to separate many papers in preclinical studies from this one: “Immunotherapy for colorectal cancer – Available clinical evidence …” This is a very useful and timely review.

Reply: We thank the reviewer for his comments. We agree that this review mostly focusses on clinical perspectives and therefore changed the title accordingly.

However, there is one major issue I hope that authors can improve it during revision. The authors have tried to cover all approaches, but not discuss some of them in great details enough. For example: Under "Checkpoint inhibition and oncolytic viruses", they only discussed one clinical trial with Pexa-Vec, which was not even in combination with checkpoint inhibitor (ref #42). They cited an old research paper about the function of GM-CSF (re #41, published in year 2000) which is useless as the readers of this paper should know the function of GM-CSF. In fact, many readers, if this is published, do not know the field of oncolytic virotherapy, now many term as "oncolytic immunotherapy", as the main mechanisms of action involve the OV-elicited antitumor immunity. In this case, it is ideal to cite one or two review articles for readers to better know the relative new filed. In this case, I would suggest to cite, (1). Lawler SE, Speranza MC, Cho CF, Chiocca EA (2017). Oncolytic Viruses in Cancer Treatment: A Review. JAMA Oncol 3: 841-849. (2). Guo ZS, Liu Z, Kowalsky S, Feist M, Kalinski P, Lu B, et al. Oncolytic immunotherapy: Conceptual evolution, current strategies, and future perspectives. Front Immunol. 2017; 8:555. The follow-up of that clinical trial is with combination with immune checkpoint inhibitor, some ongoing clinical trials. There is an abstract, if you really want to cite: MP Morelli Mp et al., A phase I/II study of pexa-vec oncolytic virus in combination with immune checkpoint inhibition in refractory colorectal cancer: Safety report. J Clin Oncol. 2019.

Reply: We thank the reviewer for this appropriate suggestion. We added one review about OVs and further added the recently published safety report about pexa-vec and immune checkpoint inhibition. However, we think the target audience of this review article and the journal rather include colorectal cancer clinicians, who may benefit from a reference paper about GM-CSF function.

Under "Adoptive cell therapy", the authors have discussed two clinical trials using TILs. The first was Ref#47, using sentinel lymph node lymphocytes, and the second one was Rosenberg's TIL again mutant KRAS. In fact, other types of immune cells have been used for adoptive cell transfer for CRC patients. For example, Ishikawa T et al. Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with IgG1 antibody in patients with gastric or colorectal cancer. Int J Cancer. 2018; 142:2599-2609.

Reply: We thank the reviewer for this suggested clinical study and added this work to our review. However, the authors are aware that not all trials using adoptive cell therapy could be mentioned and refer to other reviews in the text due to space limits and limited clinical usage.

Other minor issues are, 1. It seems that the real data generated from the experiments were presented as supplementary tables and figures? The reviewers do not have access to those data, thus make our duties much more difficult.

Reply: Indicated supplementary material was wrongly stated and refers to abstracts from conferences. The authors now tried to clarify.

2. References: Author have done very poor job in this part, and many references need updated or correct information. Please do a better job in the future. Ref #1: Name of the journal? Ref #15. Journal name? Ref #17, 18. Page number? Ref #22. Journal? Ref #26. The actual article number, (not page numbers, 1-14), is e1433981. Ref #28. The actual article number, (not page numbers, #1-7), is 202. Ref #31. Pages? Ref #33. Journal and other info? Ref #37. Volume and page numbers? Ref #38 Journal name, please?? The author list, the title of the article all contain errors. (It seems the first author is IFN-C?) Ref #39. Journal name, volume and pages?? Ref #44 and 45. Journal again? Ref #46. Journal Name and page numbers? (The name "Theranostics" is misplaced) 3. The authors like to cite many supplementary abstracts in references; Ref #16, 19, 20, 29, 32, 36 and possibly more. These information published in the abstracts were not peer reviewed with real data, and tend to be not accurate. It is a good practice not to cite too many abstracts as references in a review article. 4. There are a number of minor errors in English grammar throughout the manuscript.

Reply: We tried to correct our references thoroughly and tried to limit the number of abstracts which are of course not peer reviewed, although are highly important for the currentness of this review.

Reviewer #5: This is a comprehensive, well written and informative review. My comments are generally minor as follows. 1. It would be good to use British or American English consistently (eg tumour or tumor, but not both).

Reply: Changed to American English.

2. It is stated that ..."mainly found in the subset of mismatch repair-deficient (dMMR) or microsatellite-instability-high (MSI-H) tumors." As written, the sentence implies these are different entities. However, microsatellite instability indicates a defective mismatch repair system. It may therefore be better to clarify any distinctions between these two entities or alternatively to write....."mainly found in the subset of mismatch repair-deficient (dMMR) tumors in which high levels of microsatellite-instability (MSI-H) are found." 3. Therefor (sic) should read therefore. 4. MCR should read MCRC (page 5). 5. Constituent drugs in FOLFOX/ FOLFIRI should be spelled out at first mention (page 5).

Reply: Changed accordingly.

6. On page 9, the authors imply that targeting of MUC1 using CAR-T is "unspecific". However, this issue may be circumvented through the exploitation of binders that preferentially engage aberrantly glycosylated MUC1, as is widely found in solid tumors but not healthy tissues (Wilkie et al J Immunol Apr 1;180(7):4901-9). Targeting of MUC1 is further supported by its upregulation in tumors and owing to the fact that expression is polarized in healthy tissues (lumenal expression - inacccessible to CAR T-cells) but non-polarized in tumor cells.

Reply: We absolutely agree with the thorough comment, that targeting glycosylated MUC1 may circumvent harm of healthy tissue, however the authors only state that

targeting tumor associated antigens in CRC is still "rather" unspecific and may cause side effects as seen in clinical trials using for example CEA CAR T cells.

7. A CAR T cell trial in MCRC targeting CEA should be described (Thistlethwaite et al Cancer Immunol Immunother. 2017 Nov;66(11):1425-1436). Once again, efficacy was limited and toxicity noted as predicted by the authors. 8. The cited ErbB2 CAR T-cell study was tainted by the infusion of an excessive number of CAR T-cells. The authors may wish to note a more recent study in sarcoma in which ErbB2-specific CAR T cells proved both safe and effective (Hegde & Ahmed, Baylor College - see https://www.ascopost.com/News/59888). 9. (LAG-3) should read LAG-3.

Reply: Changed accordingly.

Reviewer #6: The authors summarized currently available evidences of immunotherapy for CRC showing the results of clinical trials. The theme and the information are really important and can be considered for the publication, however, there are points to be revised. 1. The relation between each paragraph is not clear, therefore, they should be reconstructed with the summary table showing the pros and cons of each options.

Reply: We thank this reviewer for his encouraging feedback, however the authors think all parts are ordered by subheadings and contain at least one sentence of introduction. Further, the authors tried to summarize the essence of each paragraph at its end. Therefore, the authors think a summary table is not needed.

2. The author may want to be benefitted from the language editing.

Reply: The authors tried to improve the used language throughout.

Reviewer #7: the study shows that colorectal cancer seems not to be the correct tumor type for immunotherapy. The hypermutatad colorectal cancer type is only mederately affected by immunotherapy, despite some encouraging single report, there are no randomized trials available on the efficacy of immunotherpay for colorectal cancer. the conclusion of the authors is scientifically correct and guarrantee to the paper a good scientific evaluation.

Reply: We thank the reviewer for his encouraging feedback.