ROUND 1

Reviewer #1:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (High priority)

Specific Comments to Authors: This is an original and very inspiring study regarding the biomarkers for immune checkpoint inhibitors response to gastrointestinal cancers. Even though it is in an experminetal phase, the data shows highly suggesting results for this kind of therapy. Comparing it to most of the East European countries it is an innovative approach to gastrointestinal cancers. The importance of this study is by showing the possibility to a personalized immunotherapy for GI cancers. Especially among these markers, the PD-L1 expression, has shown to be responsive to ICI in PD-L1-negative patients. Even though from these studies, PD-L1 as an independent biomarker, remains controversial. This study is very instructive according the neccessity of the biomarkers in relation to ICIs, as an important topic in the immunotherapy of gastrointestinal cancers. The limitations of the study is that lacks validations in several clinical trials.

Answer: We thank the referee for the positive comments on our work and the valuable suggestions. We still made a lot of changes to the revised manuscript to further improve its quality, and we sincerely hope that these changes will make you more satisfied. The proposed PD-L1 is also an important biomarker discussed and analyzed in this review. In the manuscript we summarized the advantages and limitations of PD-L1 as a biomarker. There is still a need for prospective studies to explain its accuracy, dynamics, and standardization, which is one of the problems to be overcome for PD-L1 as an excellent biomarker in the future.

The clinical validations mentioned by the referee were very instructive. The data related to clinical trials were not well presented in our previous manuscript. Thank you for this important suggestion. The current revisions improve upon the original with the following improvements. 1) Also based on reviewer #3'

suggestion, we have added data related to the TMB study by Marabelle et al. 2) For CNA, the original manuscript covered the work of Lu et al. but their specific data were not detailed, which we corrected and added relevant examples from smeet et al. to illustrate the feasibility of CNA as an ICI marker from a reverse perspective. 3) IFN-γ and MDM2-related data are also added in both GC and HCC based on the original manuscript. 4) Data on CD8+ TIL as a marker in CRC and esophageal cancer were added. 5) For markers worthy of reference in other tumors, we added data related to TET1, miRNA in epigenetics to supplement. 6) We have summarized some of the clinical studies currently in progress to provide a valuable reference for this purpose (Table 3). Regarding the proposed recommendations, we have modified them accordingly. We would be grateful if the results of the modifications are satisfactory.

Reviewer #2:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: Generally, this review incorporates most important Immune Checkpoint Inhibitors in gastrointestinal malignancies. Please see below my comments:

A. Even though based on some recent references, the authors did not incorporate other markers, that were published in previous excellent reviews on this topic or in recent studies. These reviews are much better than this one, regarding structure and organization of the material. Just some examples are listed below (for which, I have just the scientific interest in mentioning, as I am not the author and I have no personal relationship with their authors). 1. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nat Commun 11, 3801 (2020). 2. Darvin P, et al. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med 50, 1–11 (2018). 3. Shah, et al. Immune checkpoint inhibitors in gastrointestinal malignancies: what can we learn from experience with other tumors? Transl Gastroenterol

Hepatol 2019;4:73 4. Kourie HR, et al. Checkpoint inhibitors in gastrointestinal cancers: Expectations and reality. WJG 2017 5. Güthle M, et al. Immunotherapy in Gastrointestinal Cancers. Visc Med. 2020 Jun;36(3):231-237. 6. Mazloom A, et al. Role of Immune Checkpoint Inhibitors in Gastrointestinal Malignancies. J Clin Med. 2020 Aug 6;9(8):2533. 7. Turkes F, et al. Targeting the immune milieu in gastrointestinal cancers. J Gastroenterol 55, 909–926 (2020).

Answer: Thank you for taking the time to read our manuscript carefully and for your suggestions on contents and structure. We have made changes according to your suggestions, and more biomarkers have been presented in the manuscript, and the structure of the text as well as the tables have been extensively revised.

Firstly, The ICI biomarkers covered in the revised manuscript are mainly composed of two parts, one of which is markers that have been extensively studied and applied in GI cancers, and the other is markers involved in other tumors which are less studied and applied in GI cancers compared to others. Here we categorize and summarize them in order to provide new ideas for their research in GI cancers and the discovery of new ICI biomarkers in this field. The previous manuscript is largely complete in terms of GI-related markers, but it does have limitations in the second half. Based on your suggestions, we have revised it accordingly and added several markers of interest in epigenetics and ML, such as TET1, miRNA, IO-score.

Second, your suggestions for structural organization were also very constructive. We carefully revised our manuscript as follows. 1) The section "Factors related to patients' own characteristics" with less data on GI cancers was moved to the section " Other biomarkers of worth in GI cancers" to improve the rationalization of the article. 2) Machine learning and single cell analysis, two techniques that are important for biomarker optimization and discovery of new biomarkers, were presented and added as a stand-alone section "Emerging technologies for optimizing biomarkers" to supply structural integrity. 3) We added subheadings to some parts to increase the readability and logic of the manuscript. 4) The organization of the writing material was

reordered in some paragraphs based on the previous ones, such as paragraphs related to IFN-γ, MDM2, and ctDNA. We also added data to make the content more informative. 5) We have revised the description of the tables in the text to make the overall coherence better.

B. Also, another good paper to be mentioned would be "Lu Z, et al. Prediction of immune checkpoint inhibition with immune oncology-related gene expression in gastrointestinal cancer using a machine learning classifier. Journal for ImmunoTherapy of Cancer 2020;8:e000631.".

Answer: We thank the referee for providing information on this article. We have revised the manuscript to include this paper. Furthermore, we have now conducted a more thorough literature search and added some content as needed. For example, this paper on machine learning and other articles related to single-cell sequencing have been placed in a separate section as "Emerging technologies for optimizing biomarkers", which may be more enlightening.

C. Moreover, even though the manuscript is structured, most data are inserted in the main text, instead of being synthesized in tables (much easier to be followed). The only one table is too scarce. I would suggest data to be nicely structured in tables, instead of plain text.

Answer: That's a very good suggestion. We have revised our manuscript accordingly. We have modified Table 1 in order to provide detailed information, and have added two new tables, Table 2 and Table 3 for increased readability and better understanding. For Table 1, we also re-organized the data and summarized the overall survival and progression free survival, which were common to most of the data. In the two newly added tables, Table 2 summarizes biomarkers in other tumors that are worthy of GI cancers; Table 3 summarizes information on some of the recent ongoing clinical trials of ICI-related combination or neoadjuvant therapies in GI cancers.

D. Many references are not updated (i.e. ref 19 mentions: Published online 2019. However, it has full published data: 2019; 37(15_suppl):4021-4021). Please update data on references.

Answer: Thank you for pointing out this. We have revised the manuscript and double-checked the references to make sure there are no similar oversights.

E. Also, in order to bring something new and up-to-date, ongoing trials using ICI in gastrointestinal malignancies should be inserted in a table (https://clinicaltrials.gov).

Answer: That's a very good suggestion. The reviewer's comments were very constructive, and in fact the other two reviewers had similar suggestions. We have revised it and added Table 3 to summarize ongoing clinical trials of ICI-related combination or neoadjuvant therapies in GI cancers. However, we need to point out that these ongoing clinical trials do not specifically target one or more biomarkers to predict response to ICIs. Rather, it is more about the combination of ICIs therapy with other therapies, which may apparently have little relevance to our topic. Nonetheless, these clinical trials can provide us with a wealth of useful information and we can perform biomarker identification in subsequent data analysis.

F. Core Tip is missing from the manuscript. I read it in the Submission form. It should be inserted in the manuscript.

Answer: Thank you for pointing out this negligence. We have added Core Tip and carefully check the manuscript to avoid such oversights.

G. Format of the style requested by the journal, including references, is not adequate. Please correct.

Answer: Thank you for pointing out this. We have revised styles of the manuscript according to the journal's requirement.

H. There is no "Author contribution" section. Please add. ORCID number should also be added.

Answer: Thank you for your suggestion. We have added "Author contribution" section to clarify each author's contribution. ORCID number of each author was submitted when we first submitted the manuscript, and I suspect it will be displayed when the article is officially published. To avoid delays, we are now presenting it in the endnotes of the text.

I. There are no « Conflict-of-Interest Disclosure Form » and « Copyright License Agreement ». Please add.

Answer: Thank you for pointing out this. For this revision, the editor has sent each author an online version of the Copyright License Agreement, which each author has signed online, and I have downloaded and will submit the signed version. We will also submit Conflict-of-Interest Disclosure Form this time.

J. Revision of the English language is required (grammar, syntax and overall style).

Answer: For English writing, we realize that this's very important and that we are trying to improve it. Based on all above suggestions, we mainly check and improve from these aspects, grammatical errors, complex long sentences, formats, as well as logic, to make our manuscript concise and easy to understand. Thank you again for taking the time to provide these valuable suggestions.

Reviewer #3:
Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing**)**

Conclusion: Minor revision

1. Specific Comments to Authors: This is a mini-review about general considerations about biomarkers for ICI in gastrointestinal malignancies (considering together a group of diseases that perhaps has many intrinsic differences in terms of biology. The main topic are covered (TMB, MSI status, PDL1, tumor microenvironment and TILs role.

Answer: We thank the referee for this comment and the following accurate and positive suggestions of our work. According to the suggestions, we have made changes and would appreciate if the revisions are satisfactory.

2. Relating to TMB i suggest to cite the study by Marabelle et al (Lancet Oncol, 2020, a subgroup analysis of Keynote 158 trial, doi 10.1016/S1470-2045(20)30445-9, of which there is no mention in the text).

Answer: Thank you for providing information on this article. We have revised the manuscript to include this paper. In the prospective analysis of KEYNOTE-158, Marabella et al. assessed the association in tissue TMB (tTMB) and clinical outcome with pembrolizumab monotherapy across ten different advanced solid tumors types. We have now conducted a more thorough literature search and added some content as needed.

3. The remaining topics are well covered, however i suggest to add a specific section about single cell analysis of tumor microenvironment as a powerful tool to identify new possible biomarkers of response to immunotherapy in a large amount of tumors (only to cite one, the study by Steele et al, Nature Cancer 2021, with a complex and integrated analysis woth CYTOF and scRNAseq identified TIGIT as possible target in exhausted T cells within pancreatic cancer microenvironment .

Answer: Thank you for your suggestions on the insufficiencies of our manuscripts and for the papers you recommended. This paper inspired us to add single cell analysis to enrich our manuscript. Based on this, we have

added a new stand-alone section on "Emerging technologies for optimizing biomarkers" in conjunction with reviewer #2's suggestion of applying machine learning. This new section summarizes and discusses frontier techniques for optimizing and finding biomarkers. In the part on single-cell analysis, we describe the importance of this technique for addressing tumor heterogeneity and also present some examples, such as single-cell transcriptome analysis and single-cell mass cytometry applied in GI tumors.

4. But today there are more and more examples of multi-omics single cell analysis to identify possible new mechanisms of immunotherapy responses, in different GI tumors. I think that this should be addressed by the authors in more detail, considering that these technologies will be the future of cancer translational research).

Answer: Thank you for pointing out further directions for our revision, and this suggestion you mentioned is very applicable. Integrated multi-omics analysis can provide more comprehensive information and is important for optimization and discovery of new biomarkers. In the "Emerging technologies for optimizing biomarkers" section mentioned above, we also highlighted the importance of multi-omics in the part of single cell analysis, again presented in the context of examples in GI tumors. Thank all the reviewers again for taking the time to provide these valuable suggestions.

ROUND 2

Reviewer #1:

Scientific Quality: Grade B (Good)

Language Quality: Grade B (Minor language polishing**)**

Conclusion: Minor revision

1. Specific Comments to Authors: The authors worked hard to improve their manuscript, according to the reviewers' suggestions. I am pleased with they way it appears now. Tidy and professional, as well as up-to-date. Just a few minor corrections should be performed (mentioned in the attached file). Other than that, I noticed that Denis Kaili did not sign the Copyright License Agreement.

Answer: We thank the referee for the positive comments on our work and the valuable suggestions. We are also very appreciated to have your approval and support for our revisions. We downloaded the attached file and revised the corrections as marked. Thank you very much for marking every little question with such enthusiasm and care! In addition, the Copyright License Agreement has been emailed to Denis by the editorial team and he has clicked to sign it via email. To avoid delays, we have also prepared an electronically signed version, along with two documents, for viewing purpose. Finally, once again, thank you very much for your comments and suggestions!