Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-23-843

Reviewer A

In this article, the authors analyze the biomarkers related to the efficacy of ICIs in the treatment of malignant tumors of the digestive system. The manuscript is straightforward, well written, and concise and has clear results within the scope of a review article. Definitely deserves to be published and is a valuable contribution to the "Journ al of Gastrointestinal Oncology". The following comments need to be addressed before publication, as recommended.

[1] "#Introduction", Page 3 / Lines 1-3:
"At present, the treatment for malignant tumors of the digestive system mainly includes surgery, radiotherapy, and chemotherapy, with surgery remaining the first choice if the tumor is surgically resectable offering a chance of cure.".
The authors should make a comment about the population of elderly patients. Please, report that even though older CRC patients with pT4 disease are more prone to severe postoperative complications, there is no consensus that age affects survival outcomes. The prognosis of older patients may be confounded by differences in stage at presentation, tumor site, preexisting comorbidities, and type of treatment received.
Recommended reference: Osseis M, et al. Surgery for T4 Colorectal Cancer in Older Patients: Determinants of Outcomes. J Pers Med. 2022;12(9):1534.
Reply: Thank you for your advice. We added the content you suggested.
Changes in the text: page 3, line 2-6: And even though older CRC patients with pT4 disease are more prone to severe postoperative complications, there is no consensus that age affects survival outcomes. The prognosis of older patients may be confounded by differences in stage at presentation, tumor site, preexisting comorbidities, and type of treatment received (3).

[2] "#Introduction", Page 3 / Lines 9-11:
"At present, it has shown strong anti-tumor activity in the treatment of many tumors, such as melanoma, non-small cell lung cancer, renal cancer, bladder cancer, and triple negative breast cancer."
At that stage, the authors should mention that ICIs may be the preferred choice when aiming for sustained efficacy outcomes, while targeted therapies are primarily considered for patients in need of a relatively rapid objective response. Interestingly enough, when immunotherapy is administered to melanoma of unknown primary patients, it is likely to result in improved outcomes when contrasted with the melanoma of known primary subset. This may be attributed to their higher immunogenicity, as evidenced by immunologically mediated primary site regression.
Recommended reference: Boussios S, et al. Melanoma of unknown primary: New perspectives

for an old story. Crit Rev Oncol Hematol. 2021;158:103208.

Reply: Thank you for your advice. We added the content you suggested.

Changes in the text: page 3, line 14-20: ICIs may be the preferred choice when aiming for sustained efficacy outcomes, while targeted therapies are primarily considered for patients in need of a relatively rapid objective response. Interestingly enough, when immunotherapy is administered to melanoma of unknown primary patients, it is likely to result in improved outcomes when contrasted with the melanoma of known primary subset. This may be attributed to their higher immunogenicity, as evidenced by immunologically mediated primary site regression (4).

[3] "##Mismatch repair (MMR) defects and microsatellite instability (MSI)", Page 6 / Lines 18-19:

"Under normal circumstances, MSI has three states, namely highly unstable (MSI-H), low unstable (MSI-L) and stable (MSS).".

At that point, it should be mention that immune cell PD-L1 expression is significantly higher in MSI-H CRC as compared to MSI-L tumors, with no differences among the different MSI-H molecular subtypes. The recommended screening for defective, DNA mismatch repair includes immunohistochemistry (IHC) and/or MSI test. However, there are challenges in distilling the biological and technical heterogeneity of MSI testing down to usable data. It has been reported in the literature that IHC testing of the mismatch repair machinery may give different results for a given germline mutation and has been suggested that this may be due to somatic mutations. Recommended reference: Adeleke S, et al. Microsatellite instability testing in colorectal patients with Lynch syndrome: lessons learned from a case report and how to avoid such pitfalls. Per Med. 2022;19(4):277-286.

Reply: Thank you for your advice. We added the content you suggested.

Changes in the text: page 6, line 25-31: Immune cell PD-L1 expression is significantly higher in MSI-H CRC as compared to MSI-L tumors, with no differences among the different MSI-H molecular subtypes. The recommended screening for defective, DNA mismatch repair includes immunohistochemistry (IHC) and/or MSI test. However, there are challenges in distilling the biological and technical heterogeneity of MSI testing down to usable data. It has been reported in the literature that IHC testing of the mismatch repair machinery may give different results for a given germline mutation and has been suggested that this may be due to somatic mutations (31).

[4] "##CRC", Page 11 / Line 33 and Page 12 / Lines 1-3:

"Chen et al. reported a case of late-stage CRC with POLE mutation. After receiving pembrolizumab monotherapy, the median PFS reached 2 months, the tumor burden significantly decreased, and the microsatellite status remained stable (75).".

The authors should clarify that POLE-mutated CRC is characterized by elevated CD8+

lymphocyte infiltration and the presence of cytotoxic T-cell markers, similarly to immunogenic MSI-H cancers. POLE mutations designate a subset of CRC with more favorable outcomes, based on tumor immunogenicity. Evaluation of POLE mutation promises to refine risk stratification in CRC and may lead to identification of a subgroup of patients who will experience benefits by immune checkpoint inhibitors.

Recommended reference: Boussios S, et al. The Developing Story of Predictive Biomarkers in Colorectal Cancer. J Pers Med. 2019;9(1):12.

Reply: Thank you for your advice. We added the content you suggested.

Changes in the text: page 12, line 13-19: POLE-mutated CRC is characterized by elevated CD8+ lymphocyte infiltration and the presence of cytotoxic T-cell markers, similarly to immunogenic MSI-H cancers. POLE mutations designate a subset of CRC with more favorable outcomes, based on tumor immunogenicity. Evaluation of POLE mutation promises to refine risk stratification in CRC and may lead to identification of a subgroup of patients who will experience benefits by immune checkpoint inhibitors (79).

Reviewer B

Comprehensive review regarding looking at response to immunotherapy, overall the article is long and wordy and choppy

1. Page 5. Please discuss TPS vs. CPS score regarding PDl1

Reply: We revised this.

Changes in the text: page 6, line 13-17: (I) differences in PD-L1 evaluation (PD-L1 combined positive score (CPS) vs. tumor proportion score (TPS) vs. PD-L1 staining of tumor cells vs immune cells) among different studies. However, a previous study showed that both CPS and TPS proved to be equally predictive of response to anti-PD-1/PD-L1 therapy;

2. CRC cancer. Page 11 line 25, what do you mean better efficacy? better response, PFS, OS?

Reply: We revised this.

Changes in the text: page 12, line 5: high response to chemoradiotherapy.

3. HCC. Can you expound on why some studies PDL1 was correlated with better response rate and others worse response rate? Is it endpoint/assessment problem? Or types of immunotherapy in each trial compared to another? Be specific.

Reply: We added some contents in the paper.

Changes in the text: Based on results of studies mentioned before, we found that PDL1 was correlated with better response rate in some studies and worse in others. The underlying reason might include as follows: difference of endpoint or outcome assessment, different types of immunotherapies, difference in patients between studies.

4. This section is too short. is there evidence or lack of evidence of immunotherapy being used for high TMB MSI-stable pancreatic cancer? or PDL1+ MSI-stable pancreatic cancer? Or immunotherapy should never be used?
Reply: The evidence for PDAC is few.
Changes in the text: None.

5. Biliary tract cancer. You are noting that generally PD-L1 positivity in tumors was associated with better PFS (page 15, line 24), is it associated with OS? similarly page 15 line 28, is PDL1 associated with PFS or OS?
-page 16, line 2, higher TMB noted improved PFS, what about OS?
-page 16, line 6 and line 10 and line 13, you said favorable response immunotherapy, what does that mean? better response rate? PFS? OS? in vitro? Please be specific about what you mean.
-page 16, line 19 you said "resistance to immunotherapy" again poor response rate? poor response in vitro? PFS? OS? Please specific about what you mean.
Reply: If OS was not described in some places you mentioned, it is because no significant was observed in relevant studies.
Changes in the text: None.

6. Finally can you have a table? perhaps landmark trials in each GI cancer with immunotherapy approval along with biomarker used? and a second table of negative trials? or second table could be upcoming trials? I think you can have at least 1 table 1 figure or 2 tables that summarize the review.
Reply: Thank you for your advice. But there are too many information in this review. Tables of figures will need too many pages to cover.
Changes in the text: None.

Reviewer C

This is a well written review of biomarkers for GI cancers treated with immunotherapy. I think it is well organized and referenced. However, as a review I think it needs at least 2 tables to summarize the work being discussed. This is necessary for to make the review useful for the broader readership.

One table should summarize the relevant biomarkers, which cancers they have a role in and for each their predictive value. A separate table should summarize for each GI cancer type the types of ICI drug, the clinical study referenced in the manuscript, and associated biomarkers/significance.
Reply: Thanks. However, similar tables were already published in some else literatures.
Changes in the text: None.

Additional recommendations/clarifications:
- Page 3, line 33: Specific/clarify what is meant by liver cancer – I believe this is referring to hepatocellular cancer (HCC).
Reply: We replaced “liver cancer” as “hepatocellular cancer (HCC)”. Thanks.
Changes in the text:

- Page 8, line 12: “no quantitative relationship has been found yet” – this is unclear and contradictory, I believe you mean to say that in PD-L1 positive tumors there is not a relationship between level of expression and response; this needs to be clarified.
Reply: We revised this sentence.
Changes in the text: page 8, line 23-26: It should be noted that the main significance of these studies is to suggest that PD-L1 positive patients are more likely to benefit from ICIs treatment than PD-L1 negative patients, and there is still not a relationship between level of expression and response in PD-L1 positive tumors .