Peer Review File

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Reviewer A

I have read your article with regard to a patient with BRAF-V600E mCRC treated with Regorafenib-Tislelizumab-Crizotinib with great interest. Despite the tumor responding to targeted therapies and I think this case report could be of interest to the scientific community, several changes need to be made in order to accept this paper, particularly in the discussion, where there is a lack of relevant references with regard to biomarkers in BRAF mCRC.
Reply: We thank the reviewer for this favorable comment.

1. - Regorafenib is a multikinase inhibitor, but should not be classified as BRAF inhibitor. Please correct this in the text.
Reply: We thank the reviewer for pointing out this mistake. As the reviewer suggested, we correct this in the text in the whole text as the following **page 2 line 16, page 4 key findings, page 5 lines 75-76.**

2. - Throughout the text, check for capital letters, and spaces between words.
Reply: We apologize for this mistake. We have thoroughly corrected the capital letters, and spaces. we make sure no such errors in the revised manuscript.

3.- At the beginning of the discussion there is an important reference that needs to be included: recently, plasmatic BRAF AF has been demonstrated, and validated as an accurate prognostic factor in BRAF colorectal cancer treated with BRAF inhibitors. Thus, the following reference should be included:

Ros J, Matito J, Villacampa G, Comas R, Garcia A, Martini G, Baraibar I, Saoudi N, Salvà F, Martin Á, Antista M, Toledo R, Martinelli E, Pietrantonio F, Boccaccino A, Cremolini C, Dientsmann R, Tabernero J, Vivancos A, Elez E. Plasmatic BRAF-V600E allele fraction as a prognostic factor in metastatic colorectal cancer treated with BRAF combinatorial treatments. Ann Oncol. 2023 Jun;34(6):543-552. doi: 10.1016/j.annonc.2023.02.016. Epub 2023 Mar 14. PMID: 36921693.
Reply: We thank the reviewer for this critical comment. We agree that such statements are necessary. We have added this description **on Pages 8 lines 142-144** which are important for ctDNA guidance precision therapy of mCRC in the revised manuscript, which now reads as the following.

Recently, with the emergence of liquid biopsy as a promising method for early diagnosis, therapeutic outcome assessment and prognosis prediction of tumor, plasmatic BRAF allele fraction (AF) has been demonstrated, and validated as an accurate prognostic factor in BRAF colorectal cancer treated with BRAF inhibitors [19, 20]..

4.- Furthermore, considering the response after the dabrafenib-based treatment, the authors should also highlight the absence of RNF43 mutation in both, tissue and plasma NGS analysis, as RNF43 mutation has been demonstrated to be the unique genomic predictive biomarker in BRAF mutant mCRC. Therefore, the following references should be included:

Elez E, Ros J, Fernández J, Villacampa G, Moreno-Cárdenas AB, Arenillas C, Bernatowicz K, Comas R, Li S, Kodack DP, Fasani R, Garcia A, Gonzalo-Ruiz J, Piris-Gimenez A, Nuciforo P, Kerr G, Intini R, Montagna A, Germani MM, Randon G, Vivancos A, Smits R, Graus D, Perez-Lopez R, Cremolini C, Lonardi S, Pietrantonio F, Dienstmann R, Tabernero J, Toledo RA. RNF43 mutations predict response to anti-BRAF/EGFR combinatory therapies in BRAFV600E metastatic colorectal cancer. Nat Med. 2022 Oct;28(10):2162-2170. doi: 10.1038/s41591-022-01976-z. Epub 2022 Sep 12. PMID: 36097219; PMCID: PMC9556333.

Quintanilha JCF, Graf RP, Oxnard GR. BRAF V600E and RNF43 Co-mutations Predict Patient Outcomes With Targeted Therapies in Real-World Cases of Colorectal Cancer. Oncologist. 2023 Mar 17;28(3):e171-e174. doi: 10.1093/oncolo/oyac265. PMID: 36779536; PMCID: PMC10020799.

Reply: We thank the reviewer for this insightful advice. We added the discription of *RNF43* mutations is the unique genomic predictive biomarker of the dabrafenib-based treatment in *BRAF* V600E mCRC in **page 9 lines 156-159**, which is described below.

Several studies have shown that RNF43-mutated represents a new biomarker for its potential to help prioritize anti-EGFR/BRAF combinations in mCRC BRAF V600E patients [25, 26]. However, this case is RNF43 wild type in both, tissue and plasma NGS analysis.

5.- When talking about BRAF and MET amplification, there are only 2 cases reported in the literature, the one the authors have mentioned and the following one. Please, include also the following reference:

Ros J, Elez E. Overcoming acquired MET amplification after encorafenib-cetuximab in BRAF-V600E mutated colorectal cancer. Eur J Cancer. 2022 Sep;172:326-328. doi: 10.1016/j.ejca.2022.06.026. Epub 2022 Jul 9. PMID: 35820242.

Reply: We thank the reviewer for this constructive advice. In the revised manuscript, we added the literature description of patients with *BRAF*-mutated and *MET* amplification had benefited from *MET* inhibitor plus *BRAF* inhibitor on **page 9 lines 163-165** as follows.

Before this case, only one patient with BRAF-mutated and MET amplification had been reported, and this patient had benefited from conversion from anti-EGFR and BRAF inhibition to a MET inhibitor plus BRAF inhibitor-induced tumor response [27].

6.- Finally, in the last 2 sentences of the discussion I would suggest being more open and "soft" with the final message. Liquid biopsy may help clinicians to tailor treatment and forecast patient prognosis (however, some patients do not have ctDNA detectable) or some NGS techniques may not be enough accurate to detect it.

Reply: We thank the reviewer for this constructive advice. In the revised manuscript, In the revised manuscript, we modified the last 2 sentences of the discussion as shown as **Page 10 lines 169-172**, which now reads as.

This case uncovered liquid biopsy may help clinicians to tailor treatment and forecast patient prognosis, especially for tissue size may not be enough to conduct NGS. This case also demonstrated the importance of continuous monitoring of ctDNA during treatment, which could adjust or change the regimen based on the detection results for a better application of precision medicine.

We thank the reviewer for the whole constructive suggestion.