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Format for ANSWERING REVIEWERS

May 26th, 2016

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 25860-review.doc).

Title: Prognostic and predictive biomarkers in metastatic colorectal cancer anti-EGFR therapy

Author: Lo Nigro C, Ricci V, Vivenza D, Granetto C, Fabozzi T, Miraglio E, Merlano MC

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 25860

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated according to comments in file 25860-edited

2. Revision has been made according to the suggestions of the Reviewers:

Reviewer 1 (00505467) **commented to authors: "This review should provide explanations about the methodology applied in order the authors to perform the review. The authors should provide in the discussion section a perspective of where the research should be heading or what seems to be the pitfalls of research up until now according to their review. References must be updated i.e. reference 1 there is an updated ref from 2016 from almost the same authors".**

The Materials and methods section was added both in the Abstract (as required) and in the text in order to explain how the review process was done.

We agree with Reviewer 1 on the importance of provide a prespective of the research as well as its pitfalls up to now. We added to the Discussion "In the next future a correct analysis of multiple genes, with different mutations, is likely to be analyzed simultaneously and used for selecting patients and predicting the efficacy of anti-EGFR therapy. This analysis might be identify a subgroup of mCRC patients with distinct biological behavior and response to treatments, including anti-EGFR antibodies. All of this will be a step forward in the "personalized medicine" treatment of CRC patients".

References were updated, in particular reference 1.

Reviewer 2 (03326259) **commented to authors: "Polymorphism of certain genes has also been linked**

to the response of anti-EGFR therapy. The authors should also include that into this review".
We agree that the role of certain SNPs was not reported and we better described those identified in the FCGR2A and FCGR3A genes, related to ADCC. Moreover we added the SNP in miRNA Let7_KRAS_3UTR.

Reviewer 3 (03551372) **commented to authors: "1) The order of the biomarkers need to be adjusted. For example, it starts with KRAS mutation followed by NRAS mutation etc, and KRAS mutant clones were described before EGFR mutation. It will be better if the description of the same molecule can be grouped together. 2) This review discussed two prognostic biomarkers: BRAF mutations and ADCC activity. Why just these two? 3) In the discussion, it stated that there are ongoing studies assessing the predictive value of the number of copies of the EGFR gene, mutations in the NRAS, PI3KCA, TP53 and PTEN genes. However, there is no description on TP53 in any other sections. This needs to be added".**
We agree with the reviewer that it is better if the description of the same molecule can be grouped together, so we appropriately changed the order of the biomarkers described in the text. Actually, we added the paragraph "Acquired resistance mechanisms" where we moved the KRAS mutant clones, MET amplification and we newly described HER2 amplification and epiregulin and amphiregulin overexpression.
The prognostic role of high leves of selected miRNAs has been added in order to complete this section, beyond BRAF and ADCC.
Moreover, we completed the Predictive factors section with the description of TP53.
References were adequately positioned all over the manuscript and reordered in the Reference section.

Reviewer 4 (02570566) **commented to authors: "The authors evaluated not only the KRAS mutational status but also BRAF, NRAS, PIK3CA and PTEN alterations which might be beneficial to the selection of patients who are likely to respond to anti-EGFR therapies. However discussion section with KRAS, BRAF NRAS, PTEN is fairly explanation. The authors should extend the information in the discussion section. I provide following recommendations to improve the manuscript further. Particular Suggestions regarding the manuscript (Minor comments): 1. Perspective role and future direction in using prognostic and predictive biomarkers in response to anti-EGFR in metastatic CRC patients. 2. Actively discuss in KRAS, BRAF NRAS, PTEN mutation with anti-EGFR therapy in discuss section".**
Discussion was enriched as suggested.

Reviewer 5 (02980806) **commented to authors: "Some improvements and corrections are required: (1) As RAS mutational status was the recognized biomarker, the authors need to focus more content on RAS wild-type. There are various reviews published about the resistance mechanisms of anti-EGFR therapy, but this review didn't include the latest one, for example, HER-2 amplification, EGFR ligands amphiregulin and epiregulin, and some acquired resistance mechanisms. (2) The order to describe the biomarkers needs to be adjusted. The order in abstract, core tip, text, discussion and**

conclusion was inconsistent. In addition, the authors mentioned "TP53" in discussion, but not in any section of the review. (3) The content of abstract was too simple to summarize the review. More information was needed to provide about the significance of the review. (4)The discussion was needed to provide the clinical practice value of these biomarkers".

For the description of acquired resistance mechanisms and of TP53, please see answer to Reviewer 3.

Abstract was better structured and enriched in order to provide more impact on the significance of the review.

Discussion was better focused in order to provide the clinical practice value of these biomarkers.

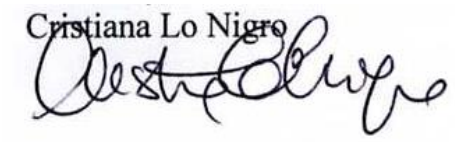
Moreover, Figure 1 was rebuilt so that fonts and lines cannot be edited or moved anymore.

Audio core tip and Data sharing statement: were also provided.

A native English speaker Professor edited the language and the grammar and a certification for class A will be provided in the next few days.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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