Format for ANSWERING REVIEWERS

Dear Editor,

Please find enclosed the edited manuscript in word format (15818-Review).

Title: Evaluation of antiangiogenic efficacy in advanced hepatocellular carcinoma: biomarkers and functional imaging

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Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 15818

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

1 Format has been updated.

2 Revision has been made according to the suggestions of the reviewer:

Reviewer 1:
Dear authors, this is a review in depth from scientists that they know the field very well. . There are some issues that need to be managed as the paragraph was deleted follows.
We would like to thank the reviewer for his/her constructive comments, and we try to answer her request and give modification accordingly.

INTRODUCTION Highlight how common and deadly is HCC (the fifth most common cancer and the second leading cause of cancer-related deaths worldwide, in each year,there are around 750,000 new cases of liver cancer and around 700,000 deaths).
Our response: changes were made, and a new paragraph was added as following: Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths worldwide [1,2]. The incidence of HCC is steadily increasing with about 625,000 new cases per year and the disease results in around 600,000 deaths yearly over the world [1,2]. Less than 30% of patients diagnosed with HCC are eligible for curative treatment [3] and during the course of the natural evolution of HCC; a significant proportion of patients are candidates for systemic therapies.

Define RECIST criteria.
Our response: Changes were made as following: Response Evaluation Criteria in Solid Tumors (RECIST) criteria [23,24] were proposed to evaluate tumor size changes during treatment in patients with cancer.
We tried to not surcharge the text because these criteria are very known and used worldwide.

Language polishing.
Our response: After the initial submission, a writing assistance and English language review were provided by Enago (www.enago.com).

BIOMARKERS
At the beginning of page 6 repetition of the aims.

Our response: The aims in the page 6 were deleted.

Too long introduction.
Our Response: The introduction was modified according to the recommendation of the reviewer and some paragraphs judged obvious or unclear were deleted.

Is it correct to consider imaging as a biomarker?
Our response: in this article, two features were discussed separately: biomarkers and imaging features. The aim was to dispose from two evaluation methods of efficacy of antiangiogenic agents. Moreover, Ludwig and Weinstein et al. reported "Biomarkers at all of these levels are now, in principle, detectable by functional molecular imaging modalities based on magnetic resonance imaging (MRI), POSITRON EMISSION TOMOGRAPHY (PET) or optical imaging". We think that is useful to discuss this feature in this paper.

CLINICAL BIOMARKERS (cutaneous adverse-events). The ideal biomarker needs also not to be detected in premalignant diseasses (eg cirrhosis).
Our response: The paragraph was added as following: Regarding HCC, biomarkers should ideally meet at least the following criteria [26,38]: 1) to be easily measurable through minimally invasive procedures, ideally using blood tests, 2) to have a prognostic value in relation to the natural history and the outcome of HCC, 3) to have a predictive value wherein its presence correlates with the clinical response to sorafenib therapy, 4) and preferably not to be detectable in premalignant diseases (e.g. cirrhosis).

Explain BCLC staging.
Our response: We added the explanation and a reference was added as following: and Barcelona-Clinic-Liver-Cancer (BCLC) staging system [47].

Too long: the paragraph was modified with no clear conclusions.
Our response: We write more concise paragraph, and we add a summary in the text as following: In summary, no clinical biomarkers of response to sorafenib were validated in clinical practice. Based on the Barcelonan prospective study, cutaneous adverse events seem to be the best track to explore in patients treated with sorafenib for advanced HCC. These results should be interpreted with caution since no untreated control arm was evaluated in this study.

CIRCULATING BIOMARKERS
ALPHA-FETOPROTEIN (AFP) Highlight that is the only biomarker marker that passed all the five phases of biomarker development It is better to refer to the phases biomarker development and classify the biomarkers accordingly (Pepe M S, Etzioni R, Feng Z, et al. J Natl Cancer Inst, 2001, 93: 1054-1061): reference added.
Our response: We added the reference as asked as following: Serum AFP is the only biomarker that passed all five phases of biomarker development as defined by Pepe el al [56].

Explain the SHARP study as it is mentioned many times in the text.
Our response: The explanation and the reference was added as following: The SHARP trial [12] is a phase 3, placebo-controlled trial that studied the benefit of sorafenib versus placebo in 602 patients with advanced HCC.

VASCULAR ENDOTHELIAL GROWTH FACTORS (VEGF) Too long.
Our response: The paragraph was modified, and we deleted unnecessary information.

TISSUE BIOMARKERS
Language polishing: ok.

Our response: Thank you for the reviewer, and we added writing assistance to improve the language quality.

IMAGING FEATURES AND FUNCTIONAL IMAGING
Language polishing.
Our response: Thank you for the reviewer, and we added writing assistance to improve the language quality

Define mRECIST criteria.
Our response: We added a paragraph as following: The modified RECIST (mRECIST) criteria are a new assessment method proposed by Leoncini and Llovet [145] to overcome the limitations of RECIST criteria. They include vascularization and tumor arterial enhancement changes of the target lesion on CT.

EPILOGUE (better use conclusion).
Our response: We changed by conclusion.

Which biomarker can be used in clinical practice (clear message) and there is no single ideal biomarker. As a result, a combination of efficient biomarkers may be a future solution.
Our response: We make modification to have a clearly message. In summary, we believe that, combining early reduction of AFP levels following sorafenib initiation with new radiological criteria could be helpful in detecting patients who might benefit from antiangiogenic treatment and to propose better tailor-made strategy management.

You can also comment on the following biomarkers: An isoform of AFP, named LENS CULINARIS AGGLUTININ REACTIVE FRACTION OF AFP (AFP-L3) associated with poor survival rate and high chance of tumor recurrence GLYPICAN-3 (GPC3) is a member of the glypican family of cell-surface heparin sulfate proteoglycans. Patients with GPC3 expression in tumors have a lower 5-year survival rate than those with no expression DES-GAMMA CARBOXY-PROTHROMBIN (DCP) is associated with advanced HCC OSTEOPONTIN (OPN) is a highly phosphorylated and glycosylated protein. It relates with metastasis and poor prognosis FIBROBLAST GROWTH FACTORS 3 AND 4 (FGF3/FGF4) can predict response to sorafenib C-MET expression relates to prognosis miRNA relates to prognosis EpCAM-positive circulating tumor cells are associated with advanced HCC CYTOKERATIN 10 AND CYTOKERATIN 19 predict the potential of metastatic HCC:
Our Response: We think the reviewer for all these pertinent biomarkers proposed. We tried to focus only biomarkers of response to antiangiogenic agents. For each factor reported in this manuscript, we give a brief report of the prognosis value for each factor in HCC in general, and we choose only those used as prognosis/predictive biomarker especially for sorafenib, which is the validated drug in HCC and evaluated in various studies. We avoided reporting all known biomarkers in HCC and those not tested with antiangiogenic agents to avoid confusion. All the cited biomarkers are of course well-described prognosis markers of HCC but were not evaluated with antiangiogenic.
We made changes as following: Japanese groups proposed the lens culinaris agglutinin reactive AFP (AFP-L3), an isoform of AFP, as a good diagnostic and prognostic biomarker for HCC [72–74]. However, few data were available regarding the value of AFP-L3 as predictive of response to antiangiogenic agents in HCC[75].
DCP is a prognostic factor of HCC as shown by Japanese works [77]. Changes in DCP plasma level were evaluated in patients treated with sorafenib [70,78,79]. Some studies reported that DCP could be an independent factor of survival in patients treated with sorafenib [78,79]. These results were not reproduced in other reports [70]. The DCP was currently mainly used in Japan and could be more investigated in western HCC population
MET expression was discussed:
Some tissue markers of response were evaluated in HCC using other antiangiogenic agents. The

tivantinib, a selective MET inhibitor, was evaluated in second line setting through a randomized, placebo-controlled phase II in patients with advanced HCC [149]. In this study, tumor expression of MET influences treatment benefit. Patient with tumor overexpression of MET clearly benefit from tivantinib treatment. High-MET tumor expression was associated with longer TTP on tivantinib compared to placebo (2.7 months versus 1.4 months; HR = 0.43, 95% CI, 0.19-0.97; p = 0.03) and OS (7.2 months versus 3.8 months, HR = 0.38, 95% CI, 0.18-0.81; p = 0.01). Interestingly, tivantinib did not show any benefit when tumor expression of MET was low [149].
Nowadays, no tissues biomarkers were able to identify patient who will respond to sorafenib. Tumor analysis data were unavailable in large clinical trial, probably because of lack of tumor samples biopsies since HCC diagnosis was frequently made according to imaging features
CK19 discussed in the tissue biomarkers.

In summary
We would like to thank the reviewer for his/her pertinent comments, and we hope that we answered adequately.

Reviewer 2:
MB et al submit an extensive review outlining several prognosis/predictive functionally biological and imaging biomarkers in hepatocellular carcinoma. Potentially these biomarkers could be more successful for monitoring treatment activity, detecting early resistance of treatment and identifying patients who will more likely benefit from treatment ideally before its initiation. The matter is such useful and interesting. However the paper suffers for some limits:

We would like the reviewer for his/her encouraging comments, and we tried to give answers according to his/her suggestions.
The overall quality of the English language remains poor, including some grammatical errors, spelling mistakes and so on.
Our response: We added after the first submission writing assistance to improve the language quality provided by Enago, USA. We correct more than 100 grammatical errors and mistakes.

Introduction needs to be rewritten. The general impression is that the introduction section, especially about sorafenib, is unnecessarily lengthy. There is too much unnecessary information, while some important terms and information are left unexplained:
Our response: We made changes for the introduction. We delete some paragraphs that seemed obvious or unnecessary. We also add a new paragraph as requested by another reviewer.

P7 line5-7," Otherwise, the absence of dermatological adverse effects could not be interpreted as treatment inefficacy since no untreated control arm was evaluated in this study", maybe the authors should provide some evidences to prove this, such as citing some ones that are most relevant and representative.
Our response: This speculation was cited and referred to the article by Reig and al. concerning cutaneous side effects and sorafenib. We deleted the paragraph from the text to avoid confusion.

P9 line5-7"However, in addition to radiological assessment, early reduction of AFP levels following sorafenib treatment could be helpful to detect patients who could benefit or not from antiangiogenic treatment and to propose better tailor-made strategy management." The authors need to describe clearly in the text, by providing better visualization of the data. Moreover, moving it to the next part "Imaging features and functional imaging" could be better: changes made:
Our response: We modified the text and moved in the part imaging and functional imaging as following:
In summary, we believe that, combining early reduction of AFP levels following sorafenib initiation with new radiological criteria could be helpful to detect patients who could benefit from antiangiogenic

treatment and to propose better tailor-made strategy management.

"Prognosis and predictive value of tissue biomarkers evaluated in hepatocellular carcinoma". The reviewer thinks this section is unnecessary. One limits is the difficulty to perform in clinical practice, because of its time-consuming and complicated, so the feasibility is very poor:
Our response: We believe that biopsies could be informative for HCC and could be encouraged for better understandings of tumor response and resistance to antiangiogenic therapy. For example, we conducted in our center a phase II trial, evaluating histological modification following sorafenib in patients undergoing surgery. A first biopsy was performed before treatment, a second one under treatment and finally we have the surgical specimen. This study was finished, and we collect all data in some weeks to publish them. We cannot mention this study for example since results were confidential currently. However, we think that biopsy should be a modern and innovative approach in patients with HCC.

The part about "Which response criteria to apply?" is too long to describe, and something should be deleted. This review aims to give a description of biomarkers instead of which criteria is better to evaluate the sorafenib or other antiangiogenic agents for advanced HCC:
Our response: We made changes in this paragraph and we write a more concise one to have a clear message as following: The modified RECIST (mRECIST) criteria are a new assessment method proposed by Leoncini and Llovet [145] to overcome the limitations of RECIST criteria. They include vascularization and tumor arterial enhancement changes of the target lesion on CT. Other new criteria including EASL criteria and Choi criteria, that evaluated tumor density changes, were also proposed to evaluate tumor response to sorafenib in patients with HCC [100,146–148]. A representative case of discrepancies between these criteria is shown in figure 1. Several studies used CT-scan evaluation to predict early response to sorafenib and to adjust treatment strategy according to the potential clinical benefit [100,147,149].
Edeline et al. [147] showed in patients treated with sorafenib for advanced HCC that overall response rate was higher when mRECIST criteria were applied compared to RECIST criteria (22.7% versus 1.9%). Interestingly, tumor response assessment according to mRECIST criteria, reclassified 22.6 % of patients as responders while they were initially categorized as having stable disease by RECIST criteria [147]. Our group found consistent results when alternative radiological criteria to RECIST were applied [100]. We evaluated early tumor response in 64 patients with advanced HCC treated with sorafenib using RECIST, mRECIST, Choi and EASL criteria [100]. These new criteria identified a higher objective response rate compared to the conventional RECIST criteria (varying from 51% for Choi to 28% and 28% for mRECIST and EASL respectively; compared to only 3% for RECIST criteria). Responder patients according to Choi criteria at the first tumor assessment had better OS compared to non-responders (22.4 versus 10.6 months, 95% CI, 0.15-086; p = 0.097) [100].
Further evaluations of these new criteria in comparison to RECIST criteria are needed in prospective clinical trials evaluating sorafenib or other antiangiogenic agents for advanced HCC.
In summary, we believe that, combining early reduction of AFP levels following sorafenib initiation with new radiological criteria could be helpful in detecting patients who might benefit from antiangiogenic treatment and to propose better tailor-made strategy management.

Description style material about the functional imaging requires structured tables & figures for better presentations, just like clinical biomarkers and circulating biomarkers above:
Our response: We added a new table as following: Table 8 Value of functional imaging in patients with hepatocellular carcinoma treated with antiangiogenic agents

Reviewer 3:
The paper is well organized. In my opinion, the paper is publishable with no significant revision.
Our response: We would like to thank the reviewer for his/her encouraging comments.

3 References and typesetting were corrected.

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

Sincerely yours,

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