April 28th, 2016

Dear Editor,

We are submitting to your attention a revised version of the review article entitled **"The challenges of advanced hepatocellular carcinoma"** to be considered for publication in the World Journal of Gastroenterology (ESPS manuscript NO: 25665).

The manuscript has been carefully revised according to the suggestions of the Editors and the Reviewers. As requested, in the annotated copy, all changes/additions are blue typed. Below, please find a point-by-point reply to all the issues raised by the Referees.

We hope that you and the Reviewer will find the revised version of our paper suitable for publication in the WJG.

On behalf of all Authors, with best regards,

Stefano Colagrande, MD

Fabio Marra, MD, PhD

Gian Giacomo Taliani, MD

Point-by-point reply to the issues raised by the Reviewers.

We would like to thank the Editors and the Reviewers for the careful evaluation of the manuscript and the useful comments.

Reviewer #1

1.1. The abbreviations should be fully spelled out when first used.

All abbreviations have been spelled out when first used.

1.2. Statistics data such as P-value should be involved when the research result were quoted in this review.

The revised versions of Tables 2 and 3 (formerly Tables 3 and 4) now include P values as reported in the quoted studies.

1.3. As we know, HBV infection may lead to HCC, in this review, the treatment of HBV induced HCC didn't mentioned.

Information on the possible significance of antiviral therapy against HBV has been added to the revised version (page 7, line 5):
As the majority of HCC develops in patients with chronic liver disease, treatment of the underlying condition and especially management of its complications, is mandatory. HBV infection accounts for about 60% of the total liver cancer in developing countries and for about 23% in developed countries [24, 25]. The benefits of antiviral nucleot(s)ide analogue therapy in improving recurrence-free survival and OS after curative treatment of HCC [26] may suggest a possible role in improving outcomes also in advanced HCC, but at this time data on this topic are lacking.

Reviewer #2

2.1- While the authors discuss at length recent negative trials, they do not try to give explanations about the cause of the failure. More importantly, they failed to discuss anti-PD1 treatment, which is currently the most promising therapy. Immunotherapy should be discussed in such a review.

We thank the reviewer for pointing this out. In revised section 3 data obtained with nivolumab and the upcoming phase 3 study have been discussed (page10, line 27):
One of the most promising areas in the field of HCC is represented by immunotherapy. Expression of PD-1 and CTLA-4 on immune cells is associated with blockade of the anti-tumor immune response, favoring the progression of cancer [46]. In a Phase I/II study recently presented in abstract form nivolumab, an anti-PD-1 monoclonal antibody, induced tumor size stabilization or reduction in 67% of the patients [47]. In addition, the effects of this treatment were durable, as previously observed in other types of cancer. A phase III study comparing the effects of sorafenib and nivolumab in advanced HCC is currently underway (Table 4).

2.2- The section 2 and 6 both discuss imaging. They should be combined.

As suggested by the Referee, sections 2 and 6 of the previous version have been combined in section 5 of the revised manuscript.

2.3- Abstract: HCC is the 3rd cause of death by cancer, not responsible for a 3rd of cancer deaths

We apologize for the mistake, which has been corrected.

2.4- Section sorafenib: The reference 12 and 13 regarding TACE focus on PVT, not all patients with advanced HCC; this should be stated.

The fact that the references indicated by the Reviewer were focused on PVT is now indicated in the revised manuscript (page 4, line 28):
Trans-arterial chemoembolization (TACE) in patients with advanced HCC due to portal vein thrombosis has been suggested to improve OS compared to patients receiving supportive care, in retrospective studies [5].

2.5- "The concentrations of alpha-fetoprotein, alkaline phosphatase, angiopoietin 2, Vascular Endothelial Growth Factor, soluble c-Kit and Hepatocyte Growth Factor have been linked to improved survival in many field practice studies[20-22] and even in the SHARP trial" ; Please make a difference between potential prognostic (AFP, Ang2, VEGF) and predictive (cKit, HGF) biomarkers.

This line of information has been included in the revised manuscript (page 6, line 23):
Numerous studies have explored the role of biochemical markers as prognostic factors or predictors of response. The concentrations of alpha-fetoprotein, alkaline phosphatase, angiopoietin 2, Vascular Endothelial Growth Factor have been linked to improved survival, while soluble c-Kit and Hepatocyte Growth Factor have been proposed as predictive markers in field practice studies[15-17] and in the SHARP trial.

2.6- Same paragraph: "This may be due to the small sample size of most studies." The biomarkers from the SHARP lack external validation, but sufficient numbers.

The sentence has been corrected as suggested (page 6, line 32):
However, despite the large numbers of studies and the interesting results, no predictors have reached enough strength to be commonly used in clinical practice, due to the small sample size of most studies or to the lack of external validation of the findings.

2.7- Section "First line"; Erlotinib: "its gene amplification has been reported in a considerable number of HCC"; according to recent large scale results (Schulze Nat Genet 2015), EGFR amplification is about 1% of cases; please correct.

New data from the Schulze's study together with the appropriate reference have been added (page 8, line 23):
Erlotinib is an orally active, potent and selective inhibitor of the human epidermal growth factor receptor, and its gene amplification has been reported in HCC [34], although recent large scale results indicate that this occurs in a limited number of cases [35].

2.8- "A phase III study is currently underway comparing sorafenib alone vs sorafenib plus doxorubicin (Sorafenib Tosylate With or Without Doxorubicin Hydrochloride in Treating Patients With Locally Advanced or Metastatic Liver Cancer, ClinicalTrials.gov Identifier:NCT01015833)" ; results were reported during ASCO GI 2016, please add the information

This line of information together with reference to the ASCO abstract book has been added (page 9, line 5):
The results of a phase III study comparing sorafenib alone vs. sorafenib plus doxorubicin have been recently presented in abstract form [38]. The addition of doxorubicin to sorafenib resulted in higher toxicity and did not improve OS or progression-free survival.

2.9- Section "second line": "In addition, in real life there are many patients for whom sorafenib is not recommended, for the presence of comorbidities, Child-Pugh status, or for economic reasons. For these patients, an alternative option is needed and second-line trials would be considered". Patients with contraindication to sorafenib could not be included in second-line trials.

The sentence has been corrected, as appropriate (page 9, line 22):
Patients who fail first-line systemic therapy are considered to have poor prognosis, and second-line trials are warranted [41] (Table 3).

2.10- please state that ramucirumab is currently being tested in patients with AFP >400ng/mL, due to positive subgroup analysis in the first phase 3 trial.

This line of information has been added (page10, line1):
However, a subgroup analysis showed that patients with elevated alpha-fetoprotein could benefit from this treatment. Therefore, a phase 3, placebo-controlled trial testing ramucirumab as a second-line treatment in patients with elevated basal alpha-fetoprotein is currently recruiting patients (NCT02435433, clinicaltrials.gov, accessed April 25, 2016).

2.11- Section "PVT": "However Jeong SW et al, in a smaller study with 33 HCC patients with PVT, shows a percentage of standard disease and disease control rate lower than in the SHARP and Asia-Pacific studies." Standard disease: correct for stable disease?

'Standard disease' has been corrected with 'stable disease'

2.12 - "No significant differences in OS has been shown between patients with thrombosis of first order branches and patients with thrombosis of the main trunk [56] ? . The authors stated the opposite at some point in the general section; it is a matter of debate, but please be consistent.

The sentence has been corrected in order to provide consistent information throughout the manuscript, as correctly pointed out by the Referee (page 12 line 24)

2.13 - "Additionally, a comparative study TACE vs. TARE demonstrated the superiority of the latter in prolonging median OS, that was reported to be 22,1 months in the advanced HCC patients [60]." The study by Salem et al showed improved TTP, but not improved OS; the results in the subgroup of advanced HCC was not statistically significant either. Please correct.

The sentence has been corrected (page 13, line 4):
During treatment, no significant differences in OS have been shown between patients with thrombosis of first order branches and those with thrombosis of the main trunk [58].

2.14 - "Only one retrospective study, with several limitations (small sample size, imbalance in baseline characteristics between the arms), compared the efficacy of TARE vs sorafenib, finding no significant differences in OS" ; a recent retrospective analysis showed benefit of TARE over sorafenib in patients with PVT (EdelineEur J Nucl Med MolecImag 2016)

The suggested line of information, together with the appropriate reference, has been added (page 13 line 12):
Additionally, a comparative study TACE vs TARE demonstrated the superiority of the latter in prolonging TTP but not in patientswith advanced HCC [62]. In a retrospective study, the efficacy of TARE has been compared to that of sorafenib, and no significant differences in OS were found [63]. It should be considered that the study had several limitations, including a small sample size, and imbalance in baseline characteristics between the arms. On the contrary TARE appears to be

particularly effective in patients with portal vein thrombosis, with a median OS ranging between 10-18 months [60, 64, 65] compared to 8,3 months in patients with portal vein thrombosis treated with sorafenib in the SHARP study [11]. Similar results showing the superiority of TARE vs sorafenib in patients with portal vein thrombosis have also been reported in another recently published study [66].

2.15 - Table 1: First column missing?

Unfortunately the first column was cut out during pdf building. The correct Table has now been included.

2.16 - Table 4: all refer

Revised Tables 2 and 3 (formerly Tables 3 and 4) include all primary references.

Reviewer #3

3.1. There is some duplication in section 2 and 6. Section 6 may be merge into a sub-topic of section 2.

As suggested by the Referee, sections 2 and 6 of the previous version have been combined in section 5 of the revised manuscript.

3.2. In the prognosis section, the reviewers mention metastatic disease without primary liver cancer were associated with a better prognosis. In addition to portal vein thrombosis (section5), intra-abdominal metastasis or distant metastasis may be reviewed in a new section.

The two sections are now clearly identified (page 17, lines 5 and 29):
- Portal vein thrombosis
- Distant metastases

3.3. How to establish a personalized therapy according to molecular pathway may be mention briefly in the conclusions and perspectives section (duplicated section number 8)

Revised section 8 (now section 7) includes a brief discussion of the need to personalized the treatment of advanced HCC(page 21, line 31):
These lines of information need to be integrated with accumulating data on the molecular heterogeneity of HCC. Collectively, these data will be instrumental to design personalized treatments, considering that HCC is one of the few solid tumors where no molecular-guided therapy exists.