Author's response to reviews

Title: Prognostic Value of alpha-Fetoprotein and Des-gamma-Carboxy Prothrombin Responses in Patients with Hepatocellular Carcinoma treated with Transarterial Chemoembolization

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Version: 5 Date: 3 August 2012

Author's response to reviews: see over
Reply to reviewers’ comments:

MS: 1654988464706614; Prognostic Value of alpha-Fetoprotein and Des-gamma -Carboxy Prothrombin Responses in Patients with Hepatocellular Carcinoma treated with Transarterial Chemoembolization. Yong Kang Lee, Seung Up Kim, Do Young Kim, Sang Hoon Ahn, Do Yun Lee, Kwang-Hyub Han, Chae Yoon Chon and Jun Yong Park

We are delighted to hear that our manuscript has been given the opportunity to be revised for publication in BMC cancer. We are thankful for the response by the editor and reviewers and appreciate reviewer's keen comments, which are indeed important points in clinical practice. We have carefully reviewed the valuable comments and suggestions of the reviewer and done our best to improve the manuscript accordingly. We have provided point-by point answers to the specific issues raised by the reviewer, and the modifications are underlined in the manuscript.

Reviewer: #1

Major compulsionry revisions

1. Because two distinct criteria (WHO and modified RECIST) have been used in the paper, the authors need to state clearly what radiologic criteria they have used for definition of progression. This will in turns affect the PFS and TTP significantly.

Response) For evaluating PFS, we use mRECIST criteria, not WHO criteria. We added this missed information in the methods section.

2. Since this is a retrospective study, all subjects may not have the same follow-up period or timing of assessment scanning. The definition of PFS is subject to bias. This is probably the reason why AFP response is not associated with PFS. The authors need to discuss this limitation in the discussion part.

Response) It is indeed an important point. We also agreed to the reviewer’s comment and discussed this issue as one of our limitations in the discussion section.

3. It is not surprising to see AFP response predicts better OS again in HCC patients (as demonstrated by Chan SL et al. JCO 09 and Riaz et al. JCO 09). Since the authors have also DCP value, I think the authors could analyze whether combined serological endpoint (AFP and/or DCP responses) will improve the prognostication. This will be the novel part of the study.

Response) According to the reviewer’s recommendation, we investigated whether the combined use of AFP and DCP responses can improve prognostication. Thus, AFP and/or DCP responders were stratified into combined tumor marker responders (cTM responders, n = 99) whereas subjects without AFP and DCP responses were stratified into cTM non-responders (n =16). PFS was similar between
cTM responders and non-responders (15.1 vs. 10.5 months; log rank test, \( P = 0.259 \)) whereas OS was significantly longer in cTM responders than non-responders (39.0 vs. 21.5 months; log rank test, \( P = 0.011 \)). In addition, cTM response was selected as one of independent predictors of OS (HR 0.312; 95% CI 0.150-0.649; \( P = 0.002 \)), together with tumor size and cirrhosis. However, cTM response did not predict PFS independently. We added these results in the results section.

4. What is the rationale or reference to support the use DCP cut-off of 20 mAU/mL? Also, what is rationale of using 50% reduction to define the DCP response?

**Response** Because it is useless to check the change in DCP levels within normal range, we selected patients with DCP level >20mAU/mL at baseline. This is based on a previous study, which calculated normal DCP level (mean 17.5 mAU/mL). In addition, our previous study can be a reference for this DCP cut-off.

Our previous study (J Gastroenterol Hepatol 2011, 27(2):313-322) defined DCP response as 20% reduction from baseline. Because all subjects in our previous study showed advanced HCC (BCLC C) and received palliative treatment such as HAIC and CCRT, we assumed that only 20% reduction might be sufficient to represent favorable treatment response and could be a useful prognostic marker. However, because our current study included mostly patient with intermediate HCC (BCLC B) undergoing more effective treatment modality (TACE) resulting in CR by mRECIST criteria in most patients, we thought that 20% reduction might not be sufficient for predicting favorable treatment outcome. Thus, we defined DCP response as 50% reduction from the baseline, similar to the definition of AFP response.

Minor Essential revisions

**Response** We added reference 24, 25.

2. In table 1, PIVKA II is used who DCP is used in the rest of article. Suggest standardization of the terms across the paper.

**Response** We revised.

**Reviewer #2**

Major points
1. The same study group has already published several reports on similar subject (prognostic values of AFP and DCP), including reference #34 and the article recently published in European Journal of Gastroenterology and Hepatology. Did the patients studied in the present study overlap those studied
in these previous studies? Are the study patients of this manuscript subpopulation of patients studied in EJGH paper? The authors should clearly describe this point and should emphasize the difference from their previous studies.

Response) The study population in our current study is different from previously published studies which investigated the predictive value of baseline AFP and DCP values (Kang SH, et al. Eur J Gastroenterol Hepatol 2012) and the same issues in advanced HCC undergoing HAIC and CCRT.(Lee MH, et al. J Gastroenterol Hepatol 2011). And reference listed as #34(revised #37) investigated by different institute (Kangdong Sacred Heart Hospital of Hallym University Medical Center) which is totally not associated with our institute.

2. Because almost patients studied were infected with HBV or HCV, antiviral treatment (nucleoside analogues for HBV patients and interferon-based therapy for HCV patients) after TACE would have influenced on OS, although it would not have influenced RFS. Especially for HBV patients who were the majority of the study population, this reviewer imagines that many patients were taking nucleoside analogues. The information on these factors should be provided. In addition, in case of patients with HBV, HBV DNA levels or HBe antigen/antibody status should be included into multivariate analysis, because these factors have reportedly been associated with OS of HBV patients with HCC.

Response) According to the reviewer’s recommendation, we sub-analyzed the influence of viral factors and antiviral treatment using nucleos(t)ide analogs on PFS and OS in patients with chronic HBV infection. However, viral factors including HBeAg positivity and HBV DNA level and anti-viral treatment using nucleos(t)ide analogs did not show independent influences on PFS and OS in multivariate analysis (all \( P > 0.05 \)). This can be explained by the appropriate suppression of viral replication using antiviral agents according to guidelines in most participants.

3. From the description of patient selection (page 9), they studied only patients with pretreatment elevation of both AFP and DCP. Therefore, the study focused on only a part of patients with HCC. This should be discussed. This reviewer think that patients with elevated AFP but DCP and patients with elevated DCP but AFP can be included into the study.

Response) We definitely agree to the reviewer’s comment. However, because we simply investigated the prognostic values of AFP and DCP response simultaneously, we inevitably selected only patients with elevated AFP and DCP in spite of a potential selection bias.

4. The authors analyzed the correlation of tumor marker response with WHO and mRECIST criteria (table 2), and compared their association with PFS and OS (table 3). However, they described in Method section that TACE was repeated with 3-4 week interval based on the radiological response evaluation (they described that TACE was repeated if viable tumor remained at CT or MRI). This
means that all patients achieved CR of mRECIST by TACE after treatment. Therefore, it is not appropriate to use objective response by mRECIST to see correlation with tumor marker response or to compare it with tumor marker response for prediction of PFS or OS.

**Response** First of all we are really sorry for confusing sentences in paragraphs described in patients and method section. We did not mean that all patients achieved CR by mRECIST. Although, at the time of best radiologic response, most of patients achieved CR (83 of 115 patients) after 2~3 session of TACE, but others (32 of 115 patients) showed PR, SD or PD after consecutive TACE. And those who showed other results than CR never reached CR after treated with additional TACE or another treatment option such as HAIC (hepatic artery infusional chemotherapy) or CCRT (concurrent chemo-radiation therapy). We revised some sentences about this issue in methods section.

5. In table 1, information on the size of tumor should be included. In addition, the number of tumors should be presented as continuous variable. These factors should be included in multivariate analyses as factors analyzed.

**Response** We added data on the size and number of measurable tumors in table 1 and 3. In multivariate regression test showed that number of tumor was one of the independent predictors of OS.

6. As the authors described in Discussion section (page 21 last part), AFP elevation was frequently observed not only by HCC but also associated with the elevation of ALT. Therefore, AFP response could be poor in patients with persistently elevated ALT. In addition, patients with persistently elevated ALT are reportedly associated with poor OS due to more rapid impairment of the liver function. This should also be discussed.

**Response** We added this issue in the discussion section.

Minor points

1. Abstract, page 5, Methods: AFP or DCP response was defined as a reduction of more than 50% from the baseline level 1 month after TACE. Why did the authors evaluate post-TACE tumor markers 1 month after treatment? Was it based on the half-lives of these tumor markers? Please provide the rationale for this timing of measurement.

**Response** Because time courses of AFP and DCP shows significant correlations with the tumor necrosis and AFP usually return to normal range within 25~35 days after tumor removal, our institute usually perform response evaluation using tumor markers and imaging at 1 month after TACE.

2. Abstract, page 5, line 16: “gamma-glutamyltransferase” should be “gamma-glutamyltranspeptidase”.
Response) We revised.

3. Introduction, page 7, lines 11-12: “Recently, the national comprehensive cancer ….” Reference should be provided for this.
Response) We added.

4. Introduction, page 8, lines 3-9: This part is not necessary and can be deleted.
Response) We deleted.

5. Patients and Methods, page 10, line 2: From this description, there were no patients with HCC less than 1 cm in diameter in study population. Was this true?
Response) As we already described in methods section, ultrasonography was repeated for HCC less than 10mm in size in 3 months. Thus, all patients in this study showed HCC larger than 10mm.

6. Statistical analysis, page 12: The factors analyzed in multivariate analyses should be listed here.
Response) We have revised all these as suggested by this reviewer.

7. Results, page 16, lines 5-10: “baseline albumin level was identified as an independent predictor of discordance ….” The result of multivariate analysis on this should be presented as a supplemental table. The Kaplan-Meier curves of tumor marker responder/non-radiologic responder and non-tumor marker responder/radiologic responder for PFS and OS should be presented as a supplemental figure. In addition, Kaplan-Meier curves of tumor marker responder/radiologic responder and non-tumor marker responder/non-radiologic responder should also be presented. Were PFS and OS of tumor marker responder/non-radiologic responder and non-tumor marker responder/radiologic responder similar with tumor marker responder/radiologic responder or non-tumor marker responder/non-radiologic responder?
Response) We added supplemental table & figure for reviewer’s request. Answer for another question, PFS and OS of tumor marker responder/non-radiologic responder and non-tumor marker responder/radiologic responder (n = 20) were not similar non-tumor marker responder/non-radiologic responder (n = 4) (18.2 vs. 6.7 months; log rank test, P=0.157 for PFS and 27.8 vs. 12.9 months; log rank test, P=0.388 for OS) nor tumor marker responder/radiologic responder (n = 91) (18.7 vs. 19.0 months; log rank test, P=0.981 and 27.8 vs. 39.2 months; log rank test P=0.187)

8. Discussion, page 17, lines 10-11: “whereas radiologic response was not significant in predicting PFS and OS.” It is normal because TACE was repeated if viable HCC remains by radiologic evaluation.
Response) We have revised all these as suggested by this reviewer
9. Discussion, page 18, lines 13-18: This part should be described in Results section with multivariate analysis presenting supplemental table and figure.

10. Discussion, page 21, line 6: “because of their medical condition” Please provide details.

Response) We have revised all these (minor revision numbered 9,10) as suggested by this reviewer

Reviewer: #3

Major Compulsory Revisions

The authors stated that: "An AFP or DCP response was defined as a reduction of more than 50% from the baseline level 1 month after TACE". This is not a correct method. Indeed, the persistence of DCP serum levels after TACE could be an expression of persistence of disease and may influence, as indeed happens in the sample studied, the lower survival. Some references

Response) Because time courses of AFP and DCP shows significant correlations with the tumor necrosis and AFP usually return to normal range within 25~35 days after tumor removal, our institute usually perform response evaluation using tumor markers and imaging at 1 month after TACE.

Exactly, Baseline AFP & DCP compared to tumor marker checked at the time of best radiologic responses mostly CR (83 of 115 patients) by mRECIST after 2~3 session of TACE. So tumor marker checked at the time of best radiologic responses means nadir of tumor marker. It means that we used same method comparing baseline AFP & DCP to nadir after treatment as Riaz et al. did.

Once again, we would like to extend our cordial gratitude to the Editorial Board and reviewers, whose comments and suggestions have guided us enormously in improving this manuscript toward the scientific soundness.

Sincerely,

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