January 13, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6395-review.doc).

Title: Entecavir *vs* lamivudine therapy for naïve patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure

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On behalf of the authors I would like sincerely to thank you for your reading our manuscript and give our appreciation to the reviewers for their comments on our work.

Enclosed is our revised manuscript entitled "Entecavir *vs* lamivudine therapy for naïve patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure". The manuscript has been improved according to the suggestions of reviewers (all the revisions were highlighted in red):

1 Format has been updated
2 References and typesetting were corrected
3 Revision has been made according to the suggestions of the reviewer

Answers to the questions of reviewer 1 (2013.10.30):

Acute on chronic liver failure (ACLF) represents an intractable liver disease with a bad prognosis. Proper diagnosis and massive intervention are required to handle these patients. Even then, the prognosis of ACLF is not still satisfactory especially in developing and resource-constrained countries. The study presented here has attempted to assess the utility of two antiviral agents for management of hepatitis B virus (HBV)-related ACLF. The most important fact about ACLF is its diagnosis. ACLF represents a pathological condition in which an acute insult cause rapidly downhill course of liver disease in a patients with chronic liver diseases. The acute insult may arise may several factors. As discussed in the Background/Aim section of Abstract of this manuscript, the authors have highlighted the fact of spontaneous acute exacerbation of chronic HBV infection. Please specifically respond to the

following concerns:

Q1. "Is the acute insult of these patients was due to exacerbation of HBV? How did you confirm that? Increased HBV DNA is not a marker of acute insult in ACLF in patients with chronic HBV infection because several patients have attended the clinics with different situations. The HBV DNA may have risen due to disease process, not due to acute insult of HBV exacerbation. Did you measure anti-HBc IgM in all cases and if that was positive in all patients. " (reviewer: 1)

A1. Thank you for reviewer's reminding. The acute insult of those patients enrolled was due to spontaneous acute exacerbation (AE) of chronic hepatitis B (CHB). Although there was no uniform definition of AE of CHB, AE was reported in many studies to be closely related to the abrupt reappearance or rise of HBV DNA in the serum of a patient with previously inactivated or resolved HBV infection. In other words, increased HBV DNA might be a marker of AE of CHB (*1 Lok AS, et al. Gastroenterology 1987; 92: 1839-1846; 2 Yeo W, et al. Br J Cancer. 2004; 90: 1306-1311; 3 Akuta N, et al. J Hepatol 2003; 38: 91-97; 4 Lok AS, et al. Gastroenterology 1991; 100: 182-188*). However, it should be noted that the symptoms of AE of CHB could be very similar to those of acute hepatitis B. A recent study showed a high HBV DNA level (>10^5 copies/mL) was useful to identify AE of CHB from acute hepatitis B (*1 Kumar M, et al. Dig Dis Sci 2006; 51: 594-599*). That was the reason why we chose the pretreatment HBV DNA load. In the consensus recommendations of APASL, acute exacerbation of CHB might be either spontaneous or due to intensive chemotherapy or immunosuppressive therapy and so on. That was to say, spontaneous AE belonged to acute insult of patients with ACLF due to reactivation of CHB (*Sarin SK, et al. Hepatol Int 2009; 3: 269-282*). Meanwhile, we also measured anti-HBc IgM in all cases and added data in Table 1 (*see page 17*).

Q2. "The primary end point of the study was to evaluate survival at 60 days and 52 weeks. I am not sure if assessment of survival at 52 weeks can be a primary end point of survival of ACLF patients. These patients have been suffering from chronic HBV infection. Thus, survival after 52 weeks of starting of ACLF may be due to variable factors. Is the concept of 52 weeks survival of ACLF makes a proper scientific logic? In addition, patients were released from hospital and how the patients were followed up for 52 weeks and how the patients passed their life after initial recovery. Please explain these facts. " (reviewer 1)

A2. Thank you for reviewer's reminding. That was a good suggestion. To make the design of the trial more reasonable, we have put survival at 52 weeks into secondary endpoints in the revised manuscript (*see page 2, the middle paragraph; page 6, 4st paragraph*). Cohort study has been used in clinical trials, such as prognostic evaluation and therapeutic effect evaluation. Follow up is an important step in a cohort study, and follow up quality will directly affect the statistical analysis and drawing a conclusion. Our follow-up work was as follows. Before the start of formal experiment, we discussed the availability of the research program repeatedly, explained significance of scientific research to the patients and improved patients' compliance. Moreover, to ensure follow up quality, we sent the experienced researchers to complete the follow-up work. They adopted various methods to follow up such as letters, telephone, family visit, network and so on. Specifically speaking,

according to our program, patients were detected every 15 days in the first 60 days and then every 3 months until 52 weeks. Clinical and laboratory databases, adverse events, and compliance were monitored during the first 60 days of treatment, and adverse events and compliance information were collected every 3 months until 52 weeks.

Q3. "There was no difference in survival after 60 day but entecavir was superior to lamivudine when survival was assessed after 52 weeks. Both lamivudine and entecavir are antiviral agents for HBV. What additional capacity or property entecavir may have that may provide a better survival at 52 weeks. This point needs more discussion." (reviewer 1)

A3. Thank you for reviewer's suggestion. Our results revealed entecavir get a better survival at 52 weeks, but we did not clearly show additional capacity or property of entecavir. To make this study more reasonable, we had discussed additional property of entecavir and elaborated the possible mechanisms more in details in the revised manuscript (*see page 10, 3rd paragraph*).

Q4. "Paragraph 4 of Discussion section needs careful citations. Pathogenesis of ACLF is not related to high levels of HBV DNA, but possibly cytokine play a major role. It may not be true that lamivudine has week antiviral activity. Please check the HBV DNA load of patients who died and survived and try to show a relation with HBV DNA level and survival." (reviewer 1)

A4. Thank you for reviewer's reminding. Through checking carefully the HBV DNA load of patients who died and survived, we found that there was no significant difference (P=0.264) despite the mean of pretreatment HBV DNA load ([7.2±1.4] log10 copies/mL) in survivors was higher than that ([6.9±1.5] log10 copies/mL) in non-survivors. This might be correlated with different baseline information of subjects, sample size and study design. In order to get more precise conclusion, more studies with larger sample size, and similar subject and trial design are needed (*see page 11, 1nd paragraph*).

Q5. "Did you measure host immunity in patients with good and bad prognosis?" (reviewer 1)

A5. Thank you for reviewer's reminding. That is a good suggestion. Although many studies have proved that host immunity influenced disease progression and antiviral efficacy in humans infected with hepatitis B virus, host immune in pathogenesis of liver failure has been rarely reported (*Wang FS, et al. Expert review of gastroenterology & hepatology, 2009, 3: 499-512*). Therefore, it is very important to carry out the study. To be honest, this study mainly aimed to compare the efficacy between entecavir and lamivudine and investigate prognostic factors in clinic for patients with ACLF. Our next focus will be explore the relations between prognosis of patients and host immune predictors, especially in cellular immune such as T helper 17, regulator T cell, CTL (*see page 12, 1nd paragraph*).

Answers to the questions of reviewer 2 (2013.12.1):

In the diagnostic criteria that should be noted: ACLF diagnostic criteria of patient serum total bilirubin should be ≥ 171 mol / L, instead of ≥ 85μmol / L. Patients enrolled in the article does not match the current standard clinical diagnostic criteria. In this study, no randomization is a drawback, as the author has mentioned in the discussion section of the article. But as real life data should be allowed in clinical.

Q1. "ACLF diagnostic criteria of patient serum total bilirubin should be ≥ 171 mol / L, instead of ≥ 85 μmol / L. Patients enrolled in the article does not match the current standard clinical diagnostic criteria." (reviewer 2)

A1. Thank you for reviewer's reminding. We had updated diagnostic criteria to match the current standard clinical diagnostic criteria in China (*see page 4, 3rd paragraph*).

Answers to the questions of reviewer 3 (2013.12.4):

In this manuscrpt, Dr. Zhang and his collegues discribed their research on HBV therapy with Entecavir versus Lamivudine. The results in the manuscript may help cilinicians to select anti HBV therapy.

Q1. "But the manuscript was poorly written. The manuscript need minior revision before it is accepted for publication in The World Journal of Gastroenterology. The manuscript need to be revised by an native English speaker before it is resubmitted to the journal." (reviewer 3)

A1. Thank you for reviewer's reminding. We have made use of a copyediting service provided by Jing-Yun Ma Editorial Office. And the editing company also provided us with a recommendation letter.

Answers to the questions of reviewer 4 (2013.12.7):

This study demonstrated that treatment by entecavir could improve the prognosis of spontaneous reactivation of HBV.

*Q1. "*In CTP and MELD scores, the value of T-Bil was used and its value was high in spontaneous reactivation of HBV. Therefore, after the overcome of spontaneous reactivation of HBV, these scores would improve naturally. These scores are useful for chronic liver failure, however, not for acute liver failure*." (reviewer 4)*

A1. Thank you for reviewer's suggestion. We were not sure about that were less useless for acute liver failure. But previous studies had proved the value of for evaluation of prognosis of acute-on-chronic liver failure (*1 Cui YL, et al. Dig Dis Sci 2010; 55: 2373-2380. 2 Garg H, et al. Hepatology 2011; 53: 774-780. 3 Garg V, et al. Gastroenterology. 2012; 142: 505-512. 4 Duseja A, et al. J Dig Dis. 2013; 14: 484-490*).

Q2. "The authors should the criteria which nucleotide analogue, entecavir or lamivudine, was used." (reviewer 4)

A2. Thank you for reviewer's reminding. We have added related content in the manuscript (*see page 5, the 4th paragraph*).

Q3. "Figure 1 is very confusing, which might misunderstand that this study is RCT." (reviewer 4)

A3. Thank you for reviewer's reminding. In order to distinguish the RCT, we have revised Figure 1 (*see page 20, Figure 1*).

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

Sincerely yours,

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