Peer Review File

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Reviewer A

Guo et al. reported the efficacy and safety of lenvatinib to prevent recurrence after LT in the patients beyond Milan criteria. They concluded that lenvatinib might provide clinical benefits without severe adverse events. The concept is clear, and the paper is generally well written and easy to read. However, as the authors also mentioned, there are several significant limitations in this study, mainly as the retrospective and uncontrolled study with insufficient number of the cases. This kind of paper should be written with using propensity matching with adopting various appropriate items including patient, tumor and surgical characteristics, at least.

Response: Thank you very much for the advice. In the patients beyond Milan criteria, the baselines of the lenvatinib group and the control group have been compared and no significant difference was found. Competing risk analysis showed lenvatinib significantly prolonged the time to recurrence after liver transplantation versus the control group (sHR, 0.40, 95% CI, 0.17-0.93; P=0.031). As the reviewer A's suggestion, we additionally performed PSM and matched 99 patients in a 1:2 ratio; 33 in the lenvatinib group and 66 matched controls. The clinical features of the two groups after PSM were well balanced. As expected, competing risk analysis showed longer TTR in the lenvatinib group than the control group in the PSM cohort (sHR, 0.31; 95% CI, 0.12-0.78; P=0.013). Notably, the OS didn't differ between the two groups but had a trend of longer OS in the lenvatinib group after PSM (HR, 0.40; 95% CI, 0.13-1.19; P=0.099).

Changes in the text: we have added these data in the manuscript (see Page 15, line 17).

Comment 1. What is the indication of lenvatinib in this study? Why did the 4 cases within Milan criteria receive lenvatinib?

Response: Thank you for the question. After liver transplantation, the application of adjuvant lenvatinib was recommended for the patients with high risk of recurrence (multiple lesions, mVI, poor differentiation or postoperative AFP/PIVKA-II positive), with the intention of reducing relapse and improving survival. Consistently, the 4 patients within Milan criteria used lenvatinib because of the existence of mVI or poor differentiation.

Changes in the text: we added the explanation in the manuscript (see Page 10, line 4)

Comment 2. Not only the tumor characteristics, but surgical items (i.e., duration, blood loss, living donor or deceased donor, ischemic time) should be compared between the groups.

Response: Thanks for this valuable suggestion. According to the reviewer A's suggestion, we compared the surgical information including duration of surgery, blood loss and ischemic time, et al. and we found that there was no significant difference between the lenvatinib group and the control group. We also found that these factors were not associated with recurrence risk of patients with HCC after liver transplantation. In addition, the donor situation was not compared since all grafts used in liver transplantation were obtained from deceased donors. We added these data in the revised manuscript (see Table 1&2).

Changes in the text: we have modified our text as advised (see Table 1&2).

Reviewer B

This is a well-done study evaluating the efficacy and safety of lenvatinib for the treatment of HCC after liver transplant, in an adjuvant setting. The manuscripts address a new knowledge gap in HCC treatment and provide great evidence for the design of future research in this area. I have the following minor comments for the authors:
Comment 1. In the Introduction, 2nd line screening programs should be used rather than monitoring programs

Response: Thanks a lot for your kind comment. According to the reviewer B's suggestion, the term "monitoring" was replaced with "screening" (Page 6, line 2).

Changes in the text: we have modified our text as advised (see Page 6, line 2).

Comment 2. The liver transplant criteria for HCC vary by the country and these indications in China should be included to provide a perspective to the audience from all over the world

Response: Thanks for the meaningful suggestion. We used the Milan criteria in our manuscript because the Milan criteria is the most rigorous criteria for liver transplantation. Patients within the Milan criteria showed less recurrence and satisfactory outcomes. Hence, we investigated the application of adjuvant therapy for reducing recurrence of patients beyond the Milan criteria after liver transplantation. In fact, several criteria were developed to expand the Milan criteria, especially in China. So, we added the Fudan criteria as an indication in China into analysis (1).

Changes in the text: we have modified our text as advised (see Table 1).

Comment 3. Was lenvatinib given as offlabel? This must be clarified in the methods section?

Response: Thank you for the comment. The use of lenvatinib was indeed off-label. Hence, only the patients with high risk of recurrence (with multiple lesions, mVI, poor differentiation or postoperative AFP/PIVKA-II positive) was applied, with the intention of reducing relapse and improving survival.

Changes in the text: we added the explanation in the manuscript. (see Page 10, line 4)

Comment 4. For statistical analysis, the threshold of the p-value from univariate for inclusion in the multivariate analysis should be specified (e.g. p-value less than or equal

to 0.10)

Response: Thanks for reminding us of this issue. We used 0.05 as the threshold of the p-value in univariate analysis and those variables with P<0.05 in univariate model were selected into multivariate analysis.

Changes in the text: Variables with P<0.05 in univariate model were selected into multivariate analysis. (see Page 11, line 11)

Comment 5. line 306is a repetition from 295. Keep only of them

Response: We are sorry for the redundant description. We were supposed to explain the retrospective design of this study, which lead to similar statements in these two paragraphs. The sentence in line 295 was deleted as advised.

Changes in the text: we have modified our text as advised (see Page 20, line 3).

Comment 6. A small section discussing the efficacy of other agents such as sorafenib in the adjuvant treatment of HCC after liver tx should be presented in the discussion to allow a comparison of various agents.

Response: Thanks for this valuable comment. The application of tyrosine kinase inhibitors (TKIs) as adjuvant therapy in HCC after radical surgery remains controversial. There were several literatures demonstrated that adjuvant sorafenib improved the outcomes of HCC with high risk of recurrence after radical resection or LTx. However, the phase III STORM trial failed to identify the clinical benefit of adjuvant sorafenib. So, more evidences of adjuvant TKIs in HCC are needed to identify the efficacy.

Changes in the text: we have modified our text as advised (see Page 19, line 21).

Comment 7. How do the authors propose practitioners from other countries who use Milan criteria for liver transplants in HCC should interpret data from this study?

Response: Thank you for the constructive comment. The present study mainly focused on the patients who underwent liver transplantation with HCC beyond Milan criteria, since this subpopulation faced a relatively high risk of postoperative recurrence. The results also give some indications for patients within Milan criteria. Firstly, the application of lenvatinib after LTx is safe and didn't increase the incidence of complications, indicating that lenvatinib could be used in patients who underwent LTx.

Besides, our data showed that adjuvant lenvatinib therapy might reduce the recurrence rate of HCCs with multiple lesions, mVI, poor differentiation or postoperative AFP/PIVKA-II positive, offering the potential that lenvatinib might confer clinical benefit to those patients even though within Milan criteria. Further evidences from large-scale, prospective clinical trials are needed.

Changes in the text: we have modified our text as advised (see Page 20, line 12).

Comment 8. Minor grammar and tense mistakes throughout the manuscript. Another native English speaker revision would help with all of this.

Response: Thank you for the suggestion. We have asked native English speakers revised our manuscript and some grammar mistakes were corrected.

Comment 9. Pre-transplant treatment details such as downstaging or other adjuvant therapies should be tabulated for the patients, if available

Response: Thanks for your suggestion. We tabulated the pre-transplant treatments in Table 1. Several patients received TACE before transplantation with the intention of downstaging or reducing the recurrence risk. The pre-transplant treatment in patients beyond Milan criteria was compared and there was no significant difference between the lenvatinib group and the control group. (Table1)

Changes in the text: we have modified our text as advised (see Table1).

Reviewer C

Dr. Den-Zhen Guo et al. reported the efficacy and safety of lenvatinib for preventing tumor recurrence after liver transplantation in hepatocellular carcinoma (HCC) beyond Milan criteria using their retrospective cohort. The result has some meaning for post-transplant adjuvant chemotherapy for HCC, and it may become some reference for the subsequent clinical trial. However, the following should be revised and discussed more deeply.

Major

Comment 1. In the abstract and introduction, the authors described lenvatinib as a first-line treatment for unresectable HCC. However, the first-line treatment has already been changed to atezolizumab + bevacizumab. The authors should correct it.

Response: Thank you for the suggestion. We are sorry for the inappropriate statement. Recent years have seen emerging regimens approved as first-line treatments for unresectable HCC, including sorafinab, lenvatinib and atezolizumab + bevacizumab (2). Hence, Lenvatinib is one of the approved first-line treatments for unresectable HCC. The statement has been corrected in the manuscript.

Changes in the text: we have modified our text as advised (see Page 6, line 20).

Comment 2. The authors should explain who decided on adjuvant treatment with lenvatinib after liver transplantation in patients and the methods. They also clearly should describe the duration of treatment of lenvatinib as adjuvant treatment.

Response: Thank you for the suggestion. After liver transplantation, the application of adjuvant lenvatinib was recommended for the patients with high risk of recurrence (multiple lesions, mVI, poor differentiation or postoperative AFP/PIVKA-II positive), with the intention of reducing relapse and improving survival. Since two years after transplantation was the peak period of recurrence, the recommended duration of lenvatinib treatment was two years.

Changes in the text: we added the explanation in the manuscript. (see Page 10, line 4)

Comment 3. The style of the result is unusual. The titles of each section, such as “Adjuvant lenvatinib didn’t prevent recurrence in unstratified patients.” should be removed.

Response: Thanks a lot for the kind recommendation. We have changed the style

according to the format of ATM.

Changes in the text: we have modified our text as advised (see subtitles of Results).

Comment 4. In safety, clinicians are concerned about the increased incidence of transplant-related events such as rejection, biliary troubles, infection, etc. The authors should explain the events in the body.

Response: Thanks for this valuable comment. We have compared the incidence of transplant-related events, including infection, rejection and bile duct narrow between the lenvatinib group and the control group. Our data showed that the incidence rates of infection, rejection and stenosis of bile duct in the lenvatinib group were comparable with those in the control group (4.8% vs 4.0%, 4.8% vs 2.0% and 2.4% vs 10.0%; all $P>0.05$). These data suggested adjuvant lenvatinib didn't increase the risk of complications after LTx.

Changes in the text: we have added these data in the revised manuscript (see Page 17, line 4).

Comment 5. The authors should intensely discuss the reasons for the difference between overall survival and time to recurrence in the discussion.

Response: Thanks for the suggestion. In the present study, we showed adjuvant lenvatinib therapy significantly prolonged the time to recurrence for patients with HCC beyond Milan criteria. However, no significant difference in OS was observed between the lenvatinib group and the control group in HCC beyond Milan criteria. which might be due to the short follow-up time and relatively small sample size. Besides, the reserved liver function after liver transplantation increased the probabilities of undergoing secondary resection and other logo-regional therapy after recurrence. Notably, after PSM further balancing the baselines, there emerged a trend toward better OS for the lenvatinib group.

Changes in the text: we have modified our text as advised (see Page 18, line 14).

Minor

Comment 1. The authors should clearly describe the observation period in the result.

Response: Thank you for the comment. The median follow-up time was 18.7 (range, 3.6-35.6) months. We have described the observation period in Results.

Changes in the text: we have modified our text as advised (see Page 13, line 10).

Comment 2. Figure 1 should be modified according to the orders of results.

Response: Thanks for the suggestion. We have modified Figure 1 according to the orders of results.

Changes in the text: we have modified our text as advised (see Figure 1).

Comment 3. In the Tables, the “Child-Pugh stage” should be “Child-Pugh class.”

Response: We are sorry for the loose term. The term “Child-Pugh stage” has been corrected as “Child-Pugh class”.

Changes in the text: we have modified our text as advised (see Table 1).

Reference

1. Zhou J, Sun H, Wang Z, Cong W, Wang J, Zeng M, et al. Guidelines for the Diagnosis and Treatment of Hepatocellular Carcinoma (2019 Edition). Liver cancer. 2020;9(6):682-720.

2. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. The New England journal of medicine. 2020;382(20):1894-905.