Dear editor Xue-Jiao Wang:

Thank you very much for your letter of Nov 29, 2018, with regard to the review of the manuscript entitled "**A six-long non-coding RNA signature predicts recurrence-free survival in hepatocellular carcinoma**" for consideration for publication in ***World Journal of Gastroenterology***. We are very grateful to the reviewers' positive comments regarding our novel, significant and interesting results. Reviewer#1 said this study was interesting. Reviewer#2 commented that this was a reasonable study. And Reviewer#3 made valuable and specific comments on how to improve this work.

We also thank the reviewers for the thorough critiques that are very helpful in preparing this revised submission. We have carefully considered each of the comments from all the reviewers and the editor, performed extra analysis, and made changes to the manuscript accordingly. Below are our point-to-point responses, in which each comment from the reviewers is italicized and our responses are highlighted in blue. The revised texts in manuscript are highlighted in red.

Reviewer #1:

This is a very interesting study on the prognostic impact of non-coding RNA signature on RFS in HCC patients. The molecular approach seems to be novel and interesting.

Response: Thanks for Reviewer's recognition of this manuscript.

However, it remains to me completely unclear how the HCC sets have been treated. Are the patients receiving surgical or non-surgical treatments? More data on patients' characteristics is essential, such as neoadjuvant locoregional treatment, waiting times, tumor grading and vascular invasion.

Response: We are sorry for the incomplete description. All 108 enrolled patients in the discovery dataset received radical resection between the years 2000 and 2013 in Singapore General Hospital (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi). And the treatment data has been added in the Methods part which are as follows:

"All 108 participants underwent curative resection for HCC."

There was no records of other clinical characteristics like neoadjuvant locoregional treatment, waiting times, tumor grading or vascular invasion of this dataset (GSE76427) in GEO database.

In Addition, I would advise a multivariate analysis on prognostic factors after the study population has been clearly defined.

Response: We agree with your comment. As you suggested, we performed Cox regression analyses of all the recorded clinical characteristics in the discovery dataset (Table 1) (GSE76427) including age, gender, TNM stage and BCLC stage and TCGA dataset (Table 2). We are sorry that there are only 4 characteristics recorded of the patients in GSE76427. The results of Cox analyses are as follows:

Table 1. Univariate/multivariate COX regression analyses of clinicopathologic factors associated with RFS in the discovery cohort

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Six-lncRNA risk score	2.82(1.74-4.56)	<0.001*	—	—
Gender (male/female)	0.61(0.28-1.32)	0.21	—	—
Age (>60/≤60 years)	1.07(0.60-1.90)	0.82	—	—
TNM stage (III/II/I)	1.27(0.89-1.81)	0.19	—	—
BCLC stage (C/B/A/0)	1.36(0.90-2.07)	0.15	—	—

*: Statistically significant.

Only one factor (six-lncRNA signature) is significant through univariate analysis, so multivariate analysis cannot be done.

Table 2. Univariate and multivariate Cox regression analyses of clinicopathologic characteristics associated with early recurrence in TCGA samples

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Six-lncRNA risk score	1.341(1.110-1.620)	0.002*	1.270(1.018-1.585)	0.034*
TNM stage (IV/III/II/I)	1.727(1.441-2.070)	<0.001*	1.705(1.393-2.089)	<0.001*
Gender (male/female)	0.982(0.711-1.355)	0.911	—	—
Age (>60/≤60)	0.956(0.707-1.294)	0.773	—	—
HBV (yes/no)	0.804(0.505-1.281)	0.359	—	—
Alcohol consumption (yes/no)	1.062(0.769-1.467)	0.717	—	—
Liver cirrhosis (yes/no)	1.271(0.861-1.877)	0.228	—	—
Albumin (>3.5/≤3.5 g/dl)	0.968(0.659-1.424)	0.870	—	—
Creatinine (≥1.1/<1.1 mg/dl)	0.739(0.511-1.069)	0.109	—	—
AFP [a] (>20/≤20 ng/ml)	1.380(0.972-1.959)	0.072		
Platelet (>250/≤250×10^9/L)	1.314(0.932-1.854)	0.119	—	—
Race (Asian/White)	1.270(0.928-1.739)	0.136	—	—
BMI [b] (≥24/<24)	0.860(0.697-1.062)	0.161	—	—
Family history (yes/no)	0.920(0.655-1.292)	0.630	—	—
ECOG [c] (>0/=0)	1.858(1.329-2.598)	<0.001*	1.486(1.045-2.114)	0.028*
TP53 mutation (yes/no)	1.389(1.004-1.922)	0.047*	1.326(0.894-1.967)	0.161

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

*: Statistically significant;

[a]: Alpha-fetoprotein;

[b]: body mass index;

[c]: Eastern Cooperative Oncology Group.

Reviewer #2:

In this article, Gu JX et al. analyzed the prognostic role of lncRNA signature in HCC. Several articles using similar concept are published in other cancer types. The statistic methods are reliable. The results provide some novel information in this field. The authors also revised the method section to avoid self-plagiarism. Generally, this is a reasonable study.

Response: Special thanks for your positive comments on this manuscript.

Reviewer #3:

The manuscript by Gu JX et al. is an interesting study about the relationship between six-long non-coding RNA signature and recurrence free survival in HCC affected patients. The Authors investigated liver tissue samples from 108 patients and they constructed a six-lncRNA signature for prognosis prediction of HCC. This risk model should provide new clinical evidence for the accurate diagnosis and targeted treatment of HCC. The paper is well written, also the statistical analysis is appropriate.

Response: We are grateful to your positive suggestions on our study.

However, the Reviewer reports some weaknesses of this paper.
MAJOR 1. Among the enrolled patients, the number of females is too low.
Response: We agree with your comment. However, the incidence rate of liver cancer in men was nearly three times higher than that in women according to the statistical data from the American Cancer Society[1]. Therefore, due to the limited sample size of the enrolled patients, the number of female participants was much lower than that of male patients (22:86 in the discovery dataset and 107:227 in the validation dataset) which implied that our prognostic model was more suitable for males. And further stratified analyses validated the appropriate application of the six-lncRNA signature in male patients (Table 1). Nonetheless, risk models which can precisely evaluate the recurrence of women HCC patients or both men and women patients are needed to construct in the future using datasets involving more female patients.

1 **Siegel RL**, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67: 7-30 [PMID: 28055103 DOI: 10.3322/caac.21387]

2. HCV infection should also be searched in the patients

Response: We totally agree with Reviewer on the research of HCV infection in HCC patients. According to the suggestion, firstly, we have also included HCV infection status as a stratifying factor for stratified analysis. From the results, the HCV infection status seems to have little influence on the application of the six-lncRNA signature (*P*>0.05). The stratification results (in red) have been added in Table 1 and shown as follows:

Characteristic	High-risk / low-risk	HR (95% CI)	*P* value
Hepatitis C			
With HCV	26/25	0.964(0.466-1.997)	0.922
Without HCV	46/54	1.434(0.792-2.596)	0.224

Secondly, the association between HCV infection and HCC recurrence has been investigated as well using Cox regression method. The results show HCV was not a significant risk factor of HCC recurrence (*P*>0.05) which might be partly due to the small sample size of HCV infected patients. The Cox results (in red) have been added in Table 2 and presented as follows:

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
HCV (yes/no)	1.271(0.798-2.207)	0.313	—	—

3. How long is the follow-up? How did the Authors evaluate tumor recurrence in the patients? The characteristics of recurrent tumors should be detailed reported in the manuscript.

Response: Patients in the discovery dataset were followed up at least once every 3–6 months and the median follow-up was 1.17 years[2]. HCC recurrence was evaluated according to the established histological criteria set by International Working Party in 1995[2,3]. We have added the follow-up information and diagnostic criteria of the participants from the discovery dataset in Methods part which are presented as below in red. As GSE76427 was obtained from the public database, the contributor did not provide any more detailed information either in GEO database or in their published paper citing this dataset.

"The median follow-up was 1.17 years. HCC recurrence was diagnosed according to the established criteria reached by International Working Party."

2 **Grinchuk OV**, Yenamandra SP, Iyer R, Singh M, Lee HK, Lim KH, Chow PK, Kuznetsov VA. Tumor-adjacent tissue co-expression profile analysis reveals pro-oncogenic ribosomal gene signature for prognosis of resectable hepatocellular carcinoma. Mol Oncol 2018; 12: 89-113 [PMID: 29117471 DOI: 10.1002/1878-0261.12153]

3 **International Working Party**. Terminology of nodular hepatocellular lesions.

Hepatology 1995; 22: 983-993 [PMID: 7657307]

4. The Authors should specify which will be the concrete application of their model to the clinical practice and its cost.

Response: We agree with Reviewer's comment. From the stratified analytic results (Table 1), the best candidates for our prognostic model are relatively younger (≤60 years) male patients possessing the following characteristics: TNM staging in I/II, Asian, with family history, HBV infection, alcohol consumption, ECOG=0 and higher level of preoperative serum ALB (>3.5 g/dl) and AFP (>20 ng/ml). This prognostic model is comprised of the expression value of six lncRNAs (*MSC-AS1*, *POLR2J4*, *EIF3J-AS1*, *SERHL*, *RMST* and *PVT1*) which is detected in patients' cancerous tissue. The tumor tissue can be obtained from liver biopsy or intraoperative specimen. The specific and detailed application and the cost of the model has been described in the Results and Discussion parts in the revised manuscript which are as follows:

"The expression value of the lncRNAs constituting the risk model in patients' tumor tissue can be tested via the liver biopsy or from the surgical specimen."

"Stratified survival analysis showed this six-lncRNA signature was more suitable for the recurrence prediction of relatively younger (aged≤60 years) Asian male patients with HBV infection, family history and history of alcohol consumption who are in TNM staging I or II and better physical condition (ECOG=0 and preoperative ALB>3.5 g/dl) but with higher preoperative AFP."

MINOR 1. A few typos, for example "algorism" repeated several times, are present in the manuscript.

Response: We are sorry for the typos. We have replaced all the "algorism" with "algorithm" and gone through the whole manuscript trying to avoid other grammatical and typo errors.