Response to the Reviewer

We thank the reviewers for their constructive comments. We have made detailed modifications according to the comments of reviewers. We believe that these changes have improved the paper and we appreciate the efforts of the reviewers in this behalf. Specific point-by-point responses are below.

Reviewer 1

-Avoid abbreviations in the title of the manuscript.

We have changed the abbreviations in the title.

Abstract:

- provide the values for incidence, mortality, and recurrence rates for liver cancer.

We improved the epidemiology of liver cancer in the background, as follows.
Liver cancer is the sixth most frequently occurring cancer in the world and the fourth most common cause of cancer mortality. (Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), 394-424.)

- define HepG2 and DDP abbreviations.

We have defined the abbreviations HepG2 and DDP. HepG2 cells (Hepatoma cell line); 5-Fluorouracil combined with Cisplatin (5-fu and DDP)

- Numbers and results of comparisons must be provided in the Results section.

We have provided comparative figures and results in the results section.

Introduction:

- "Liver cancer has high morbidity and recurrence rates" please provide the numbers supported by references.

We have provided the morbidity and mortality of liver cancer in the introduction, and eliminated the recurrence rate, as follows.
Liver cancer has high morbidity rates and is one of the leading causes of cancer-related mortality worldwide, the estimated global incidence rate of liver cancer per 100,000 person-years was 9.3 while the corresponding mortality rate was 8.5.（Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), 394-424.）

- I would not say that Cur is a cytotoxic drug.

We have modified this statement in the article

- This section must clearly present state of the art. Please briefly introduce the main results reporting curcumin and/or on liver cancer.

We have briefly introduced the current situation of curcumin in the treatment of liver cancer, and cited the references, as follows.
Curcumin has been demonstrated to inhibit the proliferation of HepG2 cells (Hepatoma cell line) in a dose and time dependent manner. The current preclinical model studies in vivo and in vitro have shown that curcumin, along with other curcumi-noids, have immense potential as curative agents for liver cancer, based on its potent antioxidant and anti-inflammatory properties and its ability to regulate a variety of signaling mechanisms.

Materials and Methods:

- Is there any reason to use only male mice? Please provide an explanation in your manuscript.

Since the incidence rates among men are two to three-fold higher than rates among women in liver cancer (Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), 394-424.), hence only male nude mice were used. We have explained and cited the literature in this paper.

- Use the symbol instead of x (1x107)

We have modified it in the article.

- Provide the references for the used doses of Cur and TG.

We have provided the maximum reference dose for the use of curcumin and have described the dosage for the use of total ginsenosides, as follows.
Curcumin is safe for use in large and small animal models, with daily doses up to 12, 000mg（Lao CD , Ruffin MT , Normolle D , Heath DD , Murray SI , Bailey JM , Boggs ME , Crowell J , Rock CL , Brenner DE . Dose escalation of a curcuminoid formulation[J]. BMC complementary and alternative medicine, 2006, 6(1): 1-4.PMID: 16545122. DOI：10.1186/1472-6882-6-10.）
The initial dose of total ginsenoside was calculated as 2 times of the usual dose of ginsenoside (10g/d), which was converted to the low dose at the beginning. The formula of human and mouse body surface area was converted to 2080mg/kg/d, and the total ginsenoside extraction rate and purity of 80% were combined to calculate 104mg/kg/d.The high dose was converted to 520mg/kg/d at 5 times the low dose.

- How was the distilled water administrated?

daily oral dose of 0.2ml administered distilled water

- The rationale of using different methods of administration for the drugs between groups must be explained. The trauma associated with intraperitoneal administration is higher than that of the oral dose.

In this experiment, curcumin and ginsenoside were given in combination to test whether they have synergistic inhibitory effects on liver cancer. Meanwhile, curcumin and ginsenoside were given separately as the control group. In addition, Studies have shown that ginsenosides are dose-dependent(Jiang JW, Chen XM, Chen XH, Zheng SS. Ginsenoside Rg3 inhibit hepatocellular carcinoma growth via intrinsic apoptotic pathway[J]. World J Gastroenterol, 2011,17 (31): 3605-13.PMID: 21987607.DOI：10.3748/wjg.v17.i31.3605.), There is no optimal dose of ginsenosides to treat tumors and no reported combination with curcumin. To investigate the effect of total ginsenoside dose on combined drug use,hence total ginsenoside was given in a low-dose group and a high-dose group. In order to reduce the pain of the experimental mice and avoid the traumatic drug administration as far as possible, the total ginsenoside and curcumin are given orally. However, chemotherapy drugs of 5-Fluorouracil and Cisplatin were not suitable for oral administration in mice, 5-Fluorouracil and Cisplatin were intraperitoneally injected. The optimal dose of curcumin was selected according to the literature, but there was no optimal dose of ginsenoside to treat tumors, nor was there any report on the combination of curcumin and ginsenoside.

- Briefly present in the manuscript how the mice were euthanized.

After 21 days of treatment, test mice were euthanized by cervical dislocation according to ARRIVE guidelines.

- Only the volume was tested?

We have further elucidated the tumor measurement.

- Use the symbol instead of x in "1 x TBST"

We have modified it in the article.

- The significance level needs to be adjusted according to the number of groups (which is 7).

We have adjusted the significance level for the number of groups, which is specified in the results and figures.

Results:

In the result section, according to the opinions of reviewers, we have moved and modified the sentences which belongs to the Discussion, Methods *section and* state of the art.

- Move the following sentences to the methods section "During the experiment, the tumor volume of mice was measured every 7 days to evaluate their growth. After 21 days of treatment, the mice were sacrificed, and the tumor volume was measured." Briefly describe how the measurements were done.

During the experiment, the tumor volume of mice was measured every 3 days to evaluate their growth. Tumor sizes were measured using calipers, and their volume was calculated using the following formula: $(L \times W^2)/2$, where L and W are the length and width of the tumor, respectively.

- References are not allowed in this section.

We have removed the references from the results section and moved it to the appropriate location in the article

- Provide P-values with four decimals and use the treshold of significance after adjustement to the number of compared groups.

We have made corresponding modifications in the paper.

- Fig 1B. I think that it is correct "Tumor volume" instead of "Tumor size".

We have made corresponding modifications in the figure1B.

- Fig. 2A, 3A. It is not clear which groups were statistically significant different (*. **). Which is the significance of "#"?
- Presents the results of statistical analysis using the statistic of the test and associated significance.
- The use of column graph is misleeding because you want to demonostrate the differences between means and a mean is a poit value not a continuous one.

We have marked statistically significant differences in the groups with letters (a,b,c,d,e,f) in the figures. The results of statistical analysis are also provided.

- Discuss the limits of your study. Did you consider of using liposomal curcumin or nano-particles of curcumin to increase de disponibility?

We highly agree with the Suggestions of reviewers. Although curcumin is efficacy and safety, but its the low relative bioavailability is a major obstacle to clinical use. Many new approaches have been explored, including the use of liposomal, nanoparticles, curcumin phospholipid complexs and structural analogues of curcumin (e.g., EF-24) to enhance the bioavailability. In future studies, we will explore the use of liposomes or nanoparticles to enhance the medicinal value of curcumin.

- Discuss the practical implication of your results.

Our results show that Cur combined with TG demonstrated regulated immune escape through the PD-L1 pathway and inhibited liver cancer growth through NF-κB-mediated inflammation and angiogenesis, which offers a potential combination of drugs that could improve the effectiveness of treatment for liver cancer.

Reviewer 2

I have reviewed the Peer-Review Report and the full text of the manuscript, of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revision. Before final acceptance, the authors need to meet ethics requirement by submitting correct documents. Please send the revised manuscript to the reviewers for a second round of peer review.

The conflict-of-interest disclosure form was not proper.

We will provide the right form of conflict-of-interest disclosure.

5 Issues raised:
(1) Please write the "article highlights" section at the end of the main text.

We have put the "article highlights" section at the end of the main text.

(2) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout;

We have provided the PubMed number and DOI citation numbers to the list of references and listed all the authors of the references.

(3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

We have submitted the the original figure documents.