Reviewer 1

1. At figure 1, you wrote mTECICT should be written mRECIST.

Yes, it has been revised.

2. In Figures 2 and 4, you wrote rat in the y-axis, those should be written in rate. Please check and revise those

Yes, it has been revised.

3. In figure 3, why you did not include progression disease cases? Were there no irAEs in PD cases? Please check and revise here.

Because of the large number of patients, the picture cannot show the PD patients. I drew another waterfall plot to describe PD patient (Figure 3A).

4. In figure 4, how many cases are included this spider plot? It did not include the 99 cases, you mentioned in figure 3 legends. Please check and revise here.

A total of 25 patients with hypothyroidism were included in the spider plot, corresponding to waterfall plot, of which 4 were PD, 5 were PR, and 16 were SD.

5. Figure 4 looks complicated due to the differences in the colors. You should use the same color as the line bar and symptom. Please check and revise here.

The legend has been supplemented in the Figure 4, including color and description.

6. The number of cases with hypothyroidism was relatively small. So, that is a big limitation of this study. You already mentioned it in the limitation part. But, please check and revise that sentences.

I have revised the sentences.

Reviewer 2

1.Title is relevant Abstract: good Add to the conclusion a note on the survival. You only state a "a better therapeutic effect." Although the results mention a better OS.

Pembrolizumab-treated patients with unresectable hepatocellular carcinoma who experienced hypothyroidism during treatment had promising objective response rate, and durable response. Hypothyroidism, an immune-related

adverse event may be used as a clinical evaluation parameter of HCC response to ICIs.

2. Keywords: kindly add Pembrolizumab by name.

Yes, I have revised.

3. Please modify HCC to unresectable or advanced HCC.

Yes, I have revised.

4.Introduction: 1- The authors mentions "Locoregional therapy including radiofrequency ablation (RFA)," as one of the treatments, but I think it is more beneficial to mention resectable versus unresectable or curable versus advanced, as RFA is one of the curative measures. RFA should not be mentioned here for comparative survival except if used for the downgrading of the tumor.

Yes, I have revised the error.

5. in line 103: "autoimmunelike" please correct to "autoimmune-like"

Yes, I have revised the error.

6.Methods: 1. The authors mentioned "evaluation criteria in solid tumors criteria (mRECIST) every 3–6 weeks by contrast-enhanced CT or MRI examination.">>> does that mean for the whole duration of follow up ie two years, because the radioactive exposure will be very high. Kindly elaborate.

Most patients choose either CT or MRI. In addition, patients who achieved PD status do not have CT or MRI.

7. The authors wrote "imageological">>this could be a hard word, could you use a more common one like "imaging study"

Yes, I have revised the error.

8.Could the authors state the longest duration of follow up? (example the longest FUP was two years or 24 months) to make clear that OS here is a short one. You stated that later in days which may be confusing to the reader.

Follow-up was terminated on April 24, 2022. The mean follow-up time was 814 days (median, 748 days; range, 438–1146 days).

9.There is no mention of AFP and whether it was used in FUP, although it is

mentioned in results of univariate analysis.

Line 125: History, including age, sex, etc., laboratory examination, including levels of alanine transaminase (ALT), aspartate aminotransferase (AST), alpha fetoprotein (AFP), etc.

10. The univariate and multivariate analysis presents all factors like a correlation table, could you kindly modify the table to contain the most relevant factors (max 5-6) especially in the multivariate analysis or separate the tables.

I have revised the table.

11 It is not clear if the base Child-Pugh had a direct effect on the occurrence of adverse events or whether it changed on follow up, could you kindly clarify?

In our study, 74.7% (68/91) patients in PD status experienced Child-Pugh score increase at the end of the disease and the percentage was 20.2% (20/99) in patients with CR, PR and SD status. However, we found that the median time of irAE occurrence was 7.5weeks and much earlier than the time of Child-Pugh score increase, so we considered that Child-Pugh had has no direct effect on irAE.

12 The effect of the tumor burden and drug response on AFP, could you mention in more details

Many studies have reported that there was a positive correlation between tumor burden and AFP. [1-3]. In addition, many studies suggested that AFP level can be used as one of the evaluation indicators of drug response[4-6]. In our study, we did not analyze the relationship between tumor burden and AFP, which is one of the limitations of this paper.

13. The authors concentrated on the effect of the drug on hypothyroidism and its relation to response rate. However, there is no mention of the effect of the drug on OS and the difference from the previous RCT conducted, and whether the multivariate factors detected in the statistical analysis are different from previous studies. Kindly add to discussion.

Previous study has reported that among the irAEs manifesting as endocrine

dysfunctions, hypothyroidism (6.07%) and hyperthyroidism (2.82%) were most common[7]. Thyroid events occur in approximately 10% of patients treated with anti-PD-1/PD-L1 monotherapy[8, 9]. The median time to onset of thyroid dysfunction, most of which is hypothyroidism, is 6 weeks after ICI initiation[10]. In our study, we found that patients who developed hypothyroidism had a longer OS and TTP than those without irAE. Multivariate analysis showed that hypothyroidism was an independent prognostic factor. In addition, we found that patients with hypothyroidism had a significant reduction in TBS from baseline by treatment, which was intuitive manifestation of the effectiveness of immunotherapy. Many previous studies have also shown that patients who experienced irAEs had superior progression free survival (PFS) and OS compared to those who did not experience irAEs[11-13]. A study of 270 non-small cell lung cancer patients treated with at least one dose of anti-PD-L1 or anti-PD-1 antibodies showed that patients who experienced thyroiditis had statistically significant improvements in OS compared to patients who did not experience the endocrinopathy (P =0 .01)[14]. A meta-analysis of 12 RCTs identified 3815 metastatic head & neck and lung cancer patients treated with ICIs showed that hypothyroidism and a significant correlation between endocrine irAEs and OS was observed (P = 0.019)[15]. In addition, a retrospective study reviewed 318-patient advanced melanoma patients and found that patients who experienced irAEs had superior OS compared to those who did not experience irAEs[16].

14.Conclusion: the conclusion at the start of the manuscript is the same as the end, please rewrite, as they have to be different. Also add future aspiration in the research in this area.

In conclusion, the adverse effects of Pembrolizumab, hypothyroidism predicted a better OS and TTP. Thus, in patients with unresectable HCC treated with Pembrolizumab, not only drug response, but also the irAE of the drug should be carefully monitored. Hypothyroidism may be used as a useful clinical predictor of response to Pembrolizumab and patients' survival.

1. Tsilimigras DI, Hyer JM, Diaz A, Bagante F, Ratti F, Marques HP, Soubrane O, Lam V, Poultsides GA, Popescu I, Alexandrescu S, Martel G, Workneh A, Guglielmi A, Hugh T, Aldrighetti L, Endo I, Pawlik TM. Synergistic Impact of Alpha-Fetoprotein and Tumor Burden on Long-Term Outcomes Following Curative-Intent Resection of Hepatocellular Carcinoma. *Cancers (Basel)*. 2021;**13**[PMID:33670174 doi:10.3390/cancers13040747].

2. Lima HA, Endo Y, Alaimo L, Moazzam Z, Munir MM, Shaikh C, Resende V, Guglielmi A, Marques HP, Cauchy F, Lam V, Poultsides GA, Popescu I, Alexandrescu S, Martel G, Hugh T, Endo I, Kitago M, Shen F, Pawlik TM. Tumor Burden Score and Serum Alpha-fetoprotein Subclassify Intermediate-Stage Hepatocellular Carcinoma. *J Gastrointest Surg*. 2022;**26**:2512-2521[PMID:36171471 doi:10.1007/s11605-022-05469-9].

3. Ho SY, Liu PH, Hsu CY, Huang YH, Liao JI, Su CW, Hou MC, Huo TI. Tumor burden score as a new prognostic surrogate in patients with hepatocellular carcinoma undergoing radiofrequency ablation: role of albumin-bilirubin (ALBI) grade vs easy ALBI grade. *Expert Rev Gastroenterol Hepatol*. 2022;**16**:903-911[PMID:35999514 doi:10.1080/17474124.2022.2117156].

4. Sun X, Mei J, Lin W, Yang Z, Peng W, Chen J, Zhang Y, Xu L, Chen M. Reductions in AFP and PIVKA-II can predict the efficiency of anti-PD-1 immunotherapy in HCC patients. *BMC Cancer*. 2021;**21**:775[PMID:34218801 doi:10.1186/s12885-021-08428-w].

5. Hayakawa Y, Tsuchiya K, Kurosaki M, Yasui Y, Kaneko S, Tanaka Y, Ishido S, Inada K, Kirino S, Yamashita K, Nobusawa T, Matsumoto H, Kakegawa T, Higuchi M, Takaura K, Tanaka S, Maeyashiki C, Tamaki N, Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Okamoto R, Izumi N. Early experience of atezolizumab plus bevacizumab therapy in Japanese patients with unresectable hepatocellular carcinoma in real-world practice. *Invest New Drugs*. 2022;**40**:392-402[PMID:34586531 doi:10.1007/s10637-021-01185-4].

6. Mukund A, Bhardwaj K, Choudhury A, Sarin SK. Survival and Outcome

in Patients Receiving Drug-Eluting Beads Transarterial Chemoembolization for Large Hepatocellular Carcinoma (>5 cm). *J Clin Exp Hepatol*. 2021;**11**:674-681[PMID:34866846 doi:10.1016/j.jceh.2021.02.003].

7. Wang Y, Zhou S, Yang F, Qi X, Wang X, Guan X, Shen C, Duma N, Vera Aguilera J, Chintakuntlawar A, Price KA, Molina JR, Pagliaro LC, Halfdanarson TR, Grothey A, Markovic SN, Nowakowski GS, Ansell SM, Wang ML. Treatment-Related Adverse Events of PD-1 and PD-L1 Inhibitors in Clinical Trials: A Systematic Review and Meta-analysis. *JAMA Oncol*. 2019;**5**:1008-1019[PMID:31021376 doi:10.1001/jamaoncol.2019.0393].

8. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, Lao CD, Wagstaff J, Schadendorf D, Ferrucci PF, Smylie M, Dummer R, Hill A, Hogg D, Haanen J, Carlino MS, Bechter O, Maio M, Marquez-Rodas I, Guidoboni M, McArthur G, Lebbé C, Ascierto PA, Long GV, Cebon J, Sosman J, Postow MA, Callahan MK, Walker D, Rollin L, Bhore R, Hodi FS, Larkin J. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2017;**377**:1345-1356[PMID:28889792 doi:10.1056/NEJMoa1709684].

9. Kassi E, Angelousi A, Asonitis N, Diamantopoulos P, Anastasopoulou A, Papaxoinis G, Kokkinos M, Giovanopoulos I, Kyriakakis G, Petychaki F, Savelli A, Benopoulou O, Gogas H. Endocrine-related adverse events associated with immune-checkpoint inhibitors in patients with melanoma. *Cancer Med*. 2019;**8**:6585-6594[PMID:31518074 doi:10.1002/cam4.2533].

10. Tan MH, Iyengar R, Mizokami-Stout K, Yentz S, MacEachern MP, Shen LY, Redman B, Gianchandani R. Spectrum of immune checkpoint inhibitors-induced endocrinopathies in cancer patients: a scoping review of case reports. *Clin Diabetes Endocrinol*. 2019;**5**:1[PMID:30693099 doi:10.1186/s40842-018-0073-4].

11. Grangeon M, Tomasini P, Chaleat S, Jeanson A, Souquet-Bressand M, Khobta N, Bermudez J, Trigui Y, Greillier L, Blanchon M, Boucekine M, Mascaux C, Barlesi F. Association Between Immune-related Adverse Events

and Efficacy of Immune Checkpoint Inhibitors in Non-small-cell Lung Cancer. *Clin Lung Cancer*. 2019;**20**:201-207[PMID:30442524 doi:10.1016/j.cllc.2018.10.002].

12. Sato K, Akamatsu H, Murakami E, Sasaki S, Kanai K, Hayata A, Tokudome N, Akamatsu K, Koh Y, Ueda H, Nakanishi M, Yamamoto N. Correlation between immune-related adverse events and efficacy in non-small cell lung cancer treated with nivolumab. *Lung Cancer*. 2018;**115**:71-74[PMID:29290265 doi:10.1016/j.lungcan.2017.11.019].

13. Ricciuti B, Genova C, De Giglio A, Bassanelli M, Dal Bello MG, Metro G, Brambilla M, Baglivo S, Grossi F, Chiari R. Impact of immune-related adverse events on survival in patients with advanced non-small cell lung cancer treated with nivolumab: long-term outcomes from a multi-institutional analysis. *J Cancer Res Clin Oncol*. 2019;**145**:479-485[PMID:30506406 doi:10.1007/s00432-018-2805-3].

14. Das S, Johnson DB. Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors. *J Immunother Cancer*. 2019;**7**:306[PMID:31730012 doi:10.1186/s40425-019-0805-8].

15. Gomes-Lima CJ, Kwagyan J, King F, Fernandez SJ, Burman KD, Veytsman I. A comprehensive meta-analysis of endocrine immune-related adverse events of immune checkpoint inhibitors and outcomes in head and neck cancer and lung cancer. *Journal of Clinical Oncology*. 2019;**37**:e14096-e14096 doi:10.1200/JCO.2019.37.15_suppl.e14096].

16. Quach HT, Dewan AK, Davis EJ, Ancell KK, Fan R, Ye F, Johnson DB. Association of Anti-Programmed Cell Death 1 Cutaneous Toxic Effects With Outcomes in Patients With Advanced Melanoma. *JAMA Oncol*. 2019;**5**:906-908[PMID:30998826 doi:10.1001/jamaoncol.2019.0046].