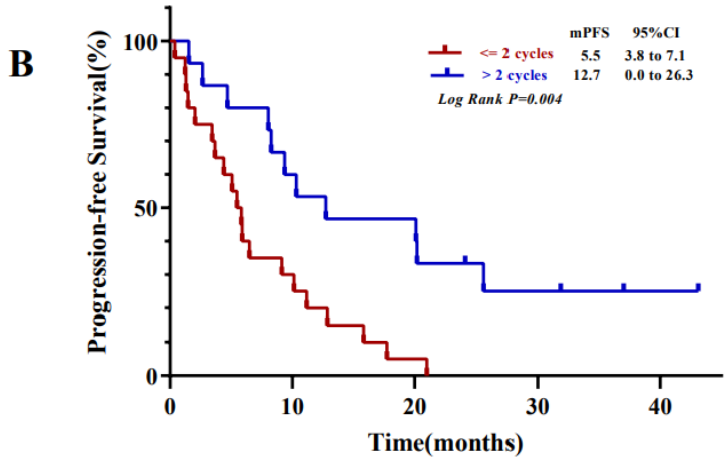
Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-23-1684

Reviewer A

General comments
The authors well summarized clinical features of ir- hepatitis in patients with advanced NSCLC. I have some questions.
Minor comments

1. Why the patients with early ir- hepatitis had poor prognosis. Are the mechanisms of ir- hepatitis different between early and late ir- hepatitis? Please explain the reason of difference of outcome between early and late ir- hepatitis because your speculation is okay.

Reply: Thank you for your kind comment. According to previous studies, we have provided a preliminary summary of the underlying mechanisms of ir-hepatitis: 1) the direct cytotoxicity of the administered antibodies via complement activation. 2) the adhesion of activated T cells to hepatic sinusoids, and apoptosis of T cells and hepatocytes. 3) ICI-induced hepatotoxicity may derive from either a hepatocellular or a cholestatic injury pattern, and so on. All of these above are mentioned in the discussion section of the manuscript. Immune activation in the immunotherapy targets not only tumor-specific T cells but also patients` cells, and the activation against hepatocytes leading to a T-cell mediated hepatitis and hepatocyte death. However, the exact mechanisms of ir-hepatitis are not clear yet, including the difference of early and late ir-hepatitis. Which still required more basic research. In the retrospective, we concluded that the early ir-hepatitis had poorer PFS significantly. In another retrospective study in our team of ir-myocarditis, we also found the earlier occurrence of myocarditis, worse PFS prognosis. From the spective from us, the reasons may as follows: Firstly, the early ir-hepatitis may loss the tolerance of hepatocytes early and activate the excessive cytokine secretion, leading to the different tumor micro environment. Secondly, the early ir-hepatitis required larger doses of steroid hormones, even immunosuppressant, which may influence the efficacy of immunotherapy. Finally, some severe and early ir-hepatitis may induce to the drug withdrawal and death, preventing the further response of immunotherapy. Our team also have carried out related basic studies to support the conclusions of early and late ir-hepatitis from the mechanism perspective. Looking forward to your further attention.



2. Line 264-265

those with earlier ir-hepatitis, developing within two treatment cycles, had prolonged survival.

Is this true? Please confirm.

Reply: Thank you for your carefulness. Due to carelessness, we have mistaken the expression in Line 264-265. And the right conclusion is that those with earlier ir-hepatitis, developing within two treatment cycles, had poorer survival, which was presented in the figure 3B. The possible reasons we have listed in the manuscript and above response. And we have revised this in the manuscript with red font ***(Page 10, Line 6)***. Thank you again for your attention.

Reviewer B

Abstract

Line 28-30 – what is the value of mentioning that AST, ALT, and Bili were significantly different with different grades of hepatitis.

Reply: Thank you for your professional review. The grades of ir-hepatitis were assessed by the serum AST or ALT. Therefore, we have revised the subscription in the abstract ***(Page 1 Line 27-28; Page 1 Line 36-37)***. Thank you again for your attention.

Line 33-35 – Is this grade 1+2 versus 3+4, this needs to be clarified.

Reply: Thank you for your valuable advice. We have added the clarified expression in the part of abstract with red font ***(Page 24, Line 25)***. Thank you again for your attention.

Line 37 – for worse PFS

Reply: Thank you for your comments. We have reviewed the expression with red font ***(Page 1, Line 38)***.

Line 38-39: “The peak median values of AST and ALT in patients with AEs provide a reference for the classification of ir-hepatitis grade” how is this helpful? This simply reflects the CTCAE grading system, it is not a conclusion.

Reply: We sincerely appreciated your review. Assuredly, the ir-hepatitis was graded referring to the CTCAE grading system, which according to the peak serum of ALT or AST. Thus, we have revised the expression in the manuscript in the light of your guide

(Page 1 Line 36-37). Thank you again!

Results: interesting the onset was median 1.6 months, 2 cycles – earlier than some other irAEs.
5 of 35 having pneumonitis as well is a high rate of co-occurrence, and interesting.
167-169: Not sure what you are reporting or its significance.
Reply: Thank you for your professional review. We have revised the expression in the manuscript with red font ***(Page 6, Line 5-9)***. Thank you again for your attention.
Discussion line 264/265 – it mentions that early hepatitis is associated with improved survival, while the results section says late hepatitis is associated with improved survival – this must be clarified.
Reply: We appreciate your carefulness. It was our mistake that caused the clerical error. And the conclusion could be drawn that the early ir-hepatitis is associated with poorer survival. We have revised the according connect in the discussion ***(Page 8, Line 13)***. Thank you again.
Conclusion
Line 284 – ALS typo.
Line 286 – Longer PFS in early hepatitis or late?
Line 289 - Hepatitis timing (development within two treatment
289 cycles) and higher severity grades were risk factors associated with PFS. This is again confusing – higher grade was a risk for reduced PFS – so if early hepatitis is associated with prolonged survival – it should not be listed this way.
The last sentence of the conclusion should be removed.
Reply: We sincerely appreciated your carefulness and profession. Due to our carelessness, we have made a series of clerical errors in the conclusion ***(Page 10, Line 6-10)***. Thank you again for your patience and apologize for any inconvenience. Thank you again for your attention.

Overall –
There is limited knowledge of irHepatitis in NSCLC, so this is a valued report – but limited by the small sample size with heterogeneity from treatment being given in different lines of therapy.

If late hepatitis is associated with longer PFS (needs clarifying in the paper) then there is also the issue that patients getting late hepatitis may have a longer PFS simply because they are still on therapy later. A patient getting irHepatitis after 5 months on therapy is biased because their PFS is > 5 months compared to someone getting it in the first month of therapy. The only way to deal with this would be to look at colitis or some other irAE and compare those who develop ‘late’ colitis with ‘late’ hepatitis. Which is beyond the scope of this paper.
Reply: Thank you for your valuable question. Firstly, we have corrected the error in the discussion and conclusion. The late hepatitis is associated with longer PFS exactly. One of the reasons indeed is the maintaining immunotherapy. However, the serve ir-hepatitis would lead to drug withdrawal and death, which did influence the efficacy of

immunotherapy. Besides, the occurrence of ir-hepatitis could change the tumor microenvironment and hepatocyte status. Then, in aspect of second question, ICIs can remain on the surface of T cells for a long time after discontinuation. A previous report (Delayed immune-related neutropenia with hepatitis by pembrolizumab) by Nakako S. et al mentioned 82% of irAE develop within 90 days after the final administration of ICIs. However, some patients show delayed onset after discontinuation, and those occurring more than 90 days are called delayed irAE. Therefore, the study involving the patients getting ir-hepatitis after 5 months on therapy, could reflect the real-world conditions better. Besides, our study mainly considered the comparison of PFS in different situations (the time of ir-hepatitis occurrence, severity, types of liver injury and whether with other irAEs), and the patient did not have an impact on our results. Therefore, we advocate that the patient still be included in the study and the data analysis. Thank you again for your professional review.

This manuscript is weakened by the fact that authors did not look at OS, although duration of follow-up would have limited the assessment, it should be examined if dates of death are accessible.
Reply: Thank you for your review. A total of 35 patients were enrolled in the study. However, util the last follow-up date, there were 15 patients still alive and 2 patients lost the specific death date. Therefore, the OS in our study was not reached, which indicated the favorable efficacy of immunotherapy in those with ir-hepatitis. We will complete the final results in the upcoming articles in this series. Thank you again.

The manuscript needs the issue timing of hepatitis and its influence on PFS addressed before an assessment can be made on whether it is suitable for publication.