Peer Review File

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Reviewer A Comments to the Author

This manuscript by Nakahama et. al. entitled "Adding SP142 to 22C3 Improves the Predictive Accuracy of Response to Immune Checkpoint Inhibitors in the Evaluation of PD-L1 Expression in Non-Small-Cell Lung Cancer" aims to evaluate whether SP142 plus 22C3 adds value for predicting response to ICI in lung cancer. Specifically, the author concluded SP142 added to 22C3 can improve the predictive accuracy of a response to ICI therapy. Generally, combined IHC staining results for immunotherapy prediction is a novel concept. However, it still should be considered about the clinical practice in the real world. Based on the study result, several suggestions and comments for the author:

1.) The most critical issue of this study is the regimen of testing. Actually, Figure 5A and Figure 5D are the most important and impressive results of this study. However, this result was highly based on 22C3 (C) staining. Additional SP142 results with TC0 or IC0 can rule out patients that may have no benefit from immunotherapy. However, even patients with SP142 positive results still had no significant benefit if their 22C3 (C) <1%. In contract, how about PFS and OS analysis compared by stratifying the 22C3 (C) results at each SP142 level? The author should analyze this comparison in parallel along with Figure 5 instead of Supplemental Figure 6. In conclusion, "adding SP142 to 22C3 improves the predictive accuracy of response to ICI…." may be a bit overly optimistic and must be met under certain conditions. It should be considered to revise the title to more fit the findings and results of the study. The author should carefully address this issue.

Response: We thank you for this thoughtful comment, which we agree with. In accordance with your suggestion, we have added the proposed analysis as Supplemental Figure 3 along with Figure 5.
(Page 14 line 240–243, Supplemental Figure 3)
Additionally, as you pointed out, the title and the conclusions were inappropriate; thus, we have changed the wording to align with the results.
(Page 1, line 2–6; Page 4, line 61–63; Page 16, line 283–288; Page 20, line 351–355)

2.) There exists a large inconsistent between 22C3 (C) and 22C3 (TMA) (Table 1). There were 108 PD-L1≧50% by 22C3 (C) while only 50 by 22C3 (TMA). There were 47 PD-L1<1 by 22C3 while 163 by 22C3 (TMA). This may lead to misinterpret the prediction of ORR by 22C3 (Figure 4 vs. Supplemental Figure 1). I think this is an important issue that the author should carefully address.

Response: We appreciate the comment, which we agree with. Following your suggestion, we have removed the Supplemental Figures about ORR, PFS and OS evaluated by 22C3 (TMA). Additionally, we have described the issue of evaluation by TMA in the limitations section.

(Page 12, line 209–212; Page 12, line 220–Page13, line 224; Page 13, line 227–236; Page 14, line 252–Page15, line 263; Page 16 line 272–274; Page 18, line 311–312; Page19, line 323–334; Supplemental Table 2)

3.) Some references were out of date such as #1. The author should cite the most recent studies precisely. For example, there did not need 6 references to support a general information of lung cancer.

Response: We thank you for your important comment. We have updated the references in the latest version and removed some of the outdated references.
(Page 4, line 69, 71; Page 5, line 73, 76, 80, 82, 87, 90; Page 6, line 94, 99, 106, 108; Page 7, line 111; Page 9, line 155, 166; Page 17, line 290, 299; Page 18, line 307, 311; Page 19, line 331; Page 20, line 344, 345; Page 22 line 386– Page 25, line 500)

4.) In the mention of Table 1, it is difficult to follow due to grid lines and background gray color. I suggest the background gray color may be differentiated according to characteristics.

Response: Thank you for your suggestion. We have changed Table 1 to make it easier to read.
(Table 1)

5.) I suggest that p-value of Kaplan-Meier survival curve should be labeled along with each curve of figure including supplemental figures.

Response: Thank you for pointing this out. We have added the p value to the figure, which did not previously show the p value.
(Supplemental Figure 1)

6.) The result of 22C3 (TMA) may be questionable when compared with 22C3 (C) in this study. Is it possible to remove results of TMA?

Response: Thank you for your important suggestion. We have only shown the positivity rate and concordance with SP142 for 22C3 (TMA) and have removed all figures regarding ORR, PFS, and OS evaluated by 22C3 (TMA).
(Page 12, line 209–212; Page 12, line 220–Page 13, line 224; Page 13, line 227–236; Page 15, line 253–263; Page 16, line 272–274)

Reviewer B Comments to the Author

All of the results and conclusions of this study depend on the information about which IHC assays were employed in this study. Was the staining done by FDA-approved kits VENTANA PD-L1 (SP142) Assay and DAKO IHC 22C3 pharmDx or by laboratory developed assays (LDTs). If LDTs were used, how were they validated?

If this is not known, all results are irrelevant. Please provide this information in order to enable proper review of this manuscript.

Response: Thank you for your important comments. The staining was performed with the FDA-approved kits VENTANA PD-L1 (SP142) Assay and DAKO IHC 22C3 pharmDx, not LDT. We have described this approach in the Methods section to make it easier to understand.
(Page 9, line 150–153)