Peer Review File

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Reviewer A

I recommend it for publication. First, the findings listed in the article are important to inform the medical community about. Secondly, the article is written in clear and precise language, that is easy to understand. Third, the sample size is significant enough to trust the information presented. Overall, great article. Great job.

[Response to reviewer A]
We wish to express our appreciation to reviewer A. Thank you for highly rating our manuscript.

Reviewer B

This is a retrospective work on a cohort of 129 consecutive patients with advanced non squamous non-small-cell lung cancer (NS-NSCLC) treated with ICI monotherapy or ICI plus chemotherapy.
The aim of the work is of interest, nevertheless, this study has several biases :
- TTF-1 negative patients have a spontaneous worse prognosis, which has been underlined in previous works in primary tumors and metastatic tumors

Reply 1: TTF-1 negative status has been reported as a poor prognostic and poor predictive for therapeutic efficacy. The treatment efficacy of ICI plus chemotherapy for TTF-1 negative NS-NSCLC is unclear and several prospective studies are ongoing. Our study is retrospective and contains some limitations as you mentioned. However, this is the first study showing non-inferior PFS and OS in TTF-1 negative versus TTF-1 positive patients. We believe this result is worth reporting. We mentioned that TTF-1 negative is an indicator of poor prognosis in the Introduction section as follows (see page 6, line 86-88).

Changes in the text: "Before ICIs were widely used, TTF-1-negative status was reported as a poor prognosis factor in both the early and advanced stages of NS-NSCLC (10)."

10. Kim JH, Kim HS, Kim BJ, et al. Prognostic Impact of TTF-1 Expression in Non-Squamous Non-Small-Cell Lung Cancer: A Meta-Analysis. J Cancer 2018;9:4279-86.

- The presentation of the results are difficult to read, especially the kaplan meier curves.
Reply 2: We have revised our figures, and have paid particular attention to the Kaplan Meier curves.

- The results for TTF-1 should be stratified according to PD-L1 expression.

Reply 3: We analyzed PFS and OS associated with ICI monotherapy and ICI + chemotherapy according to both TTF-1 and PD-L1 expression status. In ICI monotherapy, the high PD-L1 expression subgroup (PD-L1 ≥50%), had worse PFS if they were TTF-1-negative rather than TTF-1-positive. However, no statistical differences were observed in the subgroup of ICI + chemotherapy divided by PD-L1 expression. These results strengthen our conclusion that ICI plus chemotherapy is favored for TTF-1-negative patients. We have added the results in the revised manuscript as follows (see page 11, line 190-199).

Changes in the text: "*Treatment efficacy by TTF-1 expression according to PD-L1 expression status*

The relationship between TTF-1 expression status and treatment efficacy according to PD-L1 expression status is shown in Figs 5 and 6. In the ICI monotherapy group excluding EGFR and ALK mutations/translocations with PD-L1 >50% expression, TTF-1-negative patients had statistically worse PFS compared with TTF-1-positive patients (median PFS 2.1 vs. 9.4 months, P = 0.019). There were no significant differences in OS (median OS 2.6 vs. 24.7 months, P = 0.27), but mortality in the early stage of initiating treatment tended to be higher in the TTF-1-negative group. In the ICI + chemotherapy group, TTF-1-negative patients tended to have better PFS and OS than TTF-1-positive patients in the PD-L1 <1% expression group (median PFS 22.5 vs. 8.0 months, P = 0.056, median OS 32.3 vs. 16.1 months, P = 0.15)."

We added manuscripts in Discussion section as follows (see page 12, line 220-223).

"Especially in the PD-L1≥50% group, TTF-1 negative patients had significantly worse PFS than positive patients. PD-L1 high expression state is generally considered to have a favorable therapeutic efficacy of ICI. Even with high PD-L1 expression, TTF-1-negative patients may not respond to ICI monotherapy as well as TTF-1-positive patients."

- The methods for EGFR, ALK status are not describe

Reply 4: Thank you for your insightful comments. In our facility, we examined lung cancer mutation status using PCR for EGFR mutation, FISH and IHC for ALK and/or the next-generation sequencing Oncomine™ DX target test. The specific choice depended on the amount of tumor sample available to us. This information is described in the Method section as follows (see page 7, line 107-111).

Changes in the text: "Oncogenic drivers including EGFR and ALK were examined using the Cobas@ EGFR mutation test v2 (Roche, Basel, Switzerland) and FISH (Abbott Molecular, Des Plaines, IL) or IHC (Roche, Basel, Switzerland) for ALK, and/or next-generation sequencing via the Oncomine™ DX target test (Thermo Fisher, Waltham, MA). The decision of which test to use was made at the physician's discretion."

Furthermore, in accordance with the remarks of reviewer D, we described the limitation sections as follows (see page 15, line 270-276).

"A second limitation of our study was our inability to determine SRGN expression or the mutational status of *KEAP1/STK11* and other druggable mutations (with the exception of EGFR and ALK). However, neither SRGN expression nor *KEAP1/STK11* mutation routinely screened for in clinical practice, and the status of these mutations is difficult to obtain in all cases. In addition, the status of druggable mutations is not available in all cases, as it is highly dependent on the volume of tumor specimens and the clinical course."

-Tumor grade, especially for adenocarcinoma, are not given

Reply 5: We apologize that we were unable to provide this information. Since specimens are collected differently for each patient (from surgical resection, bronchoscopy, or cell blocks of pleural effusion), presenting a complete dataset on tumor grade is extremely challenging.

-The use of SPT24 antibody for TTF-1 is not recommended as far this antibody is more sensitive than 8G7/G4 clone, but is less specific and stains some squamous cell carcinomas.

Reply 6: Thank you for your insightful comments. As you mentioned, SPT24 was reported to be less specific compared with 8G7G3. However, the discuss the TTF-1 antibody in the 'limitations' section as follows (see page 15, line 276-280).

Changes in text: "The third limitation relates to the examination for TTF-1. Our institution has been using SPT24 antibody for examining tumor TTF-1 status. However, false positive signals with the SPT24 antibody have been reported in squamous cell carcinoma, and the 8G7G3 antibody has better specificity (35). Although we did not diagnose squamous cell carcinoma solely based on TTF-1 expression status, the use of different TTF-1 antibodies could impact the interpretation of our results."

35. Vidarsdottir H, Tran L, Nodin B, et al. Comparison of Three Different TTF-1 Clones in Resected Primary Lung Cancer and Epithelial Pulmonary Metastases. Am J Clin Pathol 2018;150:533-44.

- Methods section should be expanded and more precisely described.

Reply 6: We have expanded our descriptions in the Methods section as noted in Reply 4.

Reviewer C

Dr. Iso and colleagues conducted retrospective analysis of 129 patients with NS-NSCLC treated with ICI monotherapy or ICI plus chemotherapy according to TTF-1 expression. Authors found that ICI plus chemotherapy, especially ABCP is beneficial for NS-NSCLC with TTF-1 negative

cases. TTF-1 negative status in lung adenocarcinoma tends to be associated with poor prognostic, but also poor efficacy of chemotherapy or ICI monotherapy. The study focusing on the appropriate treatment for TTF-1 negative NS-NSLC is extremely important for clinical practice, and authors were able to show clear evidence to support ABCP treatment in NS-NSCLC.

[Response to reviewer C]
We wish to express our appreciation to reviewer C. The comments have helped us significantly improve the paper. Our responses to the reviewer's comments are as follows:

Major comment 1
Authors concluded that clinicians should consider ICI plus chemotherapy in TTF-1 negative patients by comparison with TTF-1 positive patients. Although the comparison between TTF-1 negative and positive is very informative, to determine whether ICI plus chemotherapy is superior to ICI monotherapy in TTF-1 negative patients, the comparative study of ICI monotherapy vs. ICI plus chemotherapy in TTF-1 negative patients is necessary.
I would recommend authors to add the analysis for ICI monotherapy vs. ICI plus chemotherapy responding Fig 1 and Fig 2.

Reply 1: **Thank you for the insightful comments. However, it is difficult to simply compare ICI monotherapy and ICI + chemotherapy in TTF-1 negative cases due to the difference in PS and line of treatment in ICI monotherapy and ICI + chemo groups. We have therefore compared OS and PFS between ICI monotherapy and ICI + chemotherapy in TTF-1 negative cases; Supplemental Figure 5 contains the results of this comparison.**

Major comment 2
According to Major comment 1, I would recommend authors to edit LINE 184-186.

"On the other hand, TTF-1-negative patients treated with ICI plus chemotherapy tended to have better PFS and a similar OS when compared with TTF-1-positive patients. Thus, clinicians should consider ICI plus chemotherapy in TTF-1-negative patients."

Reply 2: Thank you for your insightful comments. We revised the text as you suggested (see page 11-12, line 203-208).

Changes in the text: "Additionally, long-term efficacy (>2 years) and therapeutic efficacy for high PD-L1 expression of ICI monotherapy were not observed in TTF-1-negative patients. On the other hand, TTF-1-negative patients treated with ICI plus chemotherapy tended to have better PFS and a similar OS when compared with TTF-1-positive patients. As a side note that ICI plus chemotherapy showed better PFS and OS compared with ICI monotherapy in TTF-1-negative patients (*Supplemental Fig 5*)."

Minor comment 1
I would recommend adding the detail of treatment regimen (e.g., dose, cycle etc...) if possible.

Reply 3: Thank you for your insightful comments. We have added the detailed regimen information in the Results section (see page 9, line 145-156).

Changes in the text: "We separated the ICI monotherapy group across three main regimens: pembrolizumab (200 mg/body every 3 weeks), nivolumab (240 mg/body every 2 weeks or 480mg/body every 4 weeks), and atezolizumab (1200 mg/body every 3 weeks); the frequency at which these regimens were used was similar between the TTF-1-negative and -positive groups (*Table 2*). The ICI plus chemotherapy group was also separated according to three main regimens: atezolizumab (1200 mg/body) plus bevacizumab (15 mg/kg) plus carboplatin (area under the curve [AUC] 5) plus paclitaxel (175 mg/m^2) (ABCP), atezolizumab (1200 mg/body) or pembrolizumab (200 mg/body) plus carboplatin (AUC 6) plus nab-paclitaxel (100 mg/m^2), and pembrolizumab (200 mg/body) plus platinum (CDDP [75 mg/m^2] or CBDCA [AUC5]) plus pemetrexed (500 mg/m^2) every 3-4 weeks up to 4 cycles. After four cycles, the following maintenance therapies were administered. The attending physician made appropriate decisions regarding the dose reduction of chemotherapy according to the patient's condition. The frequency with which these regimens was chosen did not differ between groups (*Table 3*)."

Reviewer D

This is an informative and well written article. As far as I was able to check, there is not much and only inconsistent data about the association of TTF1 expression and efficacy of immunotherapy in literature. This retrospective analysis adds some relevant further information to this topic. However, the results from this study can only be interpreted carefully due to several limitations such as i) the retrospective design of the study ii) the limited number of patients examined iii) the heterogeneity of the patient groups (stage III, IV and recurrent). The very heterogeneous cohort is problematic, so that it is difficult to draw consistent conclusions here. I would ask the authors to make some minor corrections.

[Response to reviewer D]
We wish to express our appreciation to reviewer D. The comments have helped us significantly improve the paper. Our responses to the reviewer's comments are as follows:

1. Besides EGFR and ALK, have other driver mutations been examined and excluded in this analysis? The authors should make a short statement addressing this topic.

Reply 1: Thank you for your insightful comments. In our facility, we examined lung cancer mutation status using PCR for EGFR mutation, FISH or IHC for ALK, and/or next-generation sequencing Oncomine^{TM} DX target test. The specific choice depended on the amount of tumor sample available to us. This information is described in the Method section as follows (see page 7, line 107-111).

Changes in the text: "Oncogenic drivers including EGFR and ALK were examined using the Cobas@ EGFR mutation test v2 (Roche, Basel, Switzerland) and FISH (Abbott Molecular, Des Plaines, IL) or IHC (Roche, Basel, Switzerland) for ALK, and/or next-generation sequencing via the Oncomine™ DX target test (Thermo Fisher, Waltham, MA). The decision of which test to use was made at the physician's discretion."

In addition, we have discussed this in the 'limitations' section as follows (see page 15, line 270-276).

"A second limitation of our study was our inability to determine SRGN expression or the mutational status of *KEAP1/STK11* and other druggable mutations (with the exception of EGFR and ALK). However, neither SRGN expression nor *KEAP1/STK11* mutation routinely screened for in clinical practice, and the status of these mutations is difficult to obtain in all cases. In addition, the status of druggable mutations is not available in all cases, as it is highly dependent on the volume of tumor specimens and the clinical course."

2. For those patients with recurrent NSCLC, it would be interesting to know, if they have been treated with immunotherapy in the first-line setting or not. If so, this could be an explanation for less effectiveness of immunotherapy in those patients. The authors should add a table with first-line regimens in the cohort of recurrent NSCLC.

Reply 2: Thank you for your insightful comments. Our study included only patients receiving ICI-containing regimens for the first time. Patients received durvalumab maintenance therapy after chemoradiotherapy or adjuvant atezolizumab therapy were not included in this study.

3. Moreover, the authors should give information on patient selection criteria for the ACBP regimen, as this is not a widely used standard first-line regimen in NS-NSCLC patients. The number of patients that was treated with the ACBP regimen in this analysis appears to be relatively high. However, the authors nicely discuss possible explanations why this regimen appears to be more effective in the TTF-1-negative cohort.

Reply 3: Thank you for your insightful comments. Our institution primarily uses ABCP regimen in patients after EGFR-TKI failure, in patients with massive pleural effusions and/or multiple liver metastases, and in patients with rapidly progressing tumors. There is no deviation from the general indications. We noted this in the Discussion section as follows (see page 14, line 260-262).

Changes in the text: "Our institution used ABCP after EGFR or ALK-TKI failure, in patients with massive pleural effusions, multiple liver metastases, and/or rapidly progressing tumors. ABCP therapy may also be promising for TTF-1-negative NS-NSCLC patients."

4. Finally, the authors conclude that ICI monotherapy was not efficacious for patients with TTF-1-negative NS-NSCLC and that clinicians should consider ICI plus chemotherapy for those patients. This is consistent with already published data (Nakahama et al., 2022, Thorac Cancer, doi: 10.1111/1759-7714.14560). The authors already mention that a higher proportion of TTF-1-negative patients are PD-L1 negative whereas a higher proportion of TTF-1-positive patients are PD-L1 positive, although no significant correlation was observed. To make this statement more robust, the authors could also compare ICI monotherapy in TTF-1-negative vs. TTF-1-positive patients for each subgroup according to PD-L1 status (<1%, 1-49%, >50%). Please add this analysis to this article.

Reply 4: We analyzed PFS and OS of ICI monotherapy and ICI + chemotherapy according to the TTF-1 expression and PD-L1 expression status. In ICI monotherapy, the subgroup with high PD-L1 expression (PD-L1>50%) and TTF-1-negative status had worse PFS compared with TTF-1-positive patients. However, no statistical differences were observed between patients in the ICI + chemotherapy subgroup when they were divided based on PD-L1 expression score. These results strengthen our conclusion that ICI plus chemotherapy is favored for TTF-1-negative patients. We added the results as follows (see page 11, line 190-199).

Changes in the text:
"Treatment efficacy by TTF-1 expression according to PD-L1 expression status
The relationship between TTF-1 expression status and treatment efficacy according to PD-L1 expression status is shown in Figs 5 and 6. In the ICI monotherapy group excluding EGFR and ALK mutations/translocations with PD-L1 >50% expression, TTF-1-negative patients had statistically worse PFS compared with TTF-1-positive patients (median PFS 2.1 vs. 9.4 months, P = 0.019). There were no significant differences in OS (median OS 2.6 vs. 24.7 months, P = 0.27), but mortality in the early stage of initiating treatment tended to be higher in the TTF-1-negative group. In the ICI + chemotherapy group, TTF-1-negative patients tended to have better PFS and OS than TTF-1-positive patients in the PD-L1 <1% expression group (median PFS 22.5 vs. 8.0 months, P = 0.056, median OS 32.3 vs. 16.1 months, P = 0.15)."

We added statements to the Discussion section as follows (see page 12, line 219-223).
"Especially in the PD-L1≥50% group, TTF-1 negative patients had significantly worse PFS than positive patients. PD-L1 high expression state is generally considered to have a favorable therapeutic efficacy of ICI. Even with high PD-L1 expression, TTF-1-negative patients may not respond to ICI monotherapy as well as TTF-1-positive patients."