Peer Review File

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Response to Reviewer A

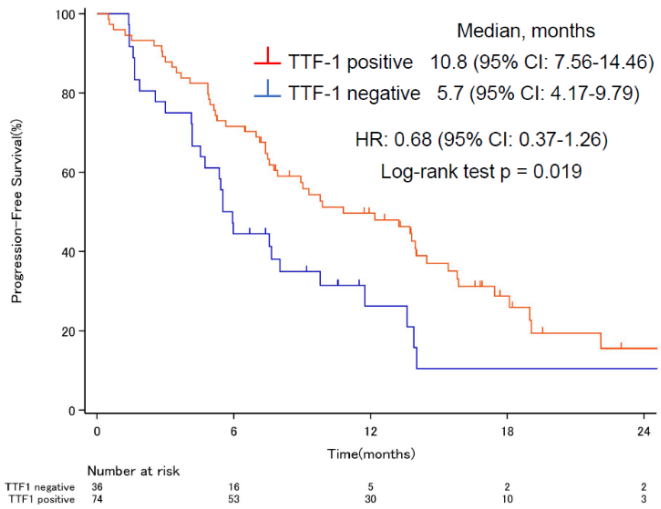
We thank the reviewer for the helpful comments on our manuscript, our specific responses to which are as follows:

1. Results: The authors should perform an additional PFS analysis strictly for cases classified as adenocarcinoma (n=110 total, 74 vs. 36) because a higher proportion of TTF1-negative cases are classified as "other". This would help rule out the possibility that the difference in PFS is strongly influenced by the presence of non-adenocarcinoma cases in the TTF1-negative cohort.

Response: As suggested, we performed an additional analysis specifically for patients with an adenocarcinoma histology (n = 110 total, 74 TTF-1 positive vs. 36 TTF-1 negative). As is now shown in the new **Supplemental Figure 2**, TTF-1–positive patients had a longer PFS compared with TTF-1–negative patients (median of 10.8 versus 5.7 months; HR of 0.68, with a 95% CI of 0.37–1.26; log-rank test p = 0.019). We have now addressed this finding in the Results (p. 11, lines 192–195) and Discussion (p. 12, lines 212– 216) sections of the revised manuscript.

Changes in the text:

Supplemental Figure 2



Results section: We also analyzed PFS specifically for patients with an adenocarcinoma histology (**Supplemental Figure 2**). This analysis also revealed that TTF-1–positive patients had a longer PFS compared with TTF-1–negative patients (median of 10.8 versus 5.7 months; HR of 0.68, with a 95% CI of 0.37–1.26; log-rank test p = 0.019).

Discussion section: A higher proportion of TTF1–negative cases than of TTF-1–positive cases was histologically classified as other than adenocarcinoma. Given that this difference might have influenced our results, we performed an additional analysis of PFS for only patients with adenocarcinoma. Even this analysis limited to adenocarcinoma, however, revealed that TTF-1 negativity was associated with a shorter PFS.

2. Discussion: The manuscript would benefit from citation of doi: 10.15252/emmm.201606711 (Guo et al, "Gene signature driving invasive mucinous adenocarcinoma of the lung"), which shows that TTF1 can activate PDL1 expression in vitro, and discussion of the implications of this regulation for the current manuscript.

Response: As suggested, we have now cited this study in the Discussion section of the revised manuscript (p. 13, lines 228–230).

Changes in the text:

Our results are consistent with recent basic research findings that TTF-1 influences the immune status of tumors, with a study of lung adenocarcinoma showing that TTF-1 is able to activate PD-L1 expression in vitro [18].

Reference: Guo M, Tomoshige K, Meister M, et al. Gene signature driving invasive mucinous adenocarcinoma of the lung. *EMBO Mol Med*. 2017; 9: 462-481.

3. Introduction: "Club cell" is now the preferred term over "Clara cell" (DOI: 10.1183/09031936.00146609).

Response: We have now changed "Clara cells" to "Club cells" in the Introduction section of the revised manuscript (p. 5, line 90).

Response to Reviewer B

We thank the reviewer for the helpful comments on our manuscript, our specific responses to which are as follows:

1. In general: The number of patients included is relatively small (n=122) and FU is short (14.6 mo.).

Response: We have now addressed this comment as a study limitation in the Discussion section of the revised manuscript (p. 15, lines 269–271).

Changes in the text:

Third, the number of patients included in the study was relatively small and the observation period was relatively short, both of which limit evaluation of the impact of TTF-1 expression on OS in particular.

2. How was progression of disease assessed? Did all CT scans undergo RECIST assessment? If yes: by whom? Was confirmation of PD mandatory?

Response: Each patient was assessed for treatment response including progression of disease by CT scans on the basis of RECIST by investigators in each hospital. We have now added this information in the Methods section (p. 7, lines 116–118).

Changes in the text:

Progression of disease had to be confirmed on the basis of assessment by investigators according to Response Evaluation Criteria in Solid Tumors version 1.1.

3. Baseline characteristics: The only relevant imbalance is found within histology. With almost 25% of TTF1-neg. patients having "other" histologies, the authors should clarify this, as one might assume that TTF1-neg. NOS might be squamous cell carcinoma.

Response: As suggested by the reviewer, we have now provided a breakdown for histology other than adenocarcinoma in the new **Supplemental Table 1** and referred to this information in the Results section (p. 9, lines 160) of the revised manuscript.

Changes in the text:

Histology other than adenocarcinoma is provided in **Supplemental Table 1**.

Supplemental Table 1 Breakdown of histology other than adenocarcinoma

Histology	TTF-1 positive (n = 1)	TTF-1 negative (n = 11)
NSCC, NOS	0	6
NSCC, favors adenocarcinoma	1	3
Large cell carcinoma, null type	0	1
Adenosquamous carcinoma	0	1

NSCC, non–small cell carcinoma; NOS, not otherwise specified.

4. Treatments: There is no clear information on the treatments used (KN189, Im130/150), this should be corrected.

Response: We have now provided details of the treatment regimens in the new **Supplemental Table 2**, which is now referred to in the Results section of the revised manuscript (p. 10, line 172-173). We also changed the sentence referring to (p. 10, line 164) and the title of Table 2, which lists the drugs administered, in order to avoid confusion.

Changes in the text:

Results section: Treatment regimens for the first-line therapy are shown in **Supplemental Table 2**.
Results section: Cancer drugs administered as first-line therapy for the study patients are shown in **Table 2**.

Supplemental Table 2 Treatment regimens for first-line therapy

	TTF-1 positive (n = 75)	TTF-1 negative (n = 47)
Treatment regimen		
Pembrolizumab + Pemetrexed + Carboplatin	32 (42.7)	20 (42.6)
Pembrolizumab + Pemetrexed + Cisplatin	18 (24.0)	11 (23.4)
Atezolizumab + Pemetrexed + Carboplatin	12 (16.0)	8 (17.0)
Atezolizumab + Pemetrexed + Carboplatin + Bevacizumab	13 (17.3)	8 (17.0)

Data are number (percent).

5. Outcome: There is no data on the efficacy of the respective treatments. Therefore, from my point of view, the only conclusion allowed is that TTF1 is prognostic.

Response: As pointed out by the reviewer, our results might reflect only the prognostic impact of TTF-1 expression, given that our study did not compare regimens. To compare PD-1/PD-L1 inhibitors plus pemetrexed and platinum chemotherapy with other regimens, we are now planning a prospective study of PD-1/PD-L1 inhibitors in combination with carboplatin plus nab-paclitaxel for patients with TTF-1–negative nonsquamous NSCLC. To avoid overstating our conclusion, we have now changed the Discussion section of the revised manuscript (p. 15, lines 261–268).

Changes in the text:

Given that our study did not compare regimens, our results might reflect only the prognostic effect of TTF-1. However, it remains possible that PD-1/PD-L1 inhibitors combined with platinum plus paclitaxel or nab-paclitaxel chemotherapy might be a better option for TTF-1–negative patients, on the basis of the suggestion that pemetrexed plus platinum chemotherapy is inferior to platinum regimens not containing pemetrexed in such patients [13]. We are planning a prospective study of PD-1/PD-L1 inhibitors in combination with carboplatin plus nab-paclitaxel for patients with TTF-1–negative nonsquamous NSCLC (jRCTs071220008).

Response to Reviewer C

We thank the reviewer for the helpful comments on our manuscript, our specific responses to which are as follows:

1. Probably worth comparing your results to the Galland et. al. paper published in OncoImmunology in May last year who have performed a similar study with similar findings.

Response: As suggested, we have now compared this previous study with our present study in the Discussion section of the revised manuscript (p. 14, lines 250–254).

Changes in the text:

A recent study also suggested that TTF-1 expression is related to PFS and OS in nonsquamous NSCLC patients treated with ICIs. However, this study differs from ours in that it included patients who received different ICIs in different lines of treatment and in that it pooled patients treated with immunotherapy alone together with those treated with a combination of ICIs plus chemotherapy [23].

Reference: Galland L, Le Page AL, Lecuelle J, et al. Prognostic value of Thyroid Transcription Factor-1 expression in lung adenocarcinoma in patients treated with anti PD-1/PD-L1. *Oncoimmunology*. 2021; 10: 1957603.

2. I'm unsure from reading your study what the overall impact on PFS is of TTF-1. If TTF-1 negativity already has negative prognostic value irrespective of treatment selected (and the strength of this association is no different irrespective of the treatment selected) then I don't think you can conclude that TTF-1 negativity can be used to guide treatment selection. Perhaps the more appropriate conclusion is that TTF-1 negative can be used to predict PFS and OS in patients treated with immune-checkpoint inhibitors.

Response: We have reexamined our conclusion and modified our text as suggested. We thus changed the Abstract (p. 4, lines 68–70) and Conclusions section (p. 16, line 277–278) of the revised manuscript.

Changes in the text:

Abstract: TTF-1 expression in advanced nonsquamous NSCLC can serve as a basis for prediction of PFS in patients treated with PD-1/PD-L1 inhibitors plus pemetrexed and platinum chemotherapy in the first-line setting.

Conclusions section: This result suggests that TTF-1 expression can serve to predict PFS in patients with advanced nonsquamous NSCLC who receive such first-line treatment.