Peer Review File

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Reviewer Comments:

Reviewer A

Comment 1: How the pleural effusion was classified as malignant is not well identified, other than looking at cytology. How many patients with cytology negative pleural effusions had pleural biopsy to confirm malignancy, as there are several other reasons which could contribute to effusion in these patients- referred to as paramalignant effusions which presumably may have better recurrence rates and prognosis.

Once this is identified and separated out, then the study may have more relevance.

Reply 1: We are grateful to the Reviewer for bringing this issue to our attention. In this article, the definition of malignant pleural effusion (MPE) is "the presence of malignant cells in pleural effusion upon cytological examination or with pleural infiltration detected by biopsy specimens. When patients were cytologically negative, researchers played judgments comprehensively via radiographic options, level of pleural tumor biomarkers, and exudates pleural fluid after testing possible reasons leading to pleural effusion". Our definition may cause several misunderstandings. So, we wanted to explain that when patients were cytologically negative and experienced biopsy specimens to ensure malignancy, researchers played judgments comprehensively via radiographic options, level of pleural tumor biomarkers, and exudates. The most important issue was to exclude other possible reasons for pleural effusion in those that were cytologically negative and experienced biopsy specimens to ensure malignancy.

Changes in the text: We deleted "When patients were cytologically negative, researchers played judgments comprehensively via radiographic options, level of pleural tumor biomarkers, and exudates pleural fluid after testing possible reasons leading to pleural effusion" in the "Definition" section to make the definition of MPE more clear.

"Definition" section (see page 5, line 107 to line 108).

Comment 2: What is meant by targeted therapy?

What are actionable mutations.

Definitions should be added within methods.

Reply 2: We are grateful to the Reviewer for bringing this issue to our attention. In our article, patients who received targeted therapy were those who received kinase inhibitors of oncogenic receptor tyrosine kinases, c-ros oncogene 1 receptor tyrosine kinase, and tropomyosin receptor kinases as well as downstream target kinases. Patients received small molecular-targeted drugs that target cancer cell-specific molecules. Actionable mutations in his article means EGFR activating mutations (EGFR Del19, L858R, T790M) or ALK rearrangement.

Changes in the text: In the “Definition” section, we added “Actionable mutations was defined as EGFR activating mutations (EGFR Del19, L858R, T790M) or ALK rearrangement. Patients who received targeted therapy were those who received kinase inhibitors of oncogenic receptor tyrosine kinases, c-ros oncogene 1 receptor tyrosine kinase, and tropomyosin receptor kinases as well as downstream target kinases.”.
“Definition” section (see page 5, line 110 to line 113).

Reviewer Comments:
Reviewer B
Comment 1: HOW MANY OF THE PATIENTS DID NOT HAVE EITHER A POSITIVE CYTOLOGY OR BIOPSY IN THE PLEURAL CAVITY? In the "Definition" section of the paper there is a mention to this point, but I could not find any precise data provided in the manuscript: A 12.3% negative cytology in 343 patients with MPE was reported in Table, which means that only 87.7% of those patients had a proven malignancy in the pleura (unless there are supplementary positive results of biopsy, not shown). To me, this information is relevant, because I do not think that patients with unproven malignancy in the pleural space should be included in this series.
Reply 1: We are grateful to the Reviewer for bringing this issue to our attention. Our definition may cause several misunderstandings. So, we wanted to explain that when patients were cytologically negative and experienced biopsy specimens to ensure malignancy, researchers played judgments comprehensively via radiographic options, level of pleural tumor biomarkers, and exudates. The most important issue was to exclude other possible reasons for pleural effusion in those that were cytologically negative and experienced biopsy specimens to ensure malignancy. Consequently, a total of 318 patients received pleural fluid cytology, among which the cytology results of 279 patients were proved to be positive and 39 patients were proved to be negative. These patients with negative cytology experienced biopsy specimens to ensure malignancy. The cytology results of the rest 25 patients were missing and they also received pleural biopsy to prove malignancy. In these patients with negative cytology or missing results, researchers exclude other possible reasons for pleural effusion.
Changes in the text: We deleted “When patients were cytologically negative, researchers played judgments comprehensively via radiographic options, level of pleural tumor biomarkers, and exudates pleural fluid after testing possible reasons leading to pleural effusion” in the “Definition” part to make the definition of MPE more clear.
“Definition” section (see page 5, line 107 to line 108).

Comment 2: ERRORS IN THE MANUSCRIPT: The quality of the manuscript is fine in general, but I found a couple of errors, as follows:
- LEGEND FOR TABLE 4: I believe that it should read "wild-type EGFR/ALK", instead of "wide--type".
- FINAL CONCLUSION IN THE MANUSCRIPT: There is an apparent contradiction

in the second line of the "Conclusion" section, where I believe it should read "more likely" than less likely, taking into account the context of the whole manuscript.

Reply 2: We are grateful to the Reviewer for bringing this issue to our attention. The errors in the manuscript have been corrected.

Changes in the text: "LEGEND FOR TABLE 4" (see page 17, line 382);
"Conclusion" section (see page 12, line 256 to line 257).

Reviewer Comments:

Reviewer C

Comment 1: The study follow up was 300 days (line 118). This is considerably shorter than the median time for cancer progression (MPE recurrence was one manifestation) described in previous literatures on lung cancer. e.g. The median PFS of Osimertinib in first line or second line settings can be up to 16-19 months (FLAURA trial). Follow up till death/censoring could be a better end point.

Reply 1: We are grateful to the Reviewer for bringing this issue to our attention. **Firstly**, the primary endpoint in the FLAURA trial was the duration of progression-free survival as determined by investigator assessments, according to RECIST, version 1.1. According to RECIST1.1, Progressive Disease (PD) was defined as "At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression)". When the patient has only non-measurable disease like pleural effusion, PD was defined as "an increase in a pleural effusion from 'trace' to 'large'". However, the recurrence of MPE could be seen as progression in cancer is not included in RECIST1.1. Consequently, it is not appropriate to evaluate the time of MPE recurrence by RECIST1.1 and the mPFS of 16-19 months in the FLAURA trial is meaningless in recurrence-free survival (RFS). **Secondly**, in our study, only 31 patients received Osimertinib or Almonertinib and 108 patients received the first-generation EGFR-TKIs. The PFS of the third-generation EGFR-TKIs is superior to the PFS of the first-generation EGFR-TKIs. It might influence the PFS and DFS in this research. **Thirdly**, 100 days was decided as the follow-up time by Schwalk et al.(1) under the background that over 50% of patients would experience recurrence within 90 days and the mPFS of those with MPE was 3-12 months. Researchers in that article believed that this was chosen as a clinically meaningful duration for the development of a recurrent, ipsilateral MPE when compared with indefinite time, given the low median survival in patients with NSCLC and MPE as well as the high expected MPE recurrence rate within 1 month of initial thoracentesis. In addition, if a patient were to have a recurrence after 100 days, this may not be considered as rapid, and management with a repeat thoracentesis would be reasonable. However, less than 40% of patients developed a recurrent MPE within 100 days in our research. **Finally**, we modulated the follow-up time according to the patient's response to targeted therapy and the time

of recurrence and survival in reality. The follow-up time of 300 days was decided and almost 60% of patients experienced MPE recurrence within 300 days.

Changes in the text: In the "Definition" section, we added "This was a meaningful duration for the recurrence of MPE according to the patients included in this study, in which over 50% of patients developed a recurrent MPE within 300 days.".

"Definition" section (see page 5, line 114 to line 115).

References:

1. Schwalk AJ, Ost DE, Saltijeral SN, et al. Risk Factors for and Time to Recurrence of Symptomatic Malignant Pleural Effusion in Patients With Metastatic Non-Small Cell Lung Cancer with EGFR or ALK Mutations. Chest 2021;159:1256-64.

Comment 2: The subjects included were at various stages of lung cancer, with or without prior cancer treatment. Those pretreated with whatsoever types of cancer treatment were likely less responding to subsequent lines of cancer treatment, and this point could lead to significant bias in RFS of MPE.

Reply 2: We are grateful to the Reviewer for bringing this issue to our attention. Whether the patient was treated before the first thoracentesis was included in the factors possibly affecting RFS of MPE and it was proved essential to the results in our univariate analysis. Furthermore, we explored the specific therapy that could influence the RFS of MPE. In the univariate analysis, chemotherapy, targeted therapy, and anti-angiogenesis therapy were proved essential. However, these factors were not included in the multivariate analysis and only receiving targeted therapy after the first thoracentesis within 30 days, lower NLR level, lower serum LDH level, and lower serum CEA level were independent protective factors. The results might be different when more patients from different hospitals were included. It has been included in the limitation.

Changes in the text: No change was made in the text.

Comment 3: The types of targeted therapy or cancer treatment were not specified. There are significant difference in anti-cancer efficacy even among the same group of target therapy. e.g. Erlotinib versus Osimertinib. Those receiving less efficacious therapy would be, as expected, prone to earlier MPE recurrence.

Reply 3: We are grateful to the Reviewer for bringing this issue to our attention. We agree that a significant difference exists in anti-cancer efficacy even among the same group of target therapy and added detailed information in the text. However, we analyzed different kinds of drugs and got negative results. There were 108 patients receiving first-generation EGFR-TKIs and 3 patients receiving second-generation EGFR-TKIs, and 31 patients receiving third-generation EGFR-TKIs. In univariate analysis, whether patients who received the first-generation EGFR-TKIs was thought of as a protective factor ($p=0.005$). However, the result of the third-generation EGFR-TKIs ($p=0.123$) was negative. Furthermore, the difference between the first-generation EGFR-TKIs and the third-generation EGFR-TKIs was not significant ($p=0.802$). Due to the lack of patients (3 patients receiving second-generation EGFR-TKIs and 31 patients receiving third-generation EGFR-TKIs), we thought that

the results might lack credibility. Consequently, we did not attach the results to the text.

Changes in the text: In the "Discussion" section, we added the limitation that "However, among the 108 patients who received the first-generation EGFR tyrosine kinase inhibitors (TKIs) and 31 patients who received the third-generation EGFR-TKIs , no significance in recurrence was found between the two groups (p=0.802). The median RFS of the two groups was both 300 days. More patients should be included and longer follow-up time should be determined to explore the difference in intrapleural effects between the different kinds of EGFR-TKIs in the future.".

"Discussion" section (see page 10, line 215 to line 220).

Comment 4: There was no clear delineation on how MPE recurrence is defined. Was it defined on the need of pleural intervention, or symptoms, or size of effusion on imaging, or a combination of these?

Reply 4: We are grateful to the Reviewer for bringing this issue to our attention. In our text, patients with recurrent MPE were regarded as those with recurrent symptomatic pleural effusion requiring the second thoracentesis to relieve symptoms and demonstrating malignancy. We want to add the explanation that patients with recurrent MPE were regarded as those experiencing the second thoracentesis and their pleural effusion was confirmed malignant.

Changes in the text: In the "Definition" part, the sentence "Patients with recurrent symptomatic pleural effusion requiring the second thoracentesis to relieve symptoms and demonstrating malignancy were regarded as those with recurrent MPE." was changed into "Patients with recurrent MPE were regarded as those experiencing the second thoracentesis and their pleural effusion was confirmed malignant.".

"Definition" section (see page 5, line 108 to line 110).

Comment 5: Overall, the study appeared to have included a heterogeneous group of patients with lung cancer with variable cancer treatment history, and control therapy for pleural effusions. The main conclusion was that cancer treatment including targeted therapy was effective in reducing recurrence, yet this is not novel.

Reply 5: We are grateful to the Reviewer for bringing this issue to our attention. Our study included 343 patients, of which 34.3% owned actionable mutations. Firstly, relatively comprehensive information was collected in our study, including baseline information, information about pleural effusion, the specific active treatment before and after the first thoracentesis, Hematic biomarkers, and Pleural biomarkers. Secondly, we gained the conclusion that cancer treatment including targeted therapy was effective in reducing recurrence. The study was supplementary material for Asian patients and proved the promising effect of targeted therapy in controlling the recurrence of pleural effusion. Thirdly, we concluded that targeted therapy combined with active intrapleural management was preferred and no difference was found between intrapleural injection and indwelling pleural catheter. We hoped that more clinical trials and comparative studies could be conducted based on the encouraging

results in the future.

Changes in the text: No change was made in the text.