Peer Review File

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Reviewer A

This is a retrospective single-center cohort study on patients with MPE due to non-small cell lung cancer upon diagnosis. I have the following queries/major concerns:

Comment 1: Three different PET-CT scanners with different reconstruction algorithm were used. This could have led to variations in scan findings.
Reply 1: Thanks for your constructive comments. We re-measure all the PET-based variables with the image pre-processing procedure "resampling" and the post-reconstruction harmonization method "Combat". Then, we re-analyze all the data Specifically, Primary, pleural, and metastatic lymph nodes' maximum standardized uptake value (SUVmax) were measured by drawing regions of interest (ROIs) in 3D semiautomatically with a threshold of 42% of SUVmax. Each ROI on PET and CT images were respectively resampled into 3×3×3 mm^3 and 1×1×1 mm^3. Furthermore, to correct for multi-scanner and multi-protocol effects, we processed PET-related parameters using a previously validated post-reconstruction harmonization approach "ComBat" in PET studies (1). Further, all ROIs were reviewed by two nuclear physicians (TL and JW) with 6 and 20 years' experience who were blinded to the endpoints.
Changes in the text: Page 10 Line 183-186; Table 2.

Comment 2: It is known that PET-CT measuring metabolic activity has poor sensitivity/specificity in diagnosing malignant nature of pleural effusion and differentiating MPE from alternative causes of 'para-malignant' pleural effusion. Reporting the lack of role of pleural MTV, TLF, SUV in predicting MPE recurrence is a repeat of the knowns.
Reply 2: Thanks for your constructive comments. We agreed that it has been pointed out that individual qualitative or semi-quantitative parameters of PET/CT might not be equipped with adequate predictive value in the differential diagnosis of malignant pleural effusion. However, the establishment of an integrated PET/CT scoring system (2) has shown well discriminative capacity for MPE with the area under the curve, sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of the PET-CT score to diagnose MPE as 0.949 (95% CI: 0.908–0.975), 83.3% (73.6%–90.6%), 92.2% (85.7%–96.4%), 10.7 (5.6–20.1), and 0.2 (0.1–0.3) respectively. Therefore, we have simplified our descriptions of the corresponding background.

Moreover, our study mainly focuses on the correlation between PET/CT-based parameters and MPE recurrence in patients already diagnosed with MPE.
Changes in the text: Page 6 Line 102-108.

Comment 3: Higher N1, N2 SUV max likely reflects higher N staging, with higher risk of cancer recurrence, irrespective of mutation status or anti-cancer treatments. Whether higher LN staging, as the authors suggest, should automatically be linked to earlier definitive MPE management is highly doubtful. And this argument is not fully supported by the study findings, as AUC is only 0.746 for N2 SUV max, which means a blanket adoption of definitive MPE treatment to all comers will lead to unnecessary treatment in 20-30% of the patients. Not to mention these more aggressive approach is not without risks.
Reply 3: Thanks for your constructive comments. In the previous version of the manuscript, the ROC curves were drawn based on dichotomous endpoints such as 300d recurrence, which might not be appropriate for our survival data. Therefore, we revised the analysis and applied the time-dependent ROC curves to evaluate the discriminative capacities. According to our predictive model for MPE recurrence, LN SUVmax>4.50 [Hazard ratio (HR), 2.54; P=0.019], female gender (HR, 0.40; P=0.011), bone metastases (HR, 3.16; P=0.001), and systemic treatment (targeted therapy vs. chemotherapy, HR, 0.32; P=0.002; immunotherapy therapy vs. chemotherapy, HR, 0.99; P=0.977) could collectively indicate MPE recurrence, with a 300d area under the curve (AUC) of 0.83 (0.73-0.93) and a 900d AUC of 0.90 (0.81-0.98). Moreover, we agreed that the single parameter such as N2 SUVmax was not well-equipped to be the decisive factor for the application of definitive MPE treatment, therefore, we adjusted our inappropriate description and revised it as promisingly, we could probably apply the non-invasive tool to identify the candidate risk factors for MPE recurrence.
Changes in the text: Page 4 Line 71, 77-78; Page 7 Line 117-120; Page 21 Line 421-424; Highlight box; Table S2.

Reviewer B

The authors present their analysis on 103 patients with metastatic NSCLC with a confirmed malignant pleural effusion on presentation. The authors analyzed PET-CT parameters and looked for associations with recurrence of the malignant pleural effusions. Interestingly, the authors found that PET-CT parameters of the pleura did not correlate with recurrence of the malignant pleural effusions. Rather, SUVmax in regional lymph nodes was significantly associated with a higher risk of recurrence of the malignant pleural effusion. The predictive ability of the presented parameters was

stronger when analyzing recurrence at 300 days and it was poor when considering the entire follow up time of the patient cohort.

MAJOR POINTS:

Comment 1: Did the authors analyze the impact of decreasing pleural or regional lymph node SUVmax after initiation of systemic therapy and how that correlated with recurrence of the malignant pleural effusion?

Reply 1: Thanks for your constructive comments. However, most participants received PET/CT merely at baseline, making it impossible to evaluate prompt therapy response and the decreased FDG uptake after initiation of systemic therapy. In the future, we hope that we could launch prospective studies ensuring comprehensive assessment including baseline and after 1-3 months to elaborate on the potential of PET/CT in the evaluation of treatment response. We have added this drawback into the limitation part of our study.

Changes in the text: Page 20 Line 414-416.

Comment 2: Was recurrence of the malignant pleural effusion considered a failure of systemic therapy? If so, was systemic therapy changed because of recurrence of the effusion?

Reply 2: Thanks for your constructive comments. This involves the choice of treatment at first MPE recurrence, however, our previous version of manuscript focused mainly on the endpoint of first recurrence thus lacking the subsequent regimens. Due to diverse systemic treatment regimens and limited sample size, we think it might be inappropriate to perform analysis as you revised based on the original cohort. Therefore, we additionally enrolled a relatively homogeneous cohort and collect the information of second MPE recurrence to make analyses as you recommended.

We recruited a cohort of treatment-naïve EGFR-mutant NSCLC patients with MPE receiving first-line TKI treatment at first onset. Eligible patients were required to satisfy the following criteria, including MPE cytologically proven by thoracentesis or histologically proven by pleural biopsy, or exudative pleural effusion highly suggestive of malignancy excluding other non-cancer reasons; pathologically proven NSCLC; MPE occurred concurrently with the diagnosis of NSCLC; with EGFR mutation and first-line EGFR-TKI treatment. Patients with prior systemic treatment or surgical resection history, lost to follow-up within 1 month and ECOG PS≥4 were ruled out. Finally, a total of 148 patients were eventually recruited during the median follow-up period of 683 (interquartile: 406-1147) days. The majority of patients received first-generation EGFR-TKI [97 (65.5%)] regimen, followed by 38 (25.7%) and 10 (6.8%) with third- and second-generation EGFR-TKI. A sum of 69 (46.6%) patients witnessed at least one MPE recurrence with a median RFS1 of 783 (interquartile: 587-NA) days,

while 35 (23.6%) recurred more than once.

In clinical scenarios, whether the first MPE recurrence without other sites' progression was regarded as a failure of systemic treatment was a comprehensive judgment according to the serum CEA level and rate of progression/recurrence established by a panel of physicians-in-charge. As you recommended, we discussed whether the first MPE recurrence regarded as the failure of systemic treatment, which in turn led to the change of systemic treatment, could influence the time from the first to second MPE recurrence. Specifically, Recurrence-free survival 1 (RFS1) was defined as the time from the MPE onset to the first MPE recurrence requiring intervention. Recurrence-free survival 2 (RFS2) was defined as the time from the first to second MPE recurrence requiring intervention. It was indicated that the change of systemic treatment was not linked with RSF2 for patients with MPE recurrence as the first sign of progression/recurrence, regardless of the presence of simultaneous progression of other sites (P=0.530; P=0.690) (Figure 3E-F). In summary, our exploratory analysis seemed to indicate that whether the change of systemic treatment at the first MPE recurrence was not crucial for second MPE recurrence. This preliminary finding should be validated in larger prospective cohorts.

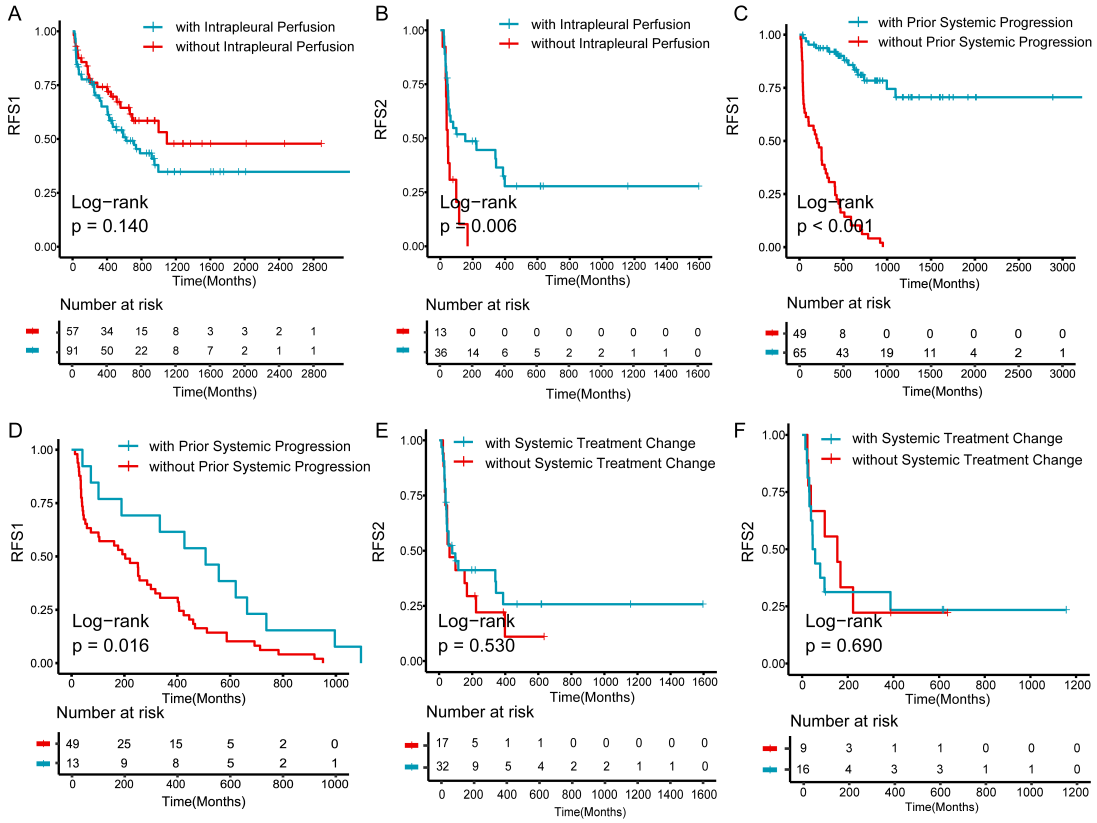

Figure 3 Survival analysis for MPE recurrence-free survival in treatment-naïve NSCLC patients with MPE and EGFR-TKI treatment. *(A) The association between intrapleural perfusion treatment at onset and RFS1. (B) The association between intrapleural perfusion treatment at first MPE recurrence and RFS2. The association*

between prior systemic progression and RFS1 for (C) all the patients and (D) the patients with MPE recurrence at least once during follow-up. The association between the change of systemic treatment at first MPE recurrence and RFS2 for (E) all the patients and (F) the patients with MPE recurrence as the first sign of progression/recurrence. ***Abbreviations:*** *MPE, malignant pleural effusion; RFS1, MPE recurrence-free survival 1, time from the onset to first MPE recurrence; RFS2, MPE recurrence-free survival 2, time from first to second MPE recurrence.*
Changes in the text: Page 8 Line 137-143; Page 9 Line 174-179; Page 12 Line 231-240; Page 13 Line 260-264; Table 1; Figure 1; Figure 3.

Comment 3: Was the recurrent malignant pleural effusion the first sign of progression after an initial response to systemic therapy? Did the authors analyze the effects of recurrence/progression in other sites and how that impacted the risk of recurrence of the malignant pleural effusion?
Reply 3: Thanks for your constructive comments. We added the baseline information including metastases site and stage (IVA or IVB) to analyze the potential effects of other sites' metastases on the risk of MPE recurrence. However, the progression/recurrence in other sites during follow-up is not foreseeable at the baseline, therefore, we think that it might be inappropriate to encompass this factor into the establishment of our predictive model as it was not happening simultaneously with other factors at the baseline. Alternatively, we took your advice by performing the Kaplan-Meier survival analysis with log-rank tests based on the above-mentioned cohort, which enrolled treatment-naïve EGFR-mutant NSCLC patients with MPE receiving first-line TKI treatment at first onset.
1. After adding the baseline parameters including bone metastases, contralateral lung metastases, liver metastases, adrenal gland metastases and stage (IVA/IVB). The univariate Cox regression analyses indicated that bone metastases were an independent risk factor for MPE recurrence [HR, 1.87; 95% CI: 1.05-3.33; P=0.033]. Moreover, multivariate analysis suggested that the LN SUVmax>4.50 [Hazard ratio (HR), 2.54; P=0.019], female gender (HR, 0.40; P=0.011), bone metastases (HR, 3.16; P=0.001), and systemic treatment (targeted therapy vs. chemotherapy, HR, 0.32; P=0.002; immunotherapy therapy vs. chemotherapy, HR, 0.99; P=0.977) could collectively indicate MPE recurrence with an optimal 300d area under the curve (AUC) of 0.83.
2. Progression patterns represented the first sign of recurrence/progression during follow-up was divided into progressive disease (PD) confirmed by Response Evaluation Criteria in Solid Tumors 1.1 criteria (RECIST 1.1) (3), MPE recurrence, both and no progression/recurrence. Moreover, patients were divided into the with and without systemic treatment change groups depending on whether the first MPE

recurrence was regarded as a failure of systemic treatment.

During the follow-up period, the majority of patients [65 (43.9%)] witnessed systemic PD as the first sign of recurrence/ progression, while merely 29 (19.6%) patients underwent single MPE recurrence as the first sign. Moreover, 20 (13.5%) patients had MPE recurrence accompanied with systemic PD simultaneously. Furthermore, we explore whether the prior history of systemic progression of other sites exerts a certain effect on MPE recurrence. It was indicated that prior systemic progression was a protective factor for time to first MPE recurrence, either for all the patients (P<0.001) or the subgroup with at least one MPE recurrence during follow-up (P<0.001; P=0.016) (Figure 3C-D). This could be attributed to the fact that the prior systemic progression would lead to the change of systemic therapy beforehand, thus promoting the control of MPE beforehand.

Changes in the text: Page 9 Line 177-179; Page 13 Line 264-268; Page 14 Line 272-276; Figure 3; Table 1.

Comment 4: The authors fail to transmit a clear message on the clinical impact of their findings. They propose more aggressive "local" therapies to address the malignant pleural effusion in patients that are considered "high risk" for recurrence. However, with the currently demonstrated poor-moderate discrimination ability of the proposed parameters, this could result in over treatment. For instance, if we consider the 300-day endpoint (where the parameters showed better AUCs), at 300 days 16/36 (44.4%) patients with N1 SUVmax >3.07, 13/33 (39.4%) patients with N2 SUVmax >3.84 would receive additional treatment without obtaining benefit during the specified period.

Reply 4: Thanks for your constructive comments. In the previous version of the manuscript, the ROC curves were drawn based on dichotomous endpoints such as 300d recurrence and 100d recurrence which might not be appropriate for our survival data. Therefore, we re-analyze and applied the time-dependent ROC curves to evaluate the discriminative capacities. Specifically, we changed the primary endpoint into the unified recurrence during the follow-up and tested our model's predictive capacity at different time points, which might be more convenient for the clinical application and decision. According to our predictive model for MPE recurrence, LN SUVmax>4.50 [Hazard ratio (HR), 2.54; P=0.019], female gender (HR, 0.40; P=0.011), bone metastases (HR, 3.16; P=0.001), and systemic treatment (targeted therapy vs. chemotherapy, HR, 0.32; P=0.002; immunotherapy therapy vs. chemotherapy, HR, 0.99; P=0.977) could collectively indicate MPE recurrence, with a 300d area under the curve (AUC) of 0.83 (0.73-0.93) and a 900d AUC of 0.90 (0.81-0.98). Moreover, although AUC is enhanced compared to the previous statistical method, we agreed that the AUC could not guarantee 100% accuracy, therefore, we adjusted our inappropriate

description of clinical impact and revised it as promisingly, we could probably apply the non-invasive tool to identify the risk factors for MPE recurrence. Also, since intrapleural perfusion therapy was merely a significant protective factor for MPE recurrence at the first recurrence, we supplemented the clinical impact of our study as we should re-consider the application of intrapleural perfusion treatment for first-onset MPE and prompt it more at the moment of recurrent MPE.
Changes in the text: Page 4 Line 77-78; Page 7 Line 117-120; Page 21 Line 421-424; Highlight box; Table S2.

Comment 5: Similarly, the authors did not demonstrate that "local" therapies had a significant impact on recurrence of the malignant pleural effusion (results shown in Table 3). Therefore, it is unclear what is the proposed strategy for patients that are classified as having a "high risk" of recurrence of the malignant pleural effusion.
Reply 5: Thanks for your constructive comments. The currently widely applied MPE control measures contained the prioritized thoracentesis and indwelling pleural catheter drainage (4). In our center, the MPE control measures within 30 days included non-hyperthermic intrapleural perfusion and indwelling pleural catheter treatment, which was implemented on 91 (61.5%) and 134 (90.5%) patients respectively. Further survival analysis indicated that neither the indwelling pleural catheter nor intrapleural perfusion therapy at baseline was associated with first MPE recurrence (P=0.712; P=0.140) (Table S1; Figure 3A), while the log-rank test indicated that intrapleural perfusion therapy at the first recurrence seemed to be a protective indicator for second MPE recurrence (P=0.006) (Figure 3B). Therefore, due to the unsatisfying role of intrapleural perfusion therapy at the baseline, we assumed that we should try alternative local interventions. According to previous studies, pleurodesis and VATS have been proven effective in improving OS and time to MPE recurrence for NSCLC patients (5-8). However, in our center, hyperthermic intrapleural perfusion, pleurodesis or VATS was not routinely carried out. Previously, in a 30-patient NSCLC cohort with MPE receiving first-line EGFR-TKI treatment, pleurodesis using sterile talc or hypotonic cisplatin was a proven protective factor for time to MPE recurrence. Another retrospective study (9) enrolled 195 non-squamous NSCLC patients with MPE who received chest tube drainage plus chemotherapy perfusion or VAST plus chemotherapy perfusion respectively, and the median OS of patients in the VATS plus chemotherapy group was higher than that of the drainage plus chemotherapy perfusion group (25 vs. 11 months, P<0.05). In summary, we should re-consider the application of intrapleural perfusion treatment for first-onset MPE and prompt it more at the moment of recurrent MPE. Moreover, we regard it might be possible to implement other local interventions initially in patients with higher recurrence risks. Based on research progress, we thought pleurodesis, video-assisted thoracic surgery or novel perfusion drugs might work.

Changes in the text: Page 4 Line 77-78; Page 7 Line 117-120; Page 21 Line 421-424; Highlight box; Table S1.

Table S1 Univariate and multivariate analyses of the factors associated with 300d MPE recurrence for 149 EGFR-mutant treatment-naïve NSCLC patients with MPE at diagnosis

	Univariate cox regression		Multivariate cox regression	
	HR (95%CI)	P value	HR (95%CI)	P value
Age	1.00 (0.98-1.02)	0.925	1.20 (0.45-3.20)	0.984
Gender (Female)	0.77 (0.46-1.29)	0.319	0.64 (0.37-1.12)	0.117
ECOG PS≥2	1.34 (0.88-2.03)	0.173	1.19 (0.76-1.87)	0.450
Liver metastases	1.61 (0.73-3.55)	0.238	1.51 (0.58-3.92)	0.395
Bone metastases	1.33 (0.79-2.24)	0.278	1.44 (0.78-2.66)	0.242
Contralateral lung metastases	0.84 (0.41-1.71)	0.637	0.83 (0.39-1.80)	0.644
Brain metastases	1.05 (0.53-2.07)	0.888	0.95 (0.44-2.04)	0.893
Adrenal gland metastases	1.15 (0.41-3.16)	0.793	0.99 (0.34-2.88)	0.986
Combined systemic treatment within 30 days				
With chemotherapy	0.25 (0.09-0.69)	***0.007***	0.21 (0.07-0.62)	***0.005***
With antiangiogenic therapy	0.95 (0.23-3.9)	0.943	1.73 (0.38-7.87)	0.475
MPE control measurements within 30 days				
Indwelling pleural catheter	1.05 (0.48-2.31)	0.893	1.20 (0.45-3.20)	0.712
Intrapleural perfusion	1.15 (0.67-1.96)	0.613	0.98 (0.56-1.72)	0.952
EGFR-activating mutations				
Exon 19 deletion	Ref.		Ref.	
Exon 21 mutation	1.09 (0.64-1.86)	0.743	1.08 (0.60-1.95)	0.804
Unknown	1.40 (0.49-3.98)	0.530	1.15 (0.35-3.77)	0.812
Generation of TKI				
First-generation	Ref.		Ref.	
Second-generation	0.90 (0.32-2.51)	0.842	1.10 (0.36-3.37)	0.870
Third-generation	0.71 (0.38-1.35)	0.302	0.84 (0.41-1.70)	0.623

† EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

MINOR POINTS:

Comment 6: Please, specify the interquartile range or range for the follow up time.

Reply 6: Thanks for your constructive comments. We have modified our text as advised.

Changes in the text: Page 12 Line 233, 241.

Comment 7: Specify absolute numbers and proportions when presenting data throughout the paper.
Reply 7: Thanks for your constructive comments. We have modified our text as advised**.**
Changes in the text: Page 12 Line 233-240; Page 13 Line 245-255, 262-264.

References:
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6. Cao Y, Zhang Q, Huang Z, et al. Better effect of intrapleural perfusion with hyperthermic chemotherapy by video-assisted thoracoscopic surgery for malignant pleural effusion treatment compared to normothermic chemoperfusion of the pleural cavity. Cancer Medicine 2022;11:348-57.
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