Peer Review File

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Reviewer A

Comment 1: Highlights: "patients who showed a favorable response to initial anti-PD-1 treatment may undergo Hyperprogressive disease when rechallenging the same immunotherapy." too general conclusion to be drawn from one case report.

Reply 1: Thank you for your important comment. We revised the original highlight more accurately as "Our study reported a patient who showed initial partial response to anti-PD-1 treatment underwent hyperprogressive disease when rechallenging the same immunotherapy. "

Changes in the text: We modified it on page 4 lines 3-5.

Comment 2: "tumor immune microenvironment can change dynamically even after cessation of anti-PD-1/PD-L1 therapy, and can cause hyperprogression during rechallenge of anti-PD-1/PD-L1 therapy". I am not sure if it is appropriate to talk about causality. It can be predictive of increased risk for hyperprogression.

Reply 2: Thank you for your help to improve the quality of this case report. As you suggested, we improved the original expression as "The tumor immune microenvironment may change dynamically even after cessation of anti-PD-1/PD-L1 therapy and may be associated with increased risk for hyperprogression during rechallenge with anti-PD-1/PD-L1 treatment."

Changes in the text: We modified this on page 4 line 25, page 5 line 1.

Comment 3: "However, the hyperprogressive phenomenon during anti-PD-1/PD-L1 rechallenge has not been reported before". "However" is not correct in this context

Reply 3: To better clarify this case report, we changed "However, the hyperprogressive phenomenon during anti-PD-1/PD-L1 rechallenge has not been reported before" into "Here, we reported the hyperprogressive phenomenon after PD-1/PD-L1 rechallenge in a patient with non-small cell lung cancer." in the Background section of the Abstract.

Changes in the text: We revised it on page 3 lines 5-7.

Comment 4: In the case report, patient's symptoms and clinical signs at diagnosis should be described. Why did he undergo a CT scan?

In which month and year did he undergo surgery? When did he relapse?

Reply 4: We added the patient's information in the case presentation section as follows:

Why did he undergo a CT scan? Patient's symptoms and clinical signs at diagnosis → CT scan was performed for the examination of cough. No respiratory sign was reported when he first visited the hospital.

In which month and year did he undergo surgery? → The patient underwent left upper

lobectomy with mediastinal lymph node dissection in September, 2018.
When did he relapse? → The disease got relapsed in January 2019.
Changes in the text: We added these information and corresponding time on page 6 lines 20-22 and page 7 lines 1, 8, 16, 17, 18.

Comment 5: Please, correctly abbreviate performance status 1 in PS ECOG 1.
Reply 5: Thank you for pointing out this. We corrected "performance status 1" as "PS ECOG 1" in the manuscript.
Changes in the text: This was revised on page 7 line 8.

Comment 6: PD-L1 expression of the primary tumor?
Reply 6: Yes, the percentage of PD-L1 expression by tumor cells was 5% in the primary tumor.
Changes in the text: We modified it on page 7 line 1-2.

Comment 7: "Partial response after pembrolizumab treatment was achieved and the disease was stable for 6 months". When was the PR achieved? After that, was it a persisting partial response (patient who is stable in the state of radiological and clinical response) or was it a proper stable disease per RECIST 1.1?
Reply 7: Thank you for helping us improving the description of the treatment result. The PR was achieved according to RECIST 1.1 in March 2019 and PR was continued for another 5 cycles of pembrolizumab treatment and 6 months after the discontinuation of pembrolizumab.
Changes in the text: We accordingly added this description on page 7 lines 10-12.

Comment 8: "A left enlarged axillary lymph node was observed in CT imaging (Figure 1D) and suspicious cancer cells were detected in pleural effusion thereafter". It has to be clear: 1) how long has pembrolizumab been stopped 2) when (month, year) the new radiological finding was evident 3) associated symptoms? PS ECOG?
Reply 8: 1) The pembrolizumab has been ceased for 6 months until the disease progressed during follow-up. 2) A left axillary lymph node was radiologically observed from chest CT scan on December 25, 2019 during follow-up and was evident in January 31, 2020. 3) The ECOG PS of this patient was 1 and no specialized respiratory symptom was reported.
Changes in the text: The relevant information were described in page 7 lines 16-21, page 7 lines 23-25.

Comment 9: Please summarize the occurrance of AEs in an apposite timeline. The text should focus only on severe AEs that led to stop or discontinuation of the drug.
Reply 9: The AEs that led to discontinuation of pembrolizumab were pneumothorax and

mediastinal emphysema for the initial treatment and hyperprogressive disease for the rechallenge of pembrolizumab, respectively.
Changes in the text: The severe AEs were added on the timeline of Figure 1.

Comment 10: Specify: S-1
Reply 10: We appreciate your comment. We specified "S-1" as tegafur/gimeracil/oteracil in the manuscript.
Changes in the text: We revised it on page 8 lines 9, 10.

Comment 11: Define hyperprogression and its radiological criteria as first thing in the discussion.
Reply 11: Thanks for your important suggestion. We summarized and discussed the definition of hyperprogressive disease and its radiological criteria at the discussion section: Hyperprogressive disease is recognized as a flair-up of tumor growth during PD-1/PD-L1 immunotherapy. Hyperprogressive disease was initially defined as disease progression at the first evaluation with an increase of tumor growth rate exceeding 100% by RECIST criteria. A recent study developed the definition of hyperprogressive disease by adding multiple new lesions into the diagnostic criteria. Compared to the CT image before pembrolizumab rechallenge in our case study, the new metastatic tumor lesion near the pleura with a rapid increase of malignant pleural effusion after the 1st cycle of pembrolizumab rechallenge and the new malignant pericardial effusion, which were detected shortly just after the 2nd cycle (the time to treatment failure was less than one month), met the diagnostic criteria of hyperprogressive disease.
Changes in the text: We added the definition of hyperprogressive disease in the Discussion section and discussed our case report diagnosis in pages 9 lines 22-25 and page 10 lines 1-7. The relevant references was also cited accordingly (References 8-10).

Comment 12: Discuss challenges for patient who stop/discontinue immunotherapy due to AEs and then progress. Rationale for rechallenge?
Reply 12: Thank you for your comments to improve the quality of our case report.
Our recent meta-analysis study (Heterogeneous Outcomes of Immune Checkpoint Inhibitor Rechallenge in Patients With NSCLC: A Systematic Review and Meta-Analysis. JTO Clin Res Rep. 2022 Mar 19;3(4):100309.) summarized the rechallenge of immune checkpoint therapy and compared the outcomes among different discontinuation reasons including immune-related adverse events (irAEs) or a planned interruption of immunotherapy after a defined number of cycles or disease progression during immunotherapy [4]. The results showed that the patients undergoing disease progression after initial discontinuation owing to irAEs or a planned interruption are more likely to benefit from the rechallenge of immunotherapy in NSCLC.

Compared to the initial immunotherapy, the rechallenge was less effective but had a lower incidence of severe irAEs. Therefore, we re-treated the patient using pembrolizumab again based on that the partial response had ever been achieved in the initial treatment of pembrolizumab.

Changes in the text: The rechallenge of checkpoint immune inhibitors was well discussed on page 9 lines 9-20.

Comment 13: Is cell block adequate for the evaluation of tumor microenvironment? Provide literature references

Reply 13: As malignant effusion is adjacent to both primary and metastatic lung tumor tissue, it is a unique peri-tumoral environment populated with tumor cells, immune cells and other immune components. Like liquid biopsy, malignant pleural effusion and pericardial effusion can be valuable sources for cytological evaluation including immunohistochemistry and flow cytometry (Principe N, et al. Malignant Pleural Effusions-A Window Into Local Anti-Tumor T Cell Immunity? Front Oncol. 2021 Apr 27;11:672747). The immunosuppressive microenvironment in malignant pleural effusion and pericardial effusion (the lower ratio of cytotoxic T cells to regulatory T cells in this case report) may partly reflected the rapid tumor progression (Huang ZY, et al. Single-cell analysis of diverse immune phenotypes in malignant pleural effusion. Nat Commun. 2021). Currently, the direct comparison of tumor microenvironment between lung cancer and malignant effusion is lacking, suggesting an interesting direction for our future study to investigate their interaction. We really appreciate your insightful comments.

Changes in the text: To clearly explain the change of immune microenvironment, the ratios of cytotoxic T cells to regulatory T cells are added in the Results section in page 8 lines 17-20.

Comment 14: "few", "many" Foxp3+.. please quantify, perform a Mann-Whitney test if they have been evaluated continuously

Reply 14: As suggested, we quantified the immune cells and performed a Mann-Whitney test to compare the ratios of $CD8^+$ T cells to $Foxp3^+$ $CD4^+$ T cells. We randomly selected 5 slides of each sample and counted the number of CD8+ T cells and $Foxp3^+$ $CD4^+$ T cells. The ratio of $CD8^+$ T cells to $Foxp3^+$ $CD4^+$ T cells was highest in the specimens before immunotherapy compared to pleural effusion and pericardial effusion (median ratio: 2.6 vs. 0.7 vs. 1.9). Among them, the ratio of $CD8^+$ T cells to $Foxp3^+$ T cells was significantly higher in the resected tumor than that in pleural effusion ($p=0.029$), although not statistically significantly higher than that in pericardial effusion ($p=0.34$).

Changes in the text: The corresponding analysis results are added in page 8 lines 17-20.

Reviewer B

Comment 1: The proposed manuscript is altogether an interesting report with valuable clinical and pathological data on the important matter of hyper progression. However, there are several key points that must be addressed before publication, especially regarding 1. hyper progression definition, 2. quantification and comparison of pathological data, 3. discussion of the alternate hypothesis of toxic effusion 4. search for similar cases in the existing literature.

Reply 1: Thank you for your comment to improve the quality of our study.

1. Hyperprogression Definition→We incorporated a paragraph to discuss the hyperprogression definition in the Discussion section as follows: Hyperprogressive disease is recognized as a flair-up of tumor growth during PD-1/PD-L1 immunotherapy [8]. Hyperprogressive disease was initially defined as disease progression at the first evaluation with an increase of tumor growth rate exceeding 100% by RECIST criteria [9]. A recent study developed the definition of hyperprogressive disease by adding multiple new lesions into the diagnostic criteria [10]. Compared to the CT image before pembrolizumab rechallenge in our case study, the new metastatic tumor lesion near the pleura with a rapid increase of malignant pleural effusion after the 1st cycle of pembrolizumab and the new malignant pericardial effusion, which were detected shortly just after the 2nd cycle of pembrolizumab, met the diagnostic criteria of hyperprogressive disease. In addition, the time to treatment failure less than one month also supported the diagnostic criteria.

2. quantification and comparison of pathological data → As suggested, we quantified the immune cells and performed a Mann-Whitney test to compare the ratios of $CD8^+$ T cells to $Foxp3^+$ $CD4^+$ T cells. We randomly selected 5 slides of each sample and counted the number of CD8+ T cells and $Foxp3^+$ $CD4^+$ T cells. The ratio of $CD8^+$ T cells to $Foxp3^+$ $CD4^+$ T cells was highest in the specimens before immunotherapy compared to pleural effusion and pericardial effusion (median ratio: 2.6 vs. 0.7 vs. 1.9). Among them, the ratio of $CD8^+$ T cells to $Foxp3^+$ T cells was significantly higher in the resected tumor than that in pleural effusion ($p=0.029$), although not statistically significantly higher than that in pericardial effusion ($p=0.34$).

3. discussion of the alternate hypothesis of toxic effusion → The same irAEs in the initial immunotherapy are more likely to occur in the patients who underwent the rechallenge of the same immune checkpoint inhibitors. A previous study analyzed the incidence of irAEs after the rechallenge of checkpoint immune inhibitors in 40 patients who discontinued initial treatment of immune checkpoint inhibitors due to irAEs (Simonaggio A, et al. Evaluation of Readministration of Immune Checkpoint Inhibitors After Immune-Related Adverse Events in Patients With Cancer. JAMA Oncol. 2019). They reported the majority experienced a recurrence of the same type of irAE or did not experience further irAEs and only 12.5% experienced a different type of irAE, suggesting the same irAE is more likely to occur in the patients undergoing the rechallenge of the same immune checkpoint inhibitors. In our case

report, the anti-PD-1 toxicity was pneumothorax and mediastinal emphysema that led to the discontinuation of the initial treatment of pembrolizumab.

In addition to the detection of malignant cells in the pleural and pericardial effusion after pembrolizumab rechallenge, the levels of glucose were lower and LDH were much higher in pleural effusion (Glu: 48, LDH: 1112) and pericardial effusion (Glu: 12, LDH: 3007) after pembrolizumab rechallenge, compared to the pleural effusion component before pembrolizumab rechallenge (Glu:104, LDH 136), suggesting that higher activity of microenvironment in malignant pleural and pericardial effusion when hyperprogressive disease occured.

Furthermore, compared to the CT image before pembrolizumab rechallenge in our case study, the new metastatic lesion near the pleura with a rapid increase of malignant pleural effusion after the 1st cycle of pembrolizumab rechallenge and the new malignant pericardial effusion just after the 2nd cycle was shortly detected. Importantly, the time to treatment failure was less than one month after the rechallenge of pembrolizumab. All above evidence supports the diagnosis of hyperprogressive disease.

4. search for similar cases in the existing literature →We searched the hyperprogressive disease under the re-administration of PD-1/PD-L1 immunotherapy in Pubmed and Embase in English, and no similar literature is found.

Changes in the text:

1. The discussion of Hyperprogression definition is added in page 9 lines 22-25 and page 10 lines 1-7. The relevant references were also cited accordingly (References 8-10).
2. The corresponding analysis results are added on page 8 lines 17-20.
3. The discussion of hyperprogressive disease was shown in page 9, lines 22-25, and page 10 lines 1-7 with updated references.
4. We discussed the search of similar literature on page 10, lines 6-7.

Comment 2: Abstract:

The rechallenge of PD-1/PD-L1 inhibitors can be a treatment option in non-small cell lung cancer patients who once responded to them."

Not clear why you the anti-PD1 is stopped in the first place: progressive disease under anti-PD1, treatment toxicity, treatment holiday? These are very different kinds of rechallenge.

Reply 2: Thank you for your important comments. Our previous study (Heterogeneous Outcomes of Immune Checkpoint Inhibitor Rechallenge in Patients With NSCLC: A Systematic Review and Meta-Analysis. JTO Clin Res Rep. 2022 Mar 19;3(4):100309.) summarized the immune checkpoint blockade discontinuation reasons including immune-related adverse events (irAEs) or disease progression during immunothrapy or stopping immunothreapy after a defined number of cycles or a long period and compared the outcomes of immunotherapeutic rechallenge among there three groups. Our analysis found that the patients undergoing disease

progression after immunotherapy cessation or discontinuation owing to irAE are more likely to benefit from the immune checkpoint inhibitor rechallenge in NSCLC.
Based on the results, we re-treated the patient using pembrolizumab again because the partial response had ever been achieved in the initial treatment, and pembrolizumab was suspended due to pneumothorax and mediastinal emphysema in the initial treatment.
Changes in the text: The rechallenge scenarios were introduced on page 6 lines 11, 12 and the cessation reason was reported on page 7 lines 16-18.

Comment 3: “although he had a favorable response”
Please be more formal about the favorable response: partial response or complete response on RECIST1.1 criteria? infra-RECIST response with reduction in the sum of target lesions <30%? dissociated response?
If it was a RECIST PR or CR you could change the title of the paper accordingly and simply say “after initial response to” rather than “after favorable response to initial”.
Reply 3: We appreciate your important comments and suggestions. The favorable response was a partial response (PR) according to RECIST1.1 criteria in the case report. So" after favorable response to initial" is replaced by " after initial response to " as suggested to accurately describe the response to initial pembrolizumab treatment.
Changes in the text: The title of the paper was revised into “Hyperprogressive disease under anti-PD-1 rechallenge after initial response to anti-PD-1 treatment for non-small cell lung cancer: a case report” on page 1 lines 1, 2.

Comment 4: “More Foxp3+ regulatory T cells were distributed in the cell blocks”
Do you mean “there was a higher concentration of Foxp3+ regulatory T cells in the samples of pleural and pericardial effusion”?
Reply 4: Thank you for pointing out this confusing description. There was a higher concentration of Foxp3+ regulatory T cells in the samples of pleural and pericardial effusion, but absolute number can not precisely reflect the immune microenvironment. As you suggested in comment 11, we used the ratio between CD8+ cells and FoxP3+ cells to measure the distribution and found that lower ratio of CD8+ T cells to Foxp3+ regulatory T cells were distributed in the cell blocks of pleural and pericardial effusion which were taken after hyperprogressive disease, compared to the resected tumor microenvironment.
Changes in the text: The corresponding results are added on page 8 lines 17-20.

Comment 5: “NLR was low in peripheral blood”
How do you define “low” NLR in the absolute? If it is just relative to the subsequent progression, prefer “lower”.
Reply 5: The NLR was longitudinally compared at different time points, so it was relative to

the subsequent progression as commented. NLR was the lowest at disease control, then rose when the disease progressed and increased dramatically when hyperprogression occurred. We replaced “low” with “lower” as suggested.

Changes in the text: It was modified on page 3 line 14.

Comment 6: Introduction:

The problem with hyper progression is that there is currently no consensus definition. Please add a sentence about this problematic, as well as the definition you choose to define your case with supporting references. Personally, I find that criteria based on the tumor growth rate are the most objective and reproducible (see Champiat et al. “Hyperprogressive Disease Is a New Pattern of Progression in Cancer Patients Treated by Anti-PD-1/PD-L1”).

Reply 6: Thanks for your comments to improve the quality of our case report. The definition of hyperprogressive disease and its radiological criteria is discussed based on the tumor growth rate as suggested: Hyperprogressive disease is recognized as a flair-up of tumor growth during PD-1/PD-L1 immunotherapy, and no consensus is currently available for its definition. Hyperprogressive disease was initially defined as disease progression at the first evaluation with an increase of tumor growth rate exceeding 100% by RECIST criteria. A recent study developed the definition of hyperprogressive disease by adding multiple new lesions into the diagnostic criteria. Compared to the CT image before pembrolizumab rechallenge in our case study, the new metastatic tumor lesion near the pleura with a rapid increase of malignant pleural effusion after the 1st cycle of pembrolizumab and the new malignant pericardial effusion, which were detected shortly just after the 2nd cycle of pembrolizumab, met the diagnostic criteria of hyperprogressive disease. In addition, the time to treatment failure less than one month also supported the diagnostic criteria.

Changes in the text: We cited the definition of hyperprogressive disease from your comments and discussed the diagnostic criteria of hyperprogressive disease in our case report in page 9 lines 22-25 and page 10 line 1-7. The relevant references was also cited accordingly (References 8-10).

Comment 7: Case presentation:

“with 5% PD-L1 expression”: please be more precise, PD-L1 expression by tumor cells (TPS), which assay?

Reply 7: Thank you for pointing out this inaccurate description. The PD-L1 expression by tumor cells of the primary tumor was 5% which was detected by 22C3 antibody with the surgically resected left upper lobe.

Changes in the text: We added the TPS on page 7 line 1-2.

Comment 8: “as the relationship between pneumothorax/mediastinal emphysema and

pembrolizumab was undeniable.": Prefer "as a causative link between pneumothorax/mediastinal emphysema and pembrolizumab was highly suspected".

Reply 8: Thanks for your kind revision. We improved this description as "as a causative link between pneumothorax/mediastinal emphysema and pembrolizumab was highly suspected", as you suggested.

Changes in the text: We modified it on page 7 lines 16-18.

Comment 9: "Partial response after pembrolizumab treatment was achieved and the disease was stable for 6 months": please precise, according to RECIST1.1.?

Reply 9: We revised the sentence as suggested: "Partial response was achieved after 2 cycles of pembrolizumab treatment according to RECIST 1.1 in March 2019." and "The patient was stable in the state of clinical partial response for 6 months after the discontinuation of pembrolizumab."

Changes in the text: The related accurate information was described on page 7 lines 10-12 and lines 20, 21.

Comment 10: "Cytology of left pleural and pericardial effusion found malignant cells. Hyperprogressive disease after pembrolizumab rechallenge was therefore identified.": Once again, briefly state what your definition criteria for hyperprogression are.

Reply 10: As suggested, the definition of hyperprogressive disease and its radiological criteria is discussed based on the tumor growth rate in the Discussion section: Hyperprogressive disease is recognized as a flair-up of tumor growth during PD-1/PD-L1 immunotherapy, and no consensus is currently available for its definition. Hyperprogressive disease was initially defined as disease progression at the first evaluation with an increase of tumor growth rate exceeding 100% by RECIST criteria. A recent study developed the definition of hyperprogressive disease by adding multiple new lesions into the diagnostic criteria. Compared to the CT image before pembrolizumab rechallenge in our case study, the new metastatic tumor lesion near the pleura with a rapid increase of malignant pleural effusion after the 1st cycle of pembrolizumab and the new malignant pericardial effusion, which were detected shortly just after the 2nd cycle of pembrolizumab, met the diagnostic criteria of hyperprogressive disease. In addition, the time to treatment failure less than one month also supported the diagnostic criteria.

Changes in the text: The discussion of Hyperprogression definition is added on page 9 lines 22-25 and page 10 line 1-7. The relevant references were also cited accordingly (References 8-10).

Comment 11: "In the tumor microenvironment of surgical specimen, the infiltration of CD8+

cells recognized as cytotoxic T cells was observed among the tumor cells (Figure 2A). Immunohistochemistry identified CD8+ cells, and more Foxp3+ cells as regulatory T cells were distributed in the cell blocks of pleural and pericardial effusion which were taken just after hyperprogressive disease": this paragraph is problematic: first, precise that immunohistochemistry was performed on samples from the previously resected primary tumor, how these samples were processed, especially for the effusions (centrifugation and FFPE embedding?) etc. Then, how do you rigorously quantify and compare the 2 kinds of immune populations in the samples? "more" is too vague. The effusion of very different kind of samples that the solid tumor, I guess that the cells present in the effusion were concentrated by centrifugation, so you cannot just compare the number of cells by unit of surface. A more satisfying measure could be for each sample (primary tumor, pleural effusion, pericardial effusion) the ratio between CD8+ cells and FoxP3+ cells.

Reply 11: We very much appreciate your important comments. Centrifugation and FFPE embedding were performed for the immune cells analysis of effusions. As suggested, we quantified the immune cells by randomly selecting 5 slides of each sample and counting the number of CD8+ T cells and Foxp3+ T cells. The ratio of CD8+ T cells to Foxp3+ T cells was highest in the specimens before immunotherapy compared to pleural effusion and pericardial effusion (median ratio: 2.6 vs. 0.7 vs. 1.9). Among them, the ratio of CD8+ T cells to Foxp3+ T cells was significantly higher in the resected tumor than that in pleural effusion ($p=0.029$), although not statistically significantly higher than that in pericardial effusion ($p=0.34$).

Changes in the text: The corresponding analysis results are added on page 8 lines 17-20.

Comment 12: Discussion:

"with pleural and pericardial dissemination shortly after pembrolizumab rechallenge": the disease was already disseminated to the pleura before pembrolizumab rechallenge

Reply 12: Suspicious cancer cells were detected in pleural effusion before pembrolizumab rechallenge, although the volume of pleural effusion was much less than that after pembrolizumab rechallenge. Compared to the CT image before pembrolizumab rechallenge, the new metastatic tumor lesion near the pleura with a rapid increase of malignant pleural effusion occurred and the new malignant pericardial effusion were detected shortly after the 1st cycle of pembrolizumab. The time to treatment failure was less than one month.

Changes in the text: The exact description is added on page 8 line 3 and discussed on page 10 lines 1-8.

Comment 13: "many Foxp3+ cells as regulatory T cells were observed": As stated above, "many" and "few" is not a satisfying term, please provide more rigorous quantification and comparison.

Reply 13: As suggested, we quantified the immune cells by randomly selecting 5 slides of each

sample and counting the number of CD8+ T cells and Foxp3+ T cells. The ratio of CD8+ T cells to Foxp3+ T cells was highest in the specimens before immunotherapy compared to pleural effusion and pericardial effusion (median ratio: 2.6 vs. 0.7 vs. 1.9). Among them, the ratio of CD8+ T cells to Foxp3+ T cells was significantly higher in the resected tumor than that in pleural effusion ($p=0.029$), although not statistically significantly higher than that in pericardial effusion ($p=0.34$).

Changes in the text: These results were added on page 8 lines 17-20.

Comment 14: Please discuss the fact that pleural and pericardial effusion can also occur as anti-PD1 toxicity, independently from tumor progression, and explain how you differentiate hyper progression from this kind of toxicity in your case. There might have already been tumor cells present in the pleural effusion, and maybe a infraclinical pericardial metastasis, so the fact that you found two more cells in the effusions after pembrolizumab rechallenge does not rule out the alternate hypothesis of an inflammatory effusion due to a specific toxicities of anti-PD1. The fact that he experienced prior pulmonary anti-PD1 toxicity is also consistent with this alternate hypothesis.

Reply 14: Thank you for pointing out this important issue.

The same irAEs in the initial immunotherapy are more likely to occur in the patients who underwent the rechallenge of the same immune checkpoint inhibitors. A previous study analyzed the incidence of irAEs after the rechallenge of checkpoint immune inhibitors in 40 patients who discontinued initial treatment of immune checkpoint inhibitors due to irAEs (Simonaggio A, et al. Evaluation of Readministration of Immune Checkpoint Inhibitors After Immune-Related Adverse Events in Patients With Cancer. JAMA Oncol. 2019). They reported the majority experienced a recurrence of the same type of irAE or did not experience further irAEs and only 12.5% experienced a different type of irAE, suggesting the same irAE is more likely to occur in the patients undergoing the rechallenge of the same immune checkpoint inhibitors. In our case report, the anti-PD-1 toxicity was pneumothorax and mediastinal emphysema that led to the discontinuation of the initial treatment of pembrolizumab.

In addition to the detection of malignant cells in the pleural and pericardial effusion after pembrolizumab rechallenge, the levels of glucose were lower and LDH were much higher in pleural effusion (Glu: 48, LDH: 1112) and pericardial effusion (Glu: 12, LDH: 3007) after pembrolizumab rechallenge, compared to the pleural effusion component before pembrolizumab rechallenge (Glu:104, LDH 136), suggesting that higher activity of microenvironment in malignant pleural and pericardial effusion when hyperprogressive disease occured.

Furthermore, compared to the CT image before pembrolizumab rechallenge in our case study, the new metastatic lesion near the pleura with a rapid increase of malignant pleural effusion after the 1st cycle of pembrolizumab rechallenge and the new malignant pericardial effusion

just after the 2nd cycle was shortly detected. Importantly, the time to treatment failure was less than one month after the rechallenge of pembrolizumab. All above evidence supports the diagnosis of hyperprogressive disease.
Changes in the text: The discussion of hyperprogressive disease was shown in page 9, lines 22-25, and page 10 lines 1-7 with updated references.

Comment 15: You state in the introduction that "However, the hyperprogressive phenomenon during anti-PD-1/PD-L1 rechallenge has not been reported before.", you should then report in the discussion if and how you searched the literature to ensure that it is the case.
Reply 15: We searched the hyperprogressive disease under the re-administration of PD-1/PD-L1 immunotherapy in Pubmed and Embase in English, and no similar literature is found.
Changes in the text: We discussed the search of similar literature in page 10, lines 6-7.

Comment 16: Typo
Line 8 : UICC International Cancer Control => Union for …
Line 33 : "The disease still progressed though S-1 was treated." => Please reformulate
Reply 16: Thank you for pointing out these errors. We revised them in the text.
Changes in the text: The typos were revised in page 7 line 3 and page 8 lines 9, 10, respectively.