Peer Review File

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Reviewer A

Case presentation:

Comment 1: Line 13: the mass had been revealed by CT incidentally during screening (not physical examination)

Reply 1: Thank you for your suggestion! We have deleted 'in a physical examination'.

Changes in text: We have modified our text as advised (see Page 4, line 13).

Comment 2: The authors have not provided the details regarding invasion of the tumour into the pleura (visceral or parietal) which would have altered the pathological staging.

intrao-operatively, was the pleura examined for any tethering or nodules suggesting invasion into the parietal pleura thus giving rise to a T4 and subsequently pleural effusion one month after?

Reply 2: Thank you for your quesetion. The tumor did not invade the parietal or visceral pleura according to postoperative pathological analysis. Also, no evidence of parietal pleural invasion was found during the surgery.

Changes in text: We have modified our text as advised (see Page 4, line 19-20 and 22-24).

Comment 3: One month after surgery, was the pleural effusion investigated eg cytology and was it a malignant effusion thus upstaging the cancer to Stage IV instead?

Reply 3: We appreciate your inquiry. We did conduct cytological examination when the patient developed pleural effusion, but no tumor cells were found in the pleural effusion. Therefore, we believe that crizotinib treatment is an adjuvant treatment after surgery, and pleural effusion was a *suspicious* progression.

Changes in text: We have modified our text as advised (see Page 4, line 33-34).

Comment 4: Why was Crizotinib chosen over Ceritinib considering these 2 drugs should be widely available in China?

Reply 4: We appreciate your inquiry. First and foremost, according to our organoid drug sensitivity test, Crizotinib showed a lower IC50 value when acting on tumor cells

in patients compared to Ceritinib, which suggests Crizotinib might have better efficacy for this patient. Also, Crizotinib may lead to milder adverse event in view of the results of two clinical trials ('PROFILE 1014' and 'ASCEND-4') in 2019. On top of that, price was also a factor we considered when choosing drugs.
Changes in text: None

Comment 5: More explanation is required regarding the technology behind PDOs, and waste culture medium as this is very foreign and new to the general reader. We would not know the significance of why the fusion gene was highest in the waste culture medium for example.
Reply 5: We have added some descriptions about organoid technology. First, we use PDO for sequencing to verify its genetic similarity with primary tumors. However, similar to the primary lesion, the abundance of *ALK* fusion mutations in organoids was also very low. Inspired by the recent detection of ctDNA in blood and pleural effusion which is also known as 'liquid biopsy', we hypothesized that ctDNA will also be released into the culture medium in which organoids grow. Therefore, we collected the culture medium of the patient's organoid for ctDNA testing, and indeed found the phenomenon of *LRRTM4-ALK* enrichment. This suggests that low abundance driven mutations might be revealed by detecting organoids' waste culture medium.
Changes in text: We have modified our text as advised (see Page 5, line 7-11; Page 7, line 9-12).

Comment 6: Perhaps a better term to use would be DFS (Disease free survival) over RFS
Reply 6: Thank you for your suggestion. We have changed this term throughout the entire article.
Changes in text: We have modified our text as advised (see Page 2, line 22 and 27; Page 3, line 4 and 12; Page 5, line 22; Page 6, line 11).

Comment 7: Pls grade your adverse events and briefly mention what they were.
Reply 7: Thank you for your suggestion. Only grade 1 diarrhea occurred during the treatment of Crizotinib according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. We have added this information to the case report.
Changes in text: We have modified our text as advised (see Page 5, line 23-26).

Comment 8: Some of the discussion sections should be elaborated under methodology, for example in describing PDO and waste culture medium. The paragraphs and headers for the discussion do not provide a nice flow for the case report and seems to be very truncated.

Reply 8: We have added some details about organoid technology and its waste culture medium sequencing in the discussion part. As for the format of paragraphs and headers, this case report is an article participating the Multi-Disciplinary Treatment (MDT) project initiated by the AME editorial team, which encourage collaboration among multiple disciplines or technical departments to solve clinical problems. This modality of discussion is provided by the editors.

Changes in text: We have modified our text as advised (see Page 6, line 16-24 and 28-32; Page 7, line 9-12).

Comment 9: The comments for the external contributors namely Andrea and Petros should be incorporated and rewritten into the discussion section and not left as questions to be answered for this case report. The discussion section should be rewritten to make it flow seamlessly.

Reply 9: We greatly appreciate your suggestion, but there is a reason for this form of discussion. After submitting the manuscript, we were asked by the AME editorial team to raise several clinical questions about this case report, and then the AME editorial team invited two international experts to answer our questions. This form is conducive to letting readers know the focus of the author's concerns when solving clinical problems. As this template was provided by the editorial team, we may need to maintain this format.

Changes in text: None

Reviewer B

In this manuscript, the authors described that a case with novel LRRTM4-ALK fusion mutation. They further mentioned that Crizotinib was selected by patient-derived organoids (PDOs) and helped the patient achieve a more than 3-year-long recurrence-free survival (RFS), indicating the benefits of PDOs.

Although the authors need to clarify some points, I think this manuscript includes some interesting points.

Comment 1: Reviewers are encouraged to provide a concise description of patient-

derived organoids (PDOs). For instance, this might include information on how soon after surgical resection they can be utilized as organoids, or the timeframe required after resection to assess drug sensitivity using PDOs.

Reply 1: We greatly appreciate your proposal. More details about PDOs were added to the article. The patient's tumor tissue was sent to the laboratory for cultivation within 4 hours after the resection. Ten days later, tumor cells expand into organoids that can undergo drug sensitivity. We obtained the patient's drug sensitivity results approximately 3 weeks after the surgery.

Changes in text: We have modified our text as advised (see Page 6, line 16-24 and 28-32).

Comment 2: Why is Alectinib, which is characterized by low adverse events and have high efficacy, not included in drug screening with PDOs? Alectinib demonstrates higher efficacy against the EML4-ALK fusion gene compared to Crizotinib and is utilized globally as a first-line treatment. If IC50 data is available, please provide additional information. Alternatively, if it was not used, kindly specify the reason for its exclusion.

Reply 2: Thank you very much for your inquiry. We did not include Alectinib because we were unable to obtain this drug for sensitivity testing in 2019. Based on your kindly suggestion, we have added this reason in the article as a limitation.

Changes in text: We have modified our text as advised (see Page 7, line 18-20).

3．Page 5、Line 5-10 and Figure 1E.

Comment 3: As mentioned by the authors, IC50 based on the viability of PDOs for ceritinib (0.87 μM), crizotinib (0.71 μM), and brigatinib (0.96 μM) were not significantly different as compared to the disparity between positive controls (EML4-ALK fusion organoids) and negative controls (non-ALK-fusion organoids).

Is the cell viability assay result representative of a single experiment? Given the relatively close IC50 values observed for each drug, it is advisable to conduct repeated assessments or increase the sample size (N) for experimentation to enhance reliability

Reply 3: Thank you very much for your suggestion! Expanding the sample size is indeed beneficial for increasing the reliability of the result. However, due to limitations of organoid culture techniques back then, there might not be enough organoids to conduct repeated experiments. The method we use is to add a drug with different concentrations to organoids, and determine their viability. Thus, we could conduct the

curve fitting of ‘drug concentration-organoid viability’ and calculate the IC_{50} value of this drug. This is a common method for calculating IC_{50} value in organoids’ drug sensitivity test, as described in the landmark article ‘*Using patient-derived organoids to predict locally advanced or metastatic lung cancer tumor response: A real-world study*’ published in *Cell Report Medicine* (Q1, IF:14.3). At the same time, we also added this deficiency to the discussion section of the article to remind readers that the importance of organoids cultivation techniques for conducting repeated assessments and enhancing reliability.

Changes in text: We have modified our text as advised (see Page 6, line 22-24; Page 7, line 16-18).