Article information: https://dx.doi.org/10.21037/pcm-21-55

Response to Reviewer A's comments:

Comment 1: Was the TP53 mutation identified in the initial tumor tissue?

Reply 1: Thank you very much for your comment. Yes, the TP53 mutation was identified in the initial tumor tissue. We added in the text as follows (See Page 5, line 10-11).

Changes in the text:

FoundationOne Liquid by clinical trial testing showed BRAF V600E and TP53 I255S mutations.

Comment 2: It would be interesting to have the variant allelic frequency of these alterations.

Reply 2: Thank you for your important comment. Unfortunately, we had no data of the variant allelic frequency in the initial tumor tissue because the FoundationOne Liquid was performed as a clinical trial setting.

Comment 3: Have the authors attempted to detect the KRAS mutation in the initial tumor sample, using a very sensitive approach such as digital PCR?

Reply 3: Thank you for your important question. Using the initial tumor sample, we underwent the Oncomine Dx Target Test and the FoundationOne Liquid. However, we could not perform digital PCR test for limited tumor samples. We added the FoundationOne Liquid in the text (See Page 5, line 9-10).

Changes in the text:

FoundationOne Liquid by clinical trial testing also showed BRAF V600E and TP53 I255S mutations.

Comment 4: The VAF of the mutations identified in plasma must also been reported.

Reply 4: Thank you for your important comment. We added the detail results of Guardant360 in the text (See Page 5, line 14-17).

Changes in the text:

Plasma sequencing (Guardant360® CDx) revealed the acquisition of the KRAS G12V mutation (0.3% of variant allele frequency) in addition to the original BRAF V600E and TP53 I255S mutations (0.2% for both of variant allele frequency).

Comment 5: How can the authors rule out that the KRAS mutation is not related to clonal hematopoiesis?

Reply 5: Thank you for your important comment. As you said, plasma-based NGS assays cannot rule out the clonal hematopoiesis of indeterminate potential. We could not perform tumor tissue-based assay at resistance. However, our patient had no KRAS mutation in plasma-based NGS assays before treatment, but plasma-based NGS assays at resistance detected KRAS mutation. For that, we suspected that this KRAS mutation at resistance would be not related to clonal hematopoiesis. Considering your comment, we changed below in discussion of the text (See Page 8, line 5-11).

Changes in the text:

A limitation of our case was that we could not perform molecular analysis by tumor tissue-based assay at resistance. Plasma-based NGS assays cannot rule out the clonal hematopoiesis of indeterminate potential. However, our patient had no KRAS mutation in plasma-based NGS assays before treatment, but plasma-based NGS assays at resistance detected KRAS mutation. For that, we suggest that this KRAS mutation at resistance would be not related to clonal hematopoiesis.

Response to Reviewer B's comments:

Major comments:

Comment 1: major English editing is required prior to publications. At the less the following changes are required.

Reply 1: Thank you for your comment. We changed the text below and this manuscript has been carefully reviewed by an experienced medical editor whose first language is English and who is specialized in the editing of papers written by physicians and scientists whose native language is not English.

Changes in the text:

A: Page 3, line 14-15; the response duration is limited, and the acquisition of resistance is expected.

B: Page 5, line 2-5; The patient was a 70-year-old Asian Male who was a former smoker and has been diagnosed with stage IV NSCLC, adenocarcinoma subtype. Of the left lung since 2018 with metastases to multiple mediastinal lymph nodes and a separate nodule in the counter lateral lung.

C: Page 5, line 5-6; An endobronchial ultrasound-guided transbronchial needle aspiration biopsy of the left hilar node revealed adenocarcinoma.

D: Page 5, line 6-9; Initial molecular testing was negative for epidermal growth factor mutations, anaplastic lymphoma kinase (ALK) and ROS1 rearrangements. Subsequent, tissue-based next generation sequencing with Oncomine Dx Target CDx revealed BRAF V600E mutation.

E: Page 5, line 10-12; He was treated with dabrafenib and trametinib combination therapy and achieved a response, with reduction in size of the left lower lobe primary, mediastinal nodes and multiple lung metastases.
F: Page 5, line 18-Page 6, line 1; Upon disease progression, the patient has been treated with carboplatin and pemetrexed.
G: Page 7, line 2-5; The number of NSCLC patients harbouring BRAF V600E mutation is relatively small, and only a few cases report on acquired resistance mechanisms have been reported. The identification of recurrent molecular aberrations will lead to the development of new therapeutic strategies for this patient population.
H: Page 7, line 6-7; Similar to other advanced NSCLC harbouring actionable mutations treated with targeted agents, resistance almost invariably develop.
I: Page 7, line 7-12; Multiple mechanisms of resistance to combined BRAF and MEK inhibitors have been reported in BRAF mutant melanoma. These include secondary mutations of the RAS/RAF/MAPK pathway such as BRAF amplification, BRAF splicing variants and gain-of-function mutations in KRAS and NRAS (10). KRAS is upstream from BRAF, and its activation may lead to activation of the PI3K/AKT/mTOR pathway, leading to tumour progression.
J: Page 7, line 13-15; secondary KRAS G12V mutation was identified as mechanism of resistance to combination therapy with dabrafenib and trametinib in our BRAF V600E mutation positive NSCLC patients.

Comment 2: The authors should provide some preliminary response data for carboplatin and pemetrexed in this patient.
Reply 2: Thank you for your comment. We added the response data for carboplatin and pemetrexed in the text as follows (See Page 6, line 1-2).
Changes in the text:
The response for carboplatin and pemetrexed was partial response with the primary tumour shrinkage.

Comment 3: I would suggest the authors to consider providing a summary of non-RAS/RAF/MAPK aberrations as resistance mechanisms.
Reply 3: Thank you for your comment. We added a summary of non-RAS/RAF/MAPK aberrations as resistance mechanisms in discussion of the text as follows (See Page 7, line 17-Page 8, line 2).
Changes in the text:
In the literature review, the increased expression level of cyclin-dependent kinase (CDK) 4 and CDKN2 deletion or nonsense mutations have been reported as the resistance mechanisms of non-RAS/RAF/MAPK aberrations.

Response to Reviewer C's comments:

Comment 1: There are typographic and grammatical errors in several places – please correct.

Reply 1: Thank you for your comment. We changed the text below and this manuscript has been carefully reviewed by an experienced medical editor whose first language is English and who is specialized in the editing of papers written by physicians and scientists whose native language is not English.

Changes in the text:

A: Page 3, line 14-15; the response duration is limited, and the acquisition of resistance is expected.

B: Page 5, line 2-5; The patient was a 70-year-old Asian Male who was a former smoker and has been diagnosed with stage IV NSCLC, adenocarcinoma subtype. Of the left lung since 2018 with metastases to multiple mediastinal lymph nodes and a separate nodule in the counter lateral lung.

C: Page 5, line 5-6; An endobronchial ultrasound-guided transbronchial needle aspiration biopsy of the left hilar node revealed adenocarcinoma.

D: Page 5, line 6-9; Initial molecular testing was negative for epidermal growth factor mutations, anaplastic lymphoma kinase (ALK) and ROS1 rearrangements. Subsequent, tissue-based next generation sequencing with Oncomine Dx Target CDx revealed BRAF V600E mutation.

E: Page 5, line 10-12; He was treated with dabrafenib and trametinib combination therapy and achieved a response, with reduction in size of the left lower lobe primary, mediastinal nodes and multiple lung metastases.

F: Page 5, line 18-Page 6, line 1; Upon disease progression, the patient has been treated with carboplatin and pemetrexed.

G: Page 7, line 2-5; The number of NSCLC patients harbouring BRAF V600E mutation is relatively small, and only a few cases report on acquired resistance mechanisms have been reported. The identification of recurrent molecular aberrations will lead to the development of new therapeutic strategies for this patient population.

H: Page 7, line 6-7; Similar to other advanced NSCLC harbouring actionable mutations treated with targeted agents, resistance almost invariably develop.

I: Page 7, line 7-12; Multiple mechanisms of resistance to combined BRAF and MEK inhibitors have been reported in BRAF mutant melanoma. These include secondary mutations of the RAS/RAF/MAPK pathway such as BRAF amplification, BRAF splicing variants and gain-of-function mutations in KRAS and NRAS (10). KRAS is upstream from BRAF, and its activation may lead to activation of the PI3K/AKT/mTOR pathway, leading to tumour progression.

J: Page 7, line 13-15; secondary KRAS G12V mutation was identified as mechanism of resistance to combination therapy with dabrafenib and trametinib in our BRAF V600E mutation positive NSCLC patients.

Comment 2: I wasn't completely clear on details of technical and biological replication (in text and legends).
Reply 2: Thank you for your comment. This article is case report not including a basic experiment. I think your point is not applicable.

Comment 3: Statistical analysis seems valid, please test model assumptions.
Reply 3: Thank you for your comment. This article is case report not including a basic experiment. I think your point is not applicable.

Comment 4: Please clarify the relationship between objectives and specific hypotheses, and the predictions for these.
Reply 4: Thank you for your comment. This article is case report not including a basic experiment. I think your point is not applicable.

Comment 5: Discussion could be trimmed a little.
Reply 5: Thank you for your comment. We trimmed a little in the discussion below.
Change in the text:
Page 7, line 2-5: The number of NSCLC patients harbouring BRAF V600E mutation is relatively small, and only a few cases report on acquired resistance mechanisms have been reported. The identification of recurrent molecular aberrations will lead to the development of new therapeutic strategies for this patient population.
Page 7, line 6-7: Similar to other advanced NSCLC harbouring actionable mutations treated with targeted agents, resistance almost invariably develop.
Page 7, line 7-12: Multiple mechanisms of resistance to combined BRAF and MEK inhibitors have been reported in BRAF mutant melanoma. These include secondary mutations of the RAS/RAF/MAPK pathway such as BRAF amplification, BRAF splicing variants and gain-of-function mutations in KRAS and NRAS (10). KRAS is upstream from BRAF, and its activation may lead to activation of the PI3K/AKT/mTOR pathway, leading to tumour progression.
Page 7, line 13-15: secondary KRAS G12V mutation was identified as mechanism of resistance to combination therapy with dabrafenib and trametinib in our BRAF V600E mutation positive NSCLC patients.
Page 7, line 17-Page 8, line 2: In the literature review, the increased expression level of cyclin-dependent kinase (CDK) 4 and CDKN2 deletion or nonsense mutations have been reported as the resistance mechanisms of non-RAS/RAF/MAPK aberrations.

Comment 6: I wasn't sure if all relevant positive controls for the assays were included, check please.

Reply 6: Thank you for your comment. This article is case report not including a basic experiment. I think your point is not applicable. All molecular assays were performed as commercial based and had positive controls of manufacturers.