Peer Review File

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First round of peer review

Reviewer A

General comments

Thanks for presenting this interesting case to the community and for the time you have taken to outline it.

Comment 1: Figure 3 is very useful for understanding the timeline of the case, which is not quite as clear in the text. I appreciate there is a maximum number of words available, but can we get a bit more details in the main body of the case report as opposed to having to get them out of a figure?

Reply 1: *We thank the reviewer for this valuable feedback and have added significant more details on the timeline of the case in the body of the case presentation.*

Changes in the text: "*She was started on 1st line platinum-based doublet therapy with carboplatin and paclitaxel with an investigational agent. After induction therapy she received pemetrexed switch maintenance therapy. Her disease metastasized to the brain while receiving maintenance pemetrexed. She subsequently underwent whole-brain radiation with 30 Gy/10 fx and was treated on a clinical trial as 2nd line treatment with pembrolizumab and an investigational immunomodulatory agent. However, she developed progression within two months.*"

Comment 2: It is quite impressive that this heavily pre-treated patient still had a decent performance status at the point when she was started on osimertinib, after PD (including intracranial PD) on multiple lines of treatment. Did she have no other comorbidities and overall low overall tumour burden to justify this? Could this detail please be expanded on please. If her PS had not been maintained I would imagine you would advise best supportive care rather than embarking on one further line of non-standard targeted therapy at this stage.

Reply 2: *We thank the reviewer for this comment. She had IDDM as her only co-morbidity and overall low tumor burden and had good performance status despite prior treatments. If this would not have been the case, we would have advised best supportive care. Her co-morbidites and sites of metastatic disease have now been explained in the text.*

Changes in the text: *"A 68-year-old Caucasian female with a past medical history of insulin dependent diabetes and a 3-pack-year smoking history (quit smoking in 1971) was diagnosed*

with right lower lobe adenocarcinoma of the lung in October 2016 via CT guided core needle biopsy. Immunohistochemical testing showed that the tumor was strongly positive for TTF-1 and CK7. At diagnosis she had metastatic disease to right hilar nodes, bilateral mediastinal nodes and celiac axis nodes."

Comment 3: This lady was treated between 2017 and 2019. Trials of HER2 targeting agents which included patients with non-exon 20 insertions were active at this point in the US (Destiny-Lung01, NCT02675829; ZENITH20-2 did not include HER2 mutations other than exon 20 insertions, the Chinese pyrotinib trial did not have any trial locations active in America). Was a referral for consideration of inclusion into a clinical trial taken into consideration at any point during her disease journey?

Reply 3: *Patient was treated on a clinical trial as a second line treatment with pembrolizumab and investigational immunomodulatory agent. This information was only provided in Figure 3 but has now been included in the text.* Trials with anti-Her2 therapy were considered however she either did not qualify for those trials due to eligibility criteria or study did not have open slots when needed to enroll this patient.

Changes in the text: *"She underwent whole-brain radiation and was started on pembrolizumab as a 2nd line treatment on a clinical trial with an investigational immunomodulatory agent."*

Comment 4: While a response was obtained, this was very short lived – especially in the intracranial disease, despite the optimal CNS penetration of osimertinib. This point should be expanded in the discussion. There are very few details about response evaluation imaging – when was this performed? When was confirmatory imaging performed? Were RECIST criteria employed in determining whether there was a response? She had CNS PD at 5 months requiring SRS – was extracranial disease stable at this point?

Reply 4: *RECIST criteria was used to evaluate response. Confirmatory imaging was obtained at 3 months from starting Osimertinib, (Figure 1 and 2). She had CNS PD at 6 months (not 5 this has been corrected) and extracranial disease was stable.*

Changes in the text: "*The patient achieved a confirmed partial response (PR) to osimertinib systemically and intracranially (Figure 1 and 2)." was changes to "The patient achieved a confirmed partial response (PR) to osimertinib systemically and intracranially according to RECIST criteria (Figure 1 and 2)." We additionally added: "After 6 months on treatment with osimertinib, there was evidence of disease progression in the brain and 9 small lesions were treated with SRS. Extracranial disease was stable at this time."*

Comment 5: The discussion says very little about the case presented and goes into a lot of (possibly, excessive) details about the literature, which ARE not necessarily relevant to the case (ZENITH20-2 did not include patients with HER2 mutations other than exon 20 insertions – is

it justified to present its fine details in a paper focusing on a different type of HER2 mutation?). Maybe cut down a bit on these details and elaborate more on what the community can learn from this case? For instance, as well as being effective, Osimertinib at 80 mg OD dose was also well tolerated (vs. other HER2-targeting drugs having significant toxicity concerns); in coming years, osimertinib could be a HER2 mutant-targeted therapy to be considered sequentially to others eg T-dxd.

Reply 5: *We thank the reviewer for this excellent comment. We do think it is important for the reader to know which trials have included exon 19, p.L755P mutation and would like to keep this in the discussion. We have added more details as suggested.*

Changes in the text: *"As well as being effective, osimertinib at alternating 160/80 mg dose was also well tolerated. This is important considering toxicity concerns with some of the other HER2-targeting drugs. In the coming years, osimertinib could become a HER2 mutant targeted therapy to be considered sequentially to others*."

Comment 6. The final sentence of the abstract and of the main paper (lines 35-37, 105-107) are virtually identical. Also, both are very generic and lack critical reflection on the case. Is there anything you can do about this?

Reply 6*: We agree with the reviewer and appreciate this important feedback. These sentences have been re-written.*

Changes in the text: *The final paragraph in the paper was changed to "Osimertinib, a 3rd generation EGFR-TKI, was effective and well tolerated in a patient with stage IV NSCLC harboring ERBB2 exon 19 c.2262_2264delinsTCC, p.(L755P) mutation, including response of intracranial metastases. Future research is required to illustrate the differential effects of targeted therapies in NSCLC harboring various types of HER2 alterations to select an optimal therapy."*

Line by line comments

Comment 7: Line 33. Change to 'limited data ARE available' please.

Reply 7: *We thank the reviewer for this comment.*

Changes in the text*: Line 33 has been changed accordingly*

Comment 8: Line 34-35. Change to 'here, we present the case of a 68-year-old female with NSCLC harboring HER2 exon 19, p. L755P mutation treated with osimertinib, resulting in systemic and intracranial response.

Reply 8: *We thank the reviewer for this comment.*

Changes in the text: *Line 34-35 has been changed accordingly.*

Comment 9: Line 35-37. Change to 'future research is required to illustrate the differential effects of targeted therapies in NSCLC harboring various types of HER2 alterations to select an optimal therapy'.
Reply 9: *We thank the reviewer for this comment*.
Changes in the text: *Line 35-37 has been changed accordingly.*

Comment 10: Line 56. Remove colon.
Reply 10: *We thank the reviewer for this comment*.
Changes in the text*: Line 56 has been changed accordingly*.

Comment 11: Line 57. Please add metastatic sites present at diagnosis and specify site of primary.
Reply 11: *Sites of metastatic disease at diagnosis have been added*.
Changes in the text*: "At diagnosis she had metastatic disease to right hilar nodes, bilateral mediastinal nodes and celiac axis nodes."*

Comment 12: Lines 60-61. Please change 'Her disease progressed in the brain while receiving platinum-based 1st line therapy' to 'She experienced progression in the [?which?] brain metastases during maintenance pemetrexed treatment after completion of induction first-line platinum-based chemotherapy [was is cisplatin or carboplatin?]'.
Did she have brain metastases at baseline and did these progress at this stage, or did she develop de novo brain. Metastases at this stage?
Please add details of WBRT and specify details of chosen immunotherapy regime – was she on a clinical trial?
Reply 12: *Details of WBRT and chosen immunotherapy regimen have been added*. Detail regarding 1st line therapy were added in the text based on previous comment. Brain mets were not present at the time of diagnosis and developed during pemetrexed maintenance.
Changes in the text: *"She subsequently underwent whole-brain radiation with 30 Gy/10 fx and was treated on a clinical trial as 2nd line treatment with pembrolizumab and an investigational immunomodulatory agent."*

Comment 13: Line 65. 'She received A combination of gemcitabine and vinorelbine'. Was her disease stable for 9 months both intracranially and extracranially? If so, please specify it.
Reply 13: *Yes her disease was stable both intracranially and extracranially during this time*.
Changes in the text: *"She then received gemcitabine-vinorelbine combination resulting in stable intracranial and extracranial disease for 9-months"*

Comment 14: Line 66. Change to 'at which point, she developed disease progression in lung

[?primary? ?lung metastases?] and brain lesions'.
Reply 14: *Further details have been added to line 66.*
Changes in the text: *"Subsequently, there was disease progression in the primary lung lesion and brain."*

Comment 15: Lines 69-72. This period could do with some restructuring and rewriting. I find it quite difficult to really understand the flow of thoughts here. I think that the main take home message from the Nagano paper that should be mentioned here, rather than the description of IC50 for Osimertinib and different HER2 mutations, is the fact that Osimertinib is the agent among those examined in that paper which has the best efficacy against the L775S mutation (see figure 5 in the Nagano paper). Then you go on to mention that you chose Osimertinib as it has optimal CNS penetration – and the missing point in the text is that this lady's disease was (presumably) mainly progressing intracranially, hence such a desire.
Reply 15: *We thank the reviewer for this comment. Lines 69-72 have been restructured.*
Changes in the text*: "Nagano et al. had recently showed that among various TKIs osimertinib had the best efficacy against the HER2 exon 19, p. L755P mutation [5]. Additionally, osimertinib was known to penetrate the blood brain barrier which was important since the main site of the patient's disease progression was in the brain. Based on the findings from Nagano et al. osimertinib was commenced at a dose of 80mg OD. To maximize chances of intracranial disease control the dose of osimertinib was increased after 2 weeks of treatment to 160/80 mg alternating days and QTc monitored."*

Comment 16: Line 72. What would these 'other treatment options' you mentioned be? She had basically received all possible lines of SACT for NSCLC by this point. Do you mean other HER2 targeting options?
Reply 15: *The reviewer is right, we were referring to other HER2 targeted options as the patient had progressed on all possible lines of SACT.*
Changes in the text: *Due to reconstruction of line 72 this has been removed. See above in comment 15.*

Comment 17: Line 72-74. What does this mean? Was she originally started at 160 mg OD and then the dose was titrated down to 80 mg OD because of toxicity? Was the initial dose 80 mg and then you went up to 160 mg and then back down? How long was she on 160 mg OD for? If longer than a couple weeks one could think that the reason for intracranial progression at 5 months was because the 160 mg OD dose was needed to control intracranial disease and the dose reduction caused resistance to treatment; this point should be discussed later on.
Reply 17. *As we were trying to shorten the case report as much as we could, we tried to*

summarize her dosing however we understand that this was too confusing. We have now elaborated in detail on her exact dosing in the case presentation.
Changes in the text*: "Based on the findings from Nagano et al. osimertinib was commenced at a dose of 80mg OD. To maximize chances of intracranial disease control the dose of osimertinib was increased after 2 weeks of treatment to 160/80 mg alternating days and QTc monitored."*

Comment 18: Line 73. Spelling mistake - the odds OF, not OFF. Could this be rephrased as 'to maximise chances of intracranial disease control, osimertinib was commenced [or escalated, if that is was happened] at a dose of 160 mg OD'.
Reply 18: *We thank the reviewer for this comment.*
Changes in the text: *Spelling mistake has been corrected in line 73. The text in line 73 has been rephrased. See above reply 17.*

Comment 19: Line 74. How convincingly drug related was the G3 fatigue? Could it not have been disease-related? Did it resolve after downtitration? It would be important to point out that these dose-limiting toxicities 1. occurred at a higher-than-normal dose at which osimertinib starts to have off-target toxicity 2. completely resolved at the dose of 80 mg OD.
Reply 19: *As above, we were trying to shorten the case report as much as we could, we tried to summarize her dosing however we understand that this was too confusion.*
Changes in the text: "*Four months after starting osimertinib the patient was admitted with grade 3 fatigue and grade 2 transaminitis. It was thought that this could either have been related to osimertinib or other medications she was taking at that time (doxycycline). Osimertinib was held for 2 week and symptoms resolved and dose was restarted at 160/80 alternating days.*"

Comment 20: Line 75. Change 'systemically' to 'extracranially'.
Reply 20: *We thank the reviewer for this comment.*
Changes in the text: *Line 75 has been changed accordingly.*

Comment 21: Line 76. Change 'few' with 'several'.
Reply 21: *We thank the reviewer for this comment*
Changes in the text: *Line 76 has been changed accordingly.*

Comment 22: Line 77-78. These two lines recap quite a lot of events. I wouldn't call this 'continuation beyong progression'. I would rather you reinforced that there was ongoing extracranial response/stability of disease and that the intracranial progression was treated with a locoregional strategy eg SRS. Why did this final CNS PD quickly led to death, unlike the previous ones? Did she develop focal neurology / deteriorated clinically quickly and was deemed by a neuro-oncology MDT to have no further radiotherapy options?

Why is figure 3 quoted at this point? It doesn't appear to make sense. It would be better to either not quote it or to insert some explanation eg 'the timeline of this patient's cancer treatment journey is portrayed in figure 3'.

Reply 22: *We agree with the reviewer and have now re-written the final part of the case presentation.*

Changes in the text: *"After 6 months on treatment with osimertinib, there was evidence of disease progression in the brain and 9 small lesions were treated with SRS. Extracranial disease was stable at this time. Osimertinib dose was increased to 160mg OD for another 4.3-months, after which the patient had further CNS progression with development of lethargy and disorientation, ultimately leading to death (The timeline of this patient's cancer treatment journey is portrayed in Figure 3)."*

Comment 23: Line 87. Suggest changing 'systemic' to 'extracranial'.

Reply 23: *We thank the reviewer for this comment.*

Changes in the text: *Line 87 has been changed accordingly.*

Comment 24: Line 88. Remove comma: it should read 'the patient lived for 11 months after starting osimertinib'. This is a very short remark wish fails to consider pt's (presumably good) quality of life and maintenance of fitness during this period.

Reply 23: *We thank the reviewer for this comment.*

Changes in the text: *The comma has been removed in line 88. We have added the following sentence: "As well as being effective, osimertinib at alternating 160/80 mg daily dose was also well tolerated."*

Comment 25: Line 102. Change 'they were found to have' to 'their best response was PR and stable disease respectively'.

Reply 25: *We thank the reviewer for this comment.*

Changes in the text: *"They were found to have…" has been changed to "their best response was PR and stable disease respectively"*

Comment 26: Line 137-138. That quote is a reply to a query – I think you actually want to quote the main trial article instead? Then the quote needs changing.

Reply 26: *We thank the reviewer for noticing this error and have quoted the main article.*

Comment 27: Figure 1. The arrows mentioned in the text need adding, I can't see them. Can the caption be edited to make it understandable on its own and tell a story? Eg Serial magnetic resonance imaging shows ongoing response in the right frontal metastasis, which had not been irradiated before.

Reply 27: *We apologize for this editing issue, the figures were labeled and had arrows in the*

word format submitted as can be accessed through the TLCR author site, but it seems this did not translate with the figures. The figures have now been fixed.
Changes in the text: *Serial magnetic resonance imaging shows ongoing response in the right frontal metastasis, which had not been irradiated before.*

Comment 28: Figure 2. The arrows mentioned in the text need adding, I can't see them. Can the caption be edited to make it understandable on its own and tell a story? Eg 'Serial computer tomography imaging of the chest, showing ongoing response in the right lower lobe lung primary' (primary which has not been mentioned in the main text at any point!!!).
Reply 28: *We apologize for this editing issue, the figures were labeled and had arrows in the word format submitted as can be accessed through the TLCR author site, but it seems this did not translate with the figures. The figures have now been fixed.*
Changes in the text: *The following caption has now been added: "Serial computer tomography imaging of the chest, showing ongoing response in the right lower lobe lung primary"*

Reviewer B

This is an interesting article, detailing how the novel off-label use of an existing approved agent has delivered defined patient benefit, and as such is certainly worthy of publication as a case report. I would however suggest some slight modifications for the purposes of clarity and conformance to standard nomenclature.

Comment 1: Read in isolation (as is likely), the abstract may be confusing to some, as many readers may just dismiss any apparent association between osimertinib and HER2, as in their minds at least, it is 'only' associated with EGFR. Some brief explanation of the logic for considering the use osimertinib (an 'EGFR' TKI) in any cases with HER2 activating mutations (irrespective of what/where they are), would therefore be very useful and likely encourage full reading of the report.

Reply 1: *We thank the reviewer for this excellent feedback and have changed the abstract to explain the logic for considering the use of Osimertinib.*

Changes in the text: *"Osimertinib, a 3rd generation EGFR-TKI, has been found in pre-clinical studies to decrease growth of NSCLC with HER2 exon 19 aberrations. Here, we present a case of a 68-year-old female with stage IV NSCLC harboring ERBB2 exon 19 c.2262_2264delinsTCC, p.(L755P) mutation treated with osimertinib, resulting in extracranial and intracranial response."*

Comment 2: Within the introduction, use of the terms 'overexpression' and 'amplification' is a bit ambiguous; dysregulation may arise as a result of protein overexpression, caused by gene amplification and/or aberrations in regulatory regions, or alternatively by the acquisition of activating/gain of function coding mutations.

Reply 2: We thank the reviewer for this comment and have made these adjustments in the introduction.

Changes in the text: *"HER2 dysregulation in non-small cell lung cancer (NSCLC) may arise as a result of protein overexpression, caused by gene amplification and/or aberrations in regulatory regions, or alternatively by the acquisition of gain of function coding mutations."*

Comment 3: Line 73: "odds off controlling" should read "odds of controlling"

Reply 2: *We thank the reviewer for this comment.*

Changes in the text: *Line 73 has been changed accordingly.*

Comment 4: There is variation and also some inaccuracy in the use of nomenclature throughout the article.

a. The gene is ERBB2 and any description of mutations at the DNA level should reference this with any inferred protein changes denoted using HGVS nomenclature, e.g., ERBB2 exon 19

mutation, c.2262_2264delinsTCC, p.(L755P) is the correct nomenclature (ideally with a suitable reference sequence also being quoted).

b. When speaking of mutations at the protein level these may simply be referred to as HER2 p.L755P (without parentheses) and no exon location is required. However, I accept that many do drop the 'p.' in such a context, and in this article, periodic reference to mutations in exon 19 or 20 may still be useful to reader. In any event, use should be consistent throughout i.e. all p.L755P or just L755P.

c. 'insertions within exon 20' should be used rather than 'exon 20 insertions' as the latter may suggest duplication of the entire exon.

Reply 4: *We thank the reviewer for this detailed comment on the use of nomenclature throughout the article.*

Changes in the text: *'exon 20 insertions' has been corrected to 'insertions within exon 20' in the text. We are now consistent with using p.L775P instead of L755P throughout the article. ERBB2 is used for any description of mutations at the DNA level.*

Reviewer C

Table 1. Line corrections that will help the manuscript.

Reply to Table 1 line corrections: *We thank the reviewer for these comments, the manuscript has been adjusted according to all the comments in Table 1 below.*

Line No.	Comments	Major/Minor/Reject
11-14	Same address for different people. Use only one for everyone that is working at this address.	Minor
16	Uppercase "Activity" and "Osimertinib" for continuity	Minor
31	Her2 is a protein in this sentence. Only use italics for a gene.	Minor
39	Keyword "exon" capitalize for continuity	Minor
56	"Case Presentation" no need for the ":"	Minor
57	Caucasian should not be capitalized	Minor
71	Osimertinib does not need to be capitalized	Minor
71	"Brain substance" This is the blood brain barrier. This is a problem during drug development as the barrier is hard to pass drugs through. Please avoid substance and use the correct medical terminology	Major
62	"however" start this as a new sentence	Minor
63,66, 69, and 72	Segregate into new paragraphs. It will make the case report read	Minor

	better. Indent new paragraphs 0.5 cm for a professional finish.	
78 and 79	Space between paragraphs. Please remove this. Also indent first line 0.5 cm.	Minor

Case Presentation

Comment 1: It is unclear whether the patient was diagnosed with a brain tumor at the initial time of diagnosis. If this was the case, then you cannot say the disease progressed to the brain. Progression should be used when describing a cancer or tumor that does not respond to therapy and continues to grow and/or metastasize.
Reply 1: *The patient did not have brain tumor at the initial time of diagnosis.*
Changes in the text: *This has now been clarified in the text and exact location of the metastatic disease at diagnosis added.*

Comment 2: It seems to me that the brain tumor occurred after chemotherapy treatment. Therefore, I strongly recommend writing metastasis rather than progressed. Moreover, how was it known that the brain tumor had originated from the lung?A positive identification would have been made, please include this information as it will help the impact of the report.
Reply 2: *We thank the reviewer for this excellent comment, the brain tumor developed after chemotherapy. A re-biopsy was not performed on the brain disease.*
Changes in the text: *It is now explained in the case presentation that the patient developed metastasis to the brain while on maintenance pemetrexed.*

Comment 3: As part of the case presentation, this section would benefit using a clinicopathological feature table. This table will give valuable information such as smoking habits, pathological staining (Axl, EGFR, Kras, etc …), genomic profiling etc… In particular, because osimertinib is being used, EGFR expression and mutation status is critical. Osimertinib is an EGFR T790M mutation TKI. Therefore, this table will greatly benefit from knowing this. Please also include secondary colonization sites such as the brain tumor into the table. It will look much better if this is included.
Reply 3: *We have added details of smoking habit, morphology and genomic profiling to the text. The patient did not harbor an EGFR mutation on genomic profiling.*
Changes in the text: *"A 68-year-old Caucasian female with a past medical history of insulin dependent diabetes and a 3-pack-year smoking history (quit smoking in 1971) was diagnosed with right lower lobe adenocarcinoma of the lung in October 2016 via CT guided core needle*

biopsy. Immunohistochemical testing showed that the tumor was strongly positive for TTF-1 and CK7. At diagnosis she had metastatic disease to right hilar nodes, bilateral mediastinal nodes and celiac axis nodes. Molecular testing showed that FISH for ALK and ROS was negative. PD-L1 tumor proportion score was <1% (22C3 assay). A 50 gene custom panel was performed using Ampliseq-based next generation sequencing (NGS) on a NextSeq 550 (Illumina Inc., San Diego, CA) and showed ERBB2 exon 19 mutation, c.2262_2264delGTTinsTCC, causing leucine to proline substitution at codon 755, p.L755P. No other driver alterations were identified, including EGFR mutation. "

Comment 4: You mentioned the first line treatment was platinum-based. Could you be more specific to the therapy? Cisplatin etc… Were you able to take biopsies during treatment? If so that information would be very useful and important. It would show any alterations during treatment. Just a table would be enough and provide other physicians an overall pathological and molecular story.

Reply 4: *The first line platinum-doublet therapy was carboplatin and pemetrexed. Unfortunately no re-biopsies were taken during treatment.*

Changes in the text: *The case presentation now specific information on the type of the platinum based therapy.*

Comment 5: The question I have is why osimertinib? Would any other EGFR TKI treatment have been available erlotinib, gefitinib etc…? You will need to rationalize why osimertinib was chosen other than being able to cross the blood-brain barrier.

Reply 5: *As we describe in the main body of the text it was because of the CNS penetration and the Nagano et al. paper that showed that among various TKIs osimertinib has activity against the HER2 exon 19, p. L755P mutation. In the original manuscript we had more details on the results of that paper however this has now been removed according to feedback from reviewers.*

Discussion

Comment 6: For me, this section needs work. There are many things you can discuss. You did mention clinical studies, which was excellent. This helps other physicians if they treat patients harbouring Her2 mutated NSCLC. This is a major revision but achievable. A little more detail, perhaps a paragraph, describing the receptor expression and mutation status and how you think this will have prolonged the patient's life to 11 months following third generation EGFR-TKI treatment.

Reply 6: *We thank the reviewer for this feedback and have added a paragraph on the above.*

Changes in the text: *"The ErbB tyrosine receptor family (HER2, EGFR, HER3, and HER4) consist of a ligand-binding extracellular domain and an intracellular tyrosine kinase domain.*

The HER2 receptor does not have an endogenous ligand for its extracellular domain and heterodimerizes with other HER family receptors and causes activation of downstream signaling through the PI3K/AKT and RAS/MAP/MEK pathways. In a pre-clinical study Li et al. presented that osimbertinib had antitumor efficacy against multiple HER2 *aberrations in NSCLC, either as a single agent or in combination with JQ1, a BET inhibitor. Subsequently, Nagano et al. showed in-vitro that HER2 variants at L755 were more sensitive to osimertinib compared to other TKIs, interestingly it was not effective against common exon 20 alterations. Because of the homology between HER2 and EGFR it has been speculate that the covalent binding site for osimertinib may be C805 (analogous Cys797 to EGFR) of human HER2, although this warrants further studying. No clinical trials have tested the efficacy of osimertinib in HER2 alterations."*

Figures

Comment 7: All figures need Labelling A-D on the images. Fig 1 upper right, please remove the highlighted boarder. Arrows are missing, please include them in clear colours that do not clash with the images. Instead of using months and years (A: Jan 2019) please use (X months following platinum/afatinib/osimetinib treatment).

Reply 7: *All the figures were labelled in word but unfortunately did not translate through. This has now been fixed including the arrows.*

Changes in text: *We have included X months following Osimertinib treatment to the labelling.*

Comment 8:Fig 2 same as fig 1. Please see above. This will make the images look very nice.

Reply 8: *Refer to reply in comment 7.*

Comment 9: Fig 3. This was very hard to read. Either use larger text and higher resolution, or make a new figure with a simple timeline and drug treatment using months rather than years i.e:

Reply 9: *We have now made a new figure that is easier to read. We tried using months instead of years but this made the timeline even more difficult to read.*

Overall assessment

Comment 10: This is an interesting report. Impact value? Not very high as it stands. However, by including the information requested this report needs to be seen by other physicians and clinical researchers. In closing remarks, the paper needs to be proofread. It has a lot of grammatical errors, including medical and scientific language. Therefore, I would strongly advise revising and polishing the manuscript. In addition, including more details about the patient in a table will greatly improve the medical and scientific impact and will therefore need to be included along with figure revisions.

Reply 10: *We thank the reviewer for this feedback. We have now adjusted all sections of the paper. More detail about the patient has been added to the case presentation. Figure revisions and grammatical errors have been corrected.*

Reviewer D

Comment 1: The presented work is a very interesting case study as there are few reported cases of NSCLC patients with HER2 mutations treated with third generation TKIs. The treatments and follow-up are described correctly, however, I think that the study would need a better description of the presentation of the case: a morphological description of the type of tumor, types of interventions, analyzes performed... that are presented in a non-existent way or very succinct in the manuscript. In addition, although it is true that there are not many references on the subject, discussion and bibliography presented is scarce and limited. Finally, some small issues: the arrows are not visible in the figures, and the records presented in the checklist do not coincide with the text.

Reply 1: *We thank the reviewer for the excellent feedback. We have now added further details on the immunohistochemistry of the tumor, no further morphological description other then adenocarcinoma could be made by pathologist as tumor sample was obtained through a needle biopsy. We added intervention and analyzes performed. Bibliography and discussion has been updated as well as the Figures and checklist.*

Second round of peer review

Reviewer A

Very well done. A much, much better manuscript than last time. We thank the reviewer for this positive feedback.

Comment A: There are still quite a few spelling/grammar mistakes throughout the text. I appreciate we are all super busy and probably doing academic work at night and at weekends – but perhaps may I suggest routinely running manuscripts through an error correction software before submitting?

Reply A: We apologize and agree with the reviewer. We used an error correction software that turned out to be inadequate.

Comment B: Previously made comment no 2 has been very nicely addressed in the letter addressed to myself but not in the text. If word limit allows -> could you find a place in the text where to add those considerations? I.e. appropriate to think out of the box and go for one extra line of tx as pt remained fit despite intracranial PD and multiple prior lines of tx. A good place to add that would be in the paragraph in lines 167-168.

Reply B: We have added a discussion on this in the above paragraph.

Changes in text: "At this time the patient was still fit for treatment. Therefore, clinical trials with anti-Her2 therapy were considered however she either did not qualify for available trials due to eligibility criteria or open slots were not available when enrollment was needed."

Comment C: Lines 168-173. Description of the Nagano paper as scientific rationale for using osimertinib in this setting – I know, I'm sorry, it was me asking you to restructure its discussion. Perhaps this section now it is a tiny bit too short and could do with a little more detail on the experiments run by Nagano and colleagues – to make it plain and clear why trying osimertinib was perfectly sensible at that point. That is - if word counts allows. Page 5116 and 5118 of the Nagano paper seem helpful to write a quick summary of the data they present on sensitivity of ERBB2 p.L755P to Osimertinib.

Reply C: We have added a summary from the paper to the text.

Changes in text: "The authors demonstrated that the IC50 of osimertinib for the *HER2* exon 19, p.L755P mutation was 23.8 nmol/L and low-dose osimertinib (8 mg/kg) could inhibit the growth of tumors with this mutation."

Comment D: Osimertinib is written with a capital O in quite a few occurrences throught the text – could it be made uniform. My preference is lower case o.

Reply D: This has been made uniform with a lower case o.

Line by line comments

Comment 1: Line 25. The opening sentence of the abstract and the introduction are very, very similar. Suggest you cut out the first sentence of the abstract altogether.

Reply 1: The first sentence of the abstract has been removed.

Comment 2: Line 27. @Editor – is it acceptable to have abbreviations in brackets (NSCLC) within the abstract? If not, please cut out.

Reply 2: Please advise, we did not receive clarification if this was acceptable or not.

Comment 3: Line 33. Harbouring [mutation] – add 'a' please, the pt did harbour A mutation

Reply 3: Text has been changed accordingly.

Comment 4: Line 35. Change 'Exon 19 p.L755P mutation' to 'ERBB2 Exon 19 p.L755P mutation'

Reply 4: Text has been changed accordingly.

Comment 5: Line 96. 'Through two major pathways, the PI3K- AKT and MEK-ERK that induce cell proliferation and migration' -> change to 'through two major pathways, the PI3K-AKT and MEK-ERK pathways, which induce cell proliferation and migration.

Reply 5: Text has been changed accordingly.

Comment 6: Line 131. IDDM is not really an adequate descriptor for diabetes in 2022 – was she a type 1 diabetic or a type 2 diabetic requiring insulin therapy?

Reply 6: We thank the reviewer for this comment and have added adequate details regarding her diabetes diagnosis.

Comment 7: Lines 132-133. 'was diagnosed with right lower lobe adenocarcinoma of the lung' -> change to 'was diagnosed with adenocarcinoma of the lung (right lower lobe primary)'.

Reply 7: Text has been changed accordingly

Comment 8. Line 135 – if word count limit allows, make TNM staging explicit.

Reply 8: T3N3M1b, this information has been added to the manuscript.

Comment 9. Line 142-143. I am not clear here – was 1st line tx a clinical trial of carbo/pacli + investigational agent? Please clarify.

Reply 9: Yes this was on a clinical trial. Sentence has now been changed to " She was started on 1st line platinum-based doublet therapy with carboplatin and paclitaxel with an investigational agent through a clinical trial."

Comment 10: Lines 158-160. 'Was treated on a clinical trial as 2nd line treatment with pembrolizumab and an investigational immunomodulatory agent' - > change to 'received 2nd line treatment with pembrolizumab and an investigational immunomodulatory agent as part of a clinical trial'.

Reply 10: Text has been changed accordingly.

Comment 11: Lines 163, 'After which…' -> change to 'After this, she…'

Reply 10: Text has been changed accordingly.

Comment 12: Lines 172-173. If word count limit allows – could you clarify the process by which you managed to get her Osimertinib? Was it via compassionate access? Was it by showing her insurer the Nagano data? Some of us work in countries where access to expensive drugs out of their main indications is complicated and it would be good to know.

Reply 12: Osimertinib was provided by AZ via patient assistance program at no cost because insurance had denied it. This information has been added to the manuscript.

Comment 13: Lines 179-181. Change numbers below 10 to letter format please.

Reply 13: Text has been changed accordingly.

Comment 14: Lines 211-212. (The timeline of this patient's cancer treatment journey is portrayed in Figure 3). -> thanks for adding this based on my previous comment. Please take this out of the brackets and move it to the beginning of this section focussing on the pt's cancer journey i.e. Insert this sentence between lines 141 and 142.

Reply 14: Sentence has been inserted between lines 141 and 142.

Comment 15: Lines 225-227. 'In the coming years, osimertinib could become a HER2 mutant targeted therapy to be considered sequentially to others'. Slightly ambitious statement - ?is there any evidence of activity of osimertinib for exon 20 alterations? The Nagano paper data aren't promising for exon 20 YVMA insertions. I agree your case seems to suggest activity in exon 19 ERBB2 point mutations, so feel free to edit so that it is clear your prediction only applies to these.

Reply 15: We thank the reviewer for this excellent comment, this is of course correct that the activity wasn't promising in exon 20 alterations. We have made edits in the manuscript to clearly state this only for exon 19 ERBB2 point mutations.

Changes in manuscript: "In the coming years, osimertinib could become a HER2 mutant targeted therapy for patients harboring Exon 19 ERBB2 point mutations."

Comment 16: Lines 228-244. Why are we repeating this point made in the introduction? I think these two sentences can be cut out altogether.

Reply 16: The sentences have been removed.

Comment 17: Lines 244-246. Preclinical study -> ?cell lines, ?organoids, ?animal model –

Reply 17: We thank the reviewer for this comment. We have added to the text that this was in animal models (mice).

Changes in text: "In a pre-clinical study using animal models, Li et al. presented that osimertinib had antitumor efficacy against multiple HER2 aberrations in NSCLC, either as a single agent or in combination with JQ1, a BET inhibitor"

Comment 18: Lines 246-247. The Nagano group were working with cell lines only, right? Make it clear please.

Reply 18: The Nagano group worked with in vitro cell lines and in vivo MANO method. This has been added to the manuscript. See page 5114 in paper.

Comment 19: Lines 256-258. More info about this trial please. ?phase II presumably? ?name of trial? Either this or make description of ZENITH20-2 shorter as well.

Reply 19: We have added more info – phase II, multicenter trial to the text and removed

ZENITH20-2.

Comment 20: Lines 263. Their best responseS were PR and stable disease respectively

Reply 20: Text has been changed accordingly.

Comment 21: Line 265 E69 following lines. After discussing the relevant literature, we have now gone back to your case. This is not immediately clear from the text – can you make it clearer? @Editor – is a separate 'conclusion' paragraph needed or are you happy with this structure in which the paper ends with the discussion? If conclusion paragraph is needed then the last two sentences can be moved there.

Reply 21: The last two sentences have been removed to a conclusion paragraph.

Reviewer B

Thank you for clearly addressing all the comments raised during my (and others) initial review. Clearly a lot of work.

We thank the reviewer for this positive feedback.

I have identified only a few minor typos in the new text.

Comment 1: Line 101 - Should read "anti-HER2 .."

Reply 1: This has been changed accordingly in the text.

Comment 2: Line 179 - presumably should read "was withheld for 2.."

Reply 2: This has been changed accordingly in the text.

Comment 3: Line 249 - Should read "speculated"

Reply 2: This has been changed accordingly in the text.