Peer Review File

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Reviewer A

Comment 1: The title should be rephrased as in the present form seems to suggest that it is a single case harboring a compound exon 21 and exon 18 mutation.
Reply 1: Thank you for your comments. We have amended the title from "Case report" to "A Case Series" see page 1 line 3

Comment 2: Both of the two patients harbored a TP53 mutation. The concurrent presence of TP53 mutations is usually associated with lower EGFR TKI efficacy (Kim Y, et al. J Thorac Oncol 2019; Aggarwal C, et al. JCO Precis Oncol 2018). This should be discussed when commenting the two cases.
Reply 2: We agree with this comment and have added discussion regarding the impact of TP53 comutations into the discussion. See page 14 line 238
Changes in the text: "Of note, both patients co-exhibited *TP53* mutations which has been shown in recent literature to be associated with a poorer prognosis and reduced response to EGFR TKI therapy"

Comment 3: The authors did not report whether patient #2 was offered the opportunity of tissue re-biopsy or plasma NGS at disease progression on osimertinib. Please clarify
Reply 3: Patient #2 was not offered a rebiopsy due to rapid clinical deterioration. See Discussion Page 14, line 241

Comment 4: In the Discussion, the authors should also include the results of two recently published studies evaluating the role of osimertinib in uncommon EGFR mutations (Bar J, et al. J Thorac Oncol 2023; Okuma Y, et al. JAMA Oncol 2023)
Reply 4: This has been included in the Discussion page 12, line 186

Comment 5: The activity seen in patient #2 seems quite low as compared with that seen usually in patients with common EGFR mutations. Preclinical data suggest that exon 18 mutations are more sensitive to 2nd generation EGFR TKIs (Kobayashi Y, et al. Clin Cancer Res 2015) and some published data support the use of afatinib in this setting (Wei Y, et al. Front Oncol. 2021).
Reply 5: The authors acknowledge this comment, we hypothesise the presence of the TP53 demised the effect. Of note patient B has a mixed adenosquamous carcinoma which has a worse prognosis

Comment 6: The authors stated in the abstract that "[osimertinib activity in] exon 18 deletion-insertion mutations has never been previously reported". However, a very recent case report showed a response with osimertinib in this kind of mutation (Cekay M, et al. Front Oncol. 2023). Please revise
Reply 6: This statement in the Introduction (page 5, line 91) has been revised

Reviewer B

I have carefully reviewed the paper addressing osimertinib treatment in a patient with EGFR mutation-positive NSCLC featuring L861R and exon 18 deletion-insertion. The focus on non-exon 19 deletion and L858R positive patients with EGFR mutant NSCLC is intriguing; however, some modifications are warranted to enhance the clarity and depth of the discussion.
Major revision

Comment 1: In both presented cases, patients exhibited EGFR mutations along with TP53 mutations. Recent literature has highlighted the prevalence of TP53-positive EGFR mutations. Furthermore, it has been reported that heterogeneous gene mutations also increase in EGFR mutation-positive patients as the disease progresses. Smoking history may be related to prognosis. The observed outcomes in the showcased patients may reflect a multifaceted scenario. Beyond the impact of osimertinib on the EGFR mutation site, the complexities arise from the confluence of additional TP53-positive mutations, potential modifications induced by smoking, and the heightened heterogeneity inherent in the progression of EGFR mutation-positive NSCLC. In light of these intricate dynamics, I recommend the incorporation of an addendum that extends the discussion beyond EGFR gene mutations to encompass these various contributory factors. This broader perspective will provide a more comprehensive understanding of the nuanced influences shaping the treatment outcomes.

Jamal-Hanjani M, et al. N Engl J Med . 2017. PMID: 28445112.
Nishio M, et al. Clin Lung Cancer. 2023 Jul;24(5):415-428. doi: 10.1016/j.cllc.2023.02.010.

Le X, et al. Cancers (Basel). 2022 Dec 12;14(24):6127. doi: 10.3390/cancers14246127.
Reply 1: Thank you for your feedback. We have added the impact of TP53 comutations to the Discussion acknowledging that TP53 comutations in EGFR mut NSCLC confers a poorer prognosis (See Page 14, line 238)
Changes in the text: “Of note, both patients co-exhibited *TP53* mutations which has been shown in recent literature to be associated with a poorer prognosis and reduced response to EGFR TKI therapy”

Comment 2: Furthermore, it is pertinent to acknowledge that Afatinib has been previously reported as a viable therapeutic option for the addressed issue. In order to enrich the discussion, I recommend including an exploration of the significance behind prioritizing Osimertinib over alternative agents such as Afatinib. This comparative analysis will contribute valuable insights into the rationale guiding treatment choices and provide a more nuanced understanding of the therapeutic decision-making process in the context of EGFR mutation-positive NSCLC with L861R and exon 18 deletion-insertion.Kim MH, et a.Anticancer Res. 2022 Mar;42(3):1615-1622. doi: 10.21873/anticanres.15636.
Reply 2: Thank you for the feedback, we have included an analysis of LUX-lung and UNICORN studies and our rationale for selecting osimertinib over afatinib. Page 12, Line 186

Minor revision

Comment 3: Were there no other metastatic sites for Patient 1?
Reply 3: The patient had cytology positive for a malignant left pleural effusion. Apart from the left upper lobe primary there were no other sites of metastatic disease.

Comment 4: What is the clinical Stage of Patient 2?
Reply 4: Stage IV, additional information has been included (Page 9, line 140)
Changes in the text: “lymphadenopathy as well as metastasis to T8 vertebral body, right adrenal gland and gluteus muscle”

Reviewer C

Wang et al. describes an important case report of clinical efficacy of osimertinib in two NSCLC-patients with uncommon EGFR genetic aberrations. The clinical efficacy is obvious in one of the patients harbouring an L861R mutation while some response is detected in the second patient harbouring an aberration in exon 18. The novelty of this case report in relation to the third generation EGFR TKI osimertinib and uncommon EGFR mutations merits publication.
Thank you for your review and comments.
Minor comment:

Comment 1: The authors should make sure that all figure panels are mentioned in the text, currently this is not the case.
Reply 1: This has been amended Page 9, Line 137

Reviewer D

The case report provides a concise overview of a study exploring the efficacy of the third-generation tyrosine kinase inhibitor (TKI) osimertinib in non-small cell lung cancer (NSCLC) patients with uncommon EGFR mutations, specifically L861R and exon 18 deletion-insertion mutations. While the manuscript effectively communicates the main findings, there are a few points that could be addressed for improvement:

1. Case Descriptions
Comment 1: The case descriptions are informative, but additional details on patient characteristics, underlying diseases, concurrent medications and maybe any other adverse events could enhance the depth of the findings. Including these details would provide a more comprehensive picture of the patients' experiences.
Reply 1: Thank you for your comments, past medical history of relevance has been included Page 5, line 97 and Page 9, line 137

2. Outcome Measures
Comment 2: While authors mention the duration of response for each patient, it would be beneficial to include additional relevant biomarker analyses in NGS that could potentially impact the prognosis. This would provide a more comprehensive understanding of the treatment outcomes.

Reply 2: We have included co-existing TP53 mutation in the Discussion section which has been shown to be a poor prognostic feature. (Page14, Line 236)
Changes in the text: “We hypothesise that the reason patient B experienced a relatively short PFS was due to the mixed adenosquamous histology which has been shown to confer a poorer prognosis”

3. Discussion
Comment 3: The conclusion is well-stated, emphasizing the initial clinical efficacy of first-line osimertinib in treating NSCLC with uncommon EGFR mutations. However, a brief mention of the study's limitations or avenues for future research could add depth to the conclusion.
Reply 3: Thank you, the limitations of our study include it being a case series, and the inability to re-biopsy patient B on progression to identify resistance mechanisms. (Page 14, Line 240)
Changes in the text: “Our study is limited as it is a case series and we did not attempt a re-biopsy in patient B or use ctDNA analysis at time of disease progression as he needed to promptly commence second line chemotherapy.”

Comment 4: It would be advantageous for readers to discern the distinctions inherent in these two case reports, given the evident variability in the response exhibited by each patient. The inclusion of scientific elucidations pertinent to this context, such as potential underlying diseases or relevant next-generation sequencing (NGS) results, would significantly enhance the comprehension of these cases.
Reply 4: Thank you, these have been included in the Case Report

Reviewer E
In this manuscript, the authors reported clinical efficacy of osimertinib in NSCLC harboring uncommon EGFR L861R and EGFR exon 18 deletion-insertion mutations. I think this report is well written without excesses and does not require further revision
Thank you for your feedback.

Reviewer F
The authors describe 2 patients with metastatic lung adenocarcinoma harboring uncommon EGFR mutations (patient 1: exon 21 L861R; patient 2: exon 18 deletion-insertion) who were treated with osimertinib.
While the subject matter is of interest, the manuscript could be more clearly organized:
Comment 1: The title should indicate exon 21 L861R mutation (not just L861R).
Reply 1: We have amended the title and have also added the word “EGFR” in line with suggestion Reviewer F. (Page 1 Line 3) In addition we have also reviewed the manuscript and have included the word EGFR throughout the entire manuscript

Comment 2: The introduction should provide more of a background regarding the incidence and nature of the 2 mutations described.
Reply 2: Thank you for your feedback. This has been included in the Introduction Page 5, line 87
Changes in the text: “Both EGFR exon 21 L861R and EGFR exon 18 deletion-insertion are

rare with an estimated prevalence of <1% and 0.3% respectively thus creating a challenge to determine whether these subtypes of EGFR mutations will respond to TKI therapy"

Comment 3: The conclusion should focus almost entirely on the 2 mutations being studied. The lengthy paragraphs regarding other uncommon mutations could be condensed as these are less relevant.
Reply 3: This is acknowledged by the authors.

Reviewer G
The authors wrote a short case report on osimertinib efficacy in uncommon EGFR mutations. I would recommend accepting the manuscript for publication after answering the following minor questions:

Comment 1: is there anything known on osimertinib plasma levels; has pharmacokinetic sampling been performed?
Reply 1: Thank you for your feedback. Osimertinib plasma levels are not routinely assessed in Australia as part of routine clinical practice and were not performed for these two patients and has been mentioned as a study limitation. Page 14, Line 240

Comment 2: was a biopsy done in the second patient with progression? Otherwise, circulating tumour DNA analysis? Is anything known on the resistance mechanism?
Reply 2: Due to the rapid progression of symptoms, a repeat biopsy or ctDNA analysis for patient #2 were not performed. Page 14, Line 240

Comment 3: based on what evidence was osimertinib treatment initiated? Any literature on other TKIs that were considered?
Reply 3: There have been some recent studies (Bar J, et al. J Thorac Oncol 2023; Okuma Y, et al. JAMA Oncol 2023) to support the use of Osimertinib in uncommon EGFR mutations. There has been previously reported preclinical data that may suggest exon 18 mutations are more sensitive to 2nd generation EGFR TKI Afatinib which was also considered during clinical decision making for patient #2 (Kobayashi Y, et al. Clin Cancer Res 2015). Page 12, Line 186

Comment 4: the authors conclude "Given uncommon EGFR mutations show variable sensitivity to treatment, this further highlights the importance of molecular tumor boards to assist in clinical decision making." However, with scarce evidence, what would a MTB contribute extra? Please specify.
Reply 4: Although an MTB was not utilized in either of these patients, we would suggest that for oncologists managing patients with suspected resistance to EGFR TKIs in a community setting, an MTB may give guidance on resistance mechanisms and subsequent treatment options.

In addition to the above reviews. We have updated the follow up period for Patient A. At time of writing with a follow up period of 18 months Patient A remains stable on treatment. (Abstract Page 3, Line 61)