Peer Review File

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Reviewer A
This case report describes a shared EGFR L861Q mutation between two different sites representing NSCLC and SCLC. The case report is important since it highlights the importance of multiple biopsies as well as evaluation through genetic profiling, especially given the limited treatment options for patients with SCLC. The discovery of rare genetic variants, as the one described in this case report, could open up for targeted therapy to such patients, and potentially for prolonged patient survival.

I recommend this manuscript to be accepted for publication.

Reviewer B
This case report refers to de novo transformation to SCLC in EGFR-mutated NSCLC.
We usually experience the transformation to SCLC as resistant mechanisms against prior EGFR TKI in EGFR-mutated NSCLC and rarely de novo transformation to SCLC in EGFR-mutated NSCLC. As the authors noted, the transformation to SCLC is known to be associated with some genomic alterations, including RB1 loss and p53 mutations.

Comment 1: Small cell lung cancer is known rarely to have EGFR mutation. Is the following scenario possible? Initially, combined small cell cancer with NSCLC component harboring EGFR L861Q mutation occurred, and then, the components of small cell carcinoma metastasize to the femur and the components of NSCLC to the axillary lymph node. Please consider it based on the results of molecular profiling,

Reply 1: We appreciate your comment. We agree this is a possibility, and we have now included a line in the discussion to state this possibility. However, given the rarity of EGFR mutation reported in SCLC and the known ability of NSCLC to transform, this case likely represented a transformation with the EGFR originating from the NSCLC. The presence of the rare EGFR mutation and the presence of the RB1 mutation supports the transformation theory rather than the co-occurance one.
Changes in Text: Please see section “Molecular Tumor Board Discussion” lines 97-126.

Comment 2: This patient received only carboplatin and etoposide (CE therapy) for SCLC. Is it impossible to undergo concurrent therapy of CE therapy and EGFR TKI? Please discuss the possibility of combination therapy as well as sequential therapy.
Reply 2: Data supporting combining tyrosine kinase inhibitors and chemotherapy is sparse in the context of EGFR mutation positive co-occurring NSCLC and SCLC. Many large-scale phases III randomized controlled trials have shown that adding chemotherapy to EGFR-TKIs did not improve survival benefit in NSCLC. On the contrary, recent studies have emonstrated that EGFR TKI combined with chemotherapy has superior efficacy compared to EGFR TKI alone in advanced EGFR mutated NSCLC. Therefore, adding a TKI to chemotherapy is still controversial and ongoing

trials will hopefully answer this question with more clarity. Nevertheless, the combination has been shown to be more toxic in multiple studies, compared to chemotherapy or TKI alone and for our patient with PS of 2, we wanted to make sure to provide a treatment that was urgently needed with plans to add afatinib after completion of four cycles of chemotherapy.
Changes in Text: Please see section "Molecular Tumor Board Discussion" lines 167-182.

Reviewer C
Overall, this is an interesting case of a less common EGFR mutation in both NSCLC and SCLC tumors, suggesting a common origin.

Comment 1: Whilst interesting, the case report does not maximise the opportunity for discussion. The introduction is brief with important details missing. The discussion and conclusions fail to properly consider the literature and at some points is speculative. Whilst suggesting a combined chemotherapy-EGFR TKI approach in this patient could be considered, the authors fail to fully discuss the literature around this topic or make a compelling argument for this. This is especially important considering that the patient case does not support /outline such an approach.
Reply 1: We thank you for your comments. We have made edits to our manuscript that will hopefully strengthen the learning point of this interesting case. We have elaborated on the topic of combining TKI and chemotherapy with literature support. The focus of this case was the unique presentation of concurrent NSCLC and SCLC, both harboring a rare EGFR mutation. While there is evidence both for and against concurrent use of chemotherapy and TKI in literature, to our knowledge, there is no report of combining TKI with chemotherapy for co-occurring SCLC and NSCLC harboring a rare EGFR mutation. Moreover, second generation EGFR TKIs that are effective against these alterations have not been studied in combination with chemotherapy. The recommendation of the molecular tumor board of a concurrent or sequential combination of chemotherapy and TKI, specifically afatinib was based on theoretical considerations, and adaptation of literature support for combining first and third generation TKIs with chemotherapy. We have made changes to the text to reflect this.
Changes in Text: Please see section "Molecular Tumor Board Discussion" lines 97-126 and 167-182.

Other minor comments:
Comment 2: There is inconsistency throughout the manuscript on using italics to denote EGFR. This negatively impacts the readability of the article. The authors should correct this throughout, ensuring italics are used in the context of genes and not used in instances where EGFR is referring directly to the protein (e.g. when referring to TKIs/therapy).
Reply 2: Thank you for your comment. Italics are represented for the gene and non-italic for the protein.
Changes in Text: Throughout text.

Comment 3: Introduction: the introduction is too brief and does not provide enough background to contextualize your case and discussion. Please consider explaining more clearly the array of activating mutations, including what is most common and sensitizing.

Reply 3: Thank you for this comment. Several sections were added to the manuscript in the intro and discussion section that complement each other. We decided to keep the intro relatively brief as to not duplicate what is in discussion. We added the more commonly sensitizing mutation of exon 19 deletion and exon 21 L858R point mutation.
Changes in Text: Please see "Introduction" lines 58-59.

Comment 4: Abstract line 28: "EGFR is a well-studied driver mutation"
Consider re-writing this sentence for clarity, e.g. *Mutations in EGFR are well-studied and can be targeted with...*
Reply 4: Thank you for the comment. The wording has been changed to reflect the comment.
Changes in Text: Please see "Abstract" line 27-29

Comment 5: Introduction, line 57: "and specifically L861Q has not been reported."
This is misleading, there are case reports of primary SCLC with EGFR L861Q mutation. See Takagi et al. BMC Cancer 2013, https://doi.org/10.1186/1471-2407-13-529
Reply 5: We appreciate the comment and for alerting us to this case report. We have now changed the text to reflect "rarely reported" instead of "not been."
Changes in Text: Please see **"**Introduction" line 57-58.

Comment 6: Introduction, line 57: "Transformation to SCLC"
You have described the frequency of transformation but this sentence does not read well. Please consider rewriting for clarity, explain what you mean by transformation, this most commonly happens following treatment - but you do not mention this.
Reply 6: Thank you for this comment. We will clarify this statement. As you have stated, EGFR mutated SCLC is typically described in patients who have received TKI for NSCLC. However, EGFR-mutation positive SCLC occurring without any preceding diagnosis of NSCLC has been described in literature, The presence of the same non-germline rare EGFR mutation in both NSCLC and SCLC specimens as well as the additional RB1 mutation in the SCLC tissue suggests that this is likely a transformation rather a co-occurrence.
Changes in Text: Please see section "Molecular Tumor Board Discussion" lines 97-126

Comment 7: Case description, line 64: replace "woman" with 'female'
Reply 7: Comment noted.
Changes in Text: Case description, line 68, "woman" replaced with "female"

Comment 8: Discussion, line 131-144: this section in large feels anecdotal and speculative. What is the evidence for ctDNA being used to determine disease burden? What is the evidence for using SUVmax as a marker of disease burden? (Note there are many types of SUV measurements, e.g., mean/max/peak. SUVmax is most reported but SUVpeak or SULpeak - adjusted for LBM, may be used in reporting PERCIST criteria and in clinical trials).
Reply 8: We agree with the reviewer. These statements have been removed, since they are provocative, not evidence-based and do not support the molecular concept of this article.
Changes in Text: This portion of the Discussion has been removed from the text.

Comment 9: Throughout the article... "PET" just refers to the scanner. Please clarify that you mean [18F]FDG PET/CT. Where you have used just 'FDG' this also needs to be changed to [18F]FDG. PETs and radionuclides come in a variety of forms and it is important that this is clear to the reader.
Reply 9: Comment noted
Changes in Text: We have changed all references of PET to [18F]FDG PET/CT and FDG to [18F]FDG throughout the text.

Comment 10: Discussion: You do not make comparisons to other similar case reports or provide much evidence about the treatment/potential treatment paradigms for either NSCLC/SCLC in this case. Whilst using an EGFR TKI in this patient (combination or sequential) seems academically appropriate...this case is of an elderly patient (?PS), with extensive disease including distal bone metastases, with SCLC histology.
Reply 10: Thank you for this comment. We addressed in the case summary the treatment decisions and expanded on this. Treating with a concurrent chemo/TKI approach has not shown to be beneficial and can be harmful in terms of toxicity especially in someone who does not have a robust PS. The decision was to treat sequentially however she expired prior to the start of the TKI. We have made edits to our manuscript that will hopefully strengthen the learning point of this interesting case. We have elaborated on the topic of combining TKI and chemotherapy with literature support. The focus of this case was the unique presentation of concurrent NSCLC and SCLC, both harboring a rare EGFR mutation. While there is evidence both for and against concurrent use of chemotherapy and TKI in literature, to our knowledge, there is no report of combining TKI with chemotherapy for co-occurring SCLC and NSCLC harboring a rare EGFR mutation. Moreover, second generation EGFR TKIs that are effective against these alterations have not been studied in combination with chemotherapy. The recommendation of the molecular tumor board of a concurrent or sequential combination of chemotherapy and TKI, specifically afatinib was based on theoretical considerations, and adaptation of literature support for combining first and third generation TKIs with chemotherapy. We have made changes to the text to reflect this.
Changes in Text: Please see section “Molecular Tumor Board Discussion” lines 97-126 and 167-182.

Comment 11: References within text should be denoted as brackets e.g. (1), rather than superscript, as per journal guidelines.
Reply 11: Comment noted.
Changes in Text: All references within the text have been denoted with brackets.

Comment 12: Reference list is inconsistent. Please refer to the journal guidelines.
Reply 12: References changed to Vancouver style as per Journal guidelines
Changes in Text: References have been updated to the Vancouver style.