Peer Review File

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Reviewer Comments

Reviewer A

An adequate review in ALK+ non-small cell lung cancer (NSCLC). Some observations:

1. Clarify that although 50% of Lung adenocarcinomas and/or NSCLCs harbor oncogenic drivers not all are actionable with available targeted therapies.

Reply: We agree with the Reviewer that 50% could seem an overestimation. Nevertheless, according to the paper by Thai et al Lancet 2021 to which we refer, up to 50% of the cases of non-squamous NSCLC harbor an actionable/targetable oncogenic driver. Their estimation come from the study by Singal G et al. "Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. JAMA 2019; 321: 1391–99." The estimation can be considered quite correct also if acknowledging the incidence of EGFR mutations in Asian population. The comment from the Reviewer was adequate as we had previously forgot to precise that the estimation concerns non-squamous NSCLC as a denominator (and not NSCLC globally).

2. Co-occurrence of other mutations such as TP53 is not described that could influence the prognosis and predict duration of response and/or progression-free survival.

Reply: We thank the Reviewer for this correct comment. We have now addressed the prognostic role of *ALK* fusion variants and co-occurring mutations at the end of Section 4.2.1.

3. Consider including the review article: Zhao et al. New perspectives for targeting therapy in ALK-positive human cancers. Oncogene 2023.

Reply: We read with interest this review, now included among the references.

4. Any information about chemotherapy with or without ALK inhibitors in ALK* NSCLC refractory or relapsed?

Reply: We agree on the relevance of this aspect for the clinical management of patients suffering from ALK+ NSCLC, and we approached it in Sections 4.3 e 4.4.

5. Bypass mechanisms by secondary activation of other receptor tyrosine kinases,

such as MET and EGFR that compensate for the blockade of ALK signaling should be highlighted.

Reply: We thank the Reviewer for pointing out the importance if bypass resistance mechanisms. The point number 2 of Section 4.1.2 now says “Off-target mechanisms of resistance: activation of signaling pathways by molecular events (mutations, fusions, amplifications, non-genetic functional hyper-activation) occurring as bypass (e.g. EGFR, MET activation) or downstream alterations (e.g. *RAS*, *SRC* mutations)”, and includes the reference “MET Alterations Are a Recurring and Actionable Resistance Mechanism in ALK-Positive Lung Cancer” by Dagogo-Jack et al Clin Cancer Res 2020.

6. In ALK+ NSCLC , resistance to ALK TKIs associated with the acquisition of point mutations deserve a detailed explanation including a table.

Reply: We agree with the Reviewer on the relevance of ALK mutations in conferring resistance to ALK inhibitors. Nevertheless, this Review has to deal with biology, molecular diagnostic and therapy of ALK+ NSCLC, that represent a large amount of information. We have mentioned the relevance of on-target resistance mechanisms in Section 4.1.2, and we think that providing a detailed part on ALK mutations would increase the “burden” of the Review itself. Interested readers could address to dedicated Reviews that specifically deal with resistance in this setting. If Editors consider a detailed Section on resistance mutations of interest for this Review, we will provide it.

7. See and review the above-mentioned article Zhao et al. Oncogene 2023. See figure 5 with regards to the development of ALK inhibitors and Table 1.

Reply: We found this Review of clinical interest and, besides having included it as a reference, we used it to validate our statements and considerations with regard to ALK inhibitors.

8. Different EML4-ALK variants have different predictive values. Please list the number of Known ALK fusions.

Reply: We thank the Reviewer for this consideration. EML4::ALK fusion variants have been discussed in Section 2.2 (and reported in Figure 1). According to comment number 2, we have commented about their prognostic role in a new paragraph.

9. Importantly, lack information on liquid biopsy the determination of ALK fusion in bloos assays.

Reply: We agree on the relevance of liquid biopsy in this setting, and Section 3.4 contains the paragraph “Indeed, the identification of actionable oncogenic drivers, including *ALK* gene fusions, on liquid biopsy could represent another method to investigate an alternative source of tumor tissue, particularly when patients present with a high tumor burden. Although *ALK* rearrangements on liquid biopsy does not represent a real-life practice and may lead to false negative results due to insufficient sensitivity, hybrid-capture NGS on plasma circulating free DNA has demonstrated the feasibility of this approach in determining *EML4::ALK* variants in clinical trials”.

Reviewer B

This is a comprehensive and nicely written review.

1. Some minor mistakes could be corrected, e.g. "short harm" in line 127

Reply: We thank the Reviewer for the careful reading of the manuscript. We have corrected the indicated mistake, and provided a new attentive reading to eliminate additional potential errors.

2. section 3.4: the RNA NGS should be mentioned explicitely, because it is more sensitive and actually recommended in addition to DNA NGS for better detection of oncogenic fusions (eg. PMID 34997651).

Reply: We all authors entirely agree on the Reviewer’s comment and we apologize for this missing point. Accordingly, the reference has been included in the text together with some others and a specific comment on the highlighted issue has been consistently fixed in the text. We thank the reviewer for this improving suggestion.

3. The emerging role of different ALK fusion variants, especially v3, and TP53 co-mutations could be also mentioned.

Reply: We thank the Reviewer for this point, that has been addressed also according to point 2 and 8 from Reviewer A.

Reviewer C

The review from Facchinetti et al. address the issue oa ALK inhibitors in the context of ALK+ NSCLC.

It is well written and balanced.

However it puzzle me that treatment strategy is now set in the direction of using the most potent TKI upfront instead of a sequential approach. This is the opposite of what happened for CML, where 90% of patients receive first line imatinib.

Aside from the clear superiority of 2-3 gen TKI in the treatment of CNS disease, there is no controlled study that compared a sequential approach versus the immediate use of more potent TKIs. This issue should be discussed in more details.

There is also insufficient emphasis on the durability of responses in NSCL as opposed to other ALK-driven tumors such as ALCL o IMT.

Reply: We thank the Reviewer for these considerations. In the section 4.2.1, we hve now included a paragraph “The best treatment strategy (sequential use of crizotinib and novel inhibitors versus novel inhibitors upfront) to be adopted in patients suffering from ALK-positive NSCLC has been a matter of discussion in the last years (88). Despite the methodological challenges in interpreting the OS data in ALEX, J-ALEX, ALESIA, and ALTA-1L trials (eagerly waiting for the CROWN ones, Table 2), the clinically meaningful increase in PFS, the activity against CNS disease and the better tolerability profiles favor the upfront use of the novel inhibitors.”

Reply: We feel that addressing the differences observed in ALCL and IMT with regard to the benefit from ALK inhibitor would be of interest, but it could also distract the readers from the focus of the Review (ALK+ NSCLC). The biological and clinical features of ALK-driven diseases, still sharing similarities, are largely dictated by the tissues of origin, and moving from NSCLC would be recommended for a manuscript dealing with the global role of ALK across malignancies.

Minor point.
At least the EGFR acronym should be made explicit.

Reply: We agree on this point, and we have now made the acronym explicit.