Peer Review File

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Reviewer A:
Major comments:

Comment 1: The section on IHC should be prefaced with multiple caveats. To date, there is no equivalent of the D5F3 antibody used in ALK NSCLC for diagnosing ROS1 rearrangements. While IHC is a promising screen, FISH or NGS continue remain within the NCCN Guidelines as the recommended diagnostic tests. While the authors subsequently mention the caveats in Section 2.5, these should be stated up front.

Reply 1: Thank you for your comment.
FISH and NGS effectively represent gold standards for ROS1 rearrangements detection, as discussed in the following sections, although NGS is unfortunately not routinely available in clinical practice anywhere. We have made corresponding revisions to the section on IHC, as pointed.

Comment 2: The NGS section should expand on the trade-offs versus DNA vs RNA based capture as this has relevance to the detection of challenging ROS1 fusions (e.g. ROS1-GOPC).

Reply 2: Thank you for your comment. We revised all section on NGS. We gave more attention to section FISH which is the standard diagnostic method most frequently applied as it is accessible everywhere, respect to NGS.

Comment 3: "Lorlatinib and Repotrectinib, which are potent oral inhibitors of ALK and ROS1, among other targets, achieved promising results against several ROS1 emerging mutations (G2032R, D2033N e S1986Y) resistant to Crizotinib and Ceritinib."
This is wrong. Lorlatinib does not overcome G2032R. Recommend the authors check their references and accurately summarize kinase domain mutations. A table might be helpful here. They can refer to Katayama et al Nature Communications 2019.

Reply 3: Thank you for your observation. The information was corrected, which was also correctly reported in later sections.

Comment 4: I would encourage the authors to add discussion on DS-6051b and NVL-520, as these are emerging ROS1 inhibitors

Reply 4: Thanks for the suggestion, we included a reference to preclinical data on the activity of the new ROS1 inhibitors.

Minor comments:
Comment 1: "It is known to be involved during embryonal development and to have homology with anaplastic lymphoma kinase protein (ALK) greater than 80% in the ATP binding site and kinase domains, but function and expression in adult tissue are uncertain [2]."

This statement is inaccurate. While expressed during development, ROS1 is not involved with embryonal development (in the same as ALK is involved with neural tube development).

Reply 1: Thank you for your comment. The sentence really may appear unclear, what we mean is

that there is homology between the ALK and ROS1 protein in kinase domain; however respect physiological function, while we know that ALK protein is involved in embryonic development, there is no clear information about the role of ROS1 protein. We have revised the sentence in the manuscript.

Comment 2: "FISH is traditionally considered the gold standard for ROS1 and ALK rearrangement identification in lung cancer, using the same criteria; this technique is moderately expensive."

I would disagree. The gold standard would be NGS (ideally through RNA-based capture).

Reply 2: Thank you for your comment. This is a controversial topic, especially in recent years with the increased focus on the molecular biology of lung cancer.
ESMO guidelines recommend FISH as standard approach for ROS1 rearrangement detection and as confirmatory test. NGS is high sensitivity and specificity method and is indicated in ESMO guidelines as an alternative approach for confirming gene fusions. NGS should become the gold standard in clinical practice, however is not available worldwide as first diagnostic approach. Also NCCN guidelines recommend FISH and NGS as diagnostic test for ROS1 rearrangements.

Comment 3: "NGS has high sensitivity and specificity; being able to simultaneously identify a large number of gene alterations using low amounts of samples, not only from FFPE tissue, but also starting from circulating tumor DNA or RNA."

This is incorrect. While circulating cell-free RNA technologies are being explored, to my knowledge, these are not being used in clinical practice. This should be rephrased to circulating tumor DNA.

Reply 3: Thank you very much for your comment. The sentence is effectively unclear, we corrected main text by explaining that NGS can detect a large number of molecular alterations not only from small tissue samples but also from peripheral blood, by thus circulating tumor DNA, which is the benefit of liquid biopsy.
Mention to cell-free RNA technologies is correctly a research issue, not applicable in clinical practice; we revised the main text as you suggested.

Comment 4: "Other TKIs acting against ROS1 alterations, including Brigatinib, Cabozantinib, Ceritinib, Lorlatinib and Repotrectinib have shown clinical efficacy in overcoming resistance due to novel ROS1 genetic mutations."
This sentence is confusing. Are they authors referring to the ability of these drugs to overcome kinase domain mutations?

Reply 4: Thank you for the appropriate comment. What we mean is that in addition to Crizotinib, there are other TKIs, including Brigatinib, Cabozantinib, Ceritinib, Lorlatinib and Repotrectinib, effective against ROS1 rearrangement in advanced lung cancer. Some of these have limited activity against resistant mutations of kinase domain after first line with Crizotinib. We have write more clearly this part of text.

Reviewer B:

Comment 1: 19 Authors for a narrative review on a subject that is not a novelty seems to me an aberration.

Reply 1: Thank you for your comment. We are agree with you that 19 authors is a high numbers, but we guarantee that all authors contribute to this paper

Comment 2: In the abstract, Authors state that crizotinib and entrectinib are approved. As explained in the text, this is true for EMA, not for FDA.

Reply 2: Thanks for your helpful observation; we have modified the abstract, describing EMA and FDA approval as in the text.

Comment 3: I do not like how introduction is framed. Authors do not need to make a structured introduction with background, methods, objectives etc.

Reply 3: Thank you for your observation. The journal required a 'Methods' section within the main text, so to avoid section repetition between abstract and text-introduction, we elaborated a 'Methods' sub-section outside the introduction section and revised the same introduction.

Comment 4: "The PubMed database was searched principally using the keywords "Non-small cell lung cancer", "ROS1 rearrangements". We excluded articles not published in English."
Is this a systematic review? This could be suggested by Table 1 as well.

Reply 4: This is not a systematic review, but new guideline of the journal request that also narrative review report keywords of pubmed research.

Comment 5: ROS1 Biological signaling pathway and detection: this chapter is a mix of biology-clinical features-pathological features-coalterations etc.
The title should represents this.

Reply 5: Thank you for the observation. We revised the title of the paragraph, to reflect more the content of the text.

Comment 6: I am quite skeptical regarding the non-mutual exclusivity of ROS1 rearrangements with other molecular driver events. I consider the reference 15: ROS1 Fusions Rarely Overlap with Other 453 Oncogenic Drivers in Non–Small Cell Lung Cancer. Lin JJ et al JTO 2017 as crucial in this case.
I interpreted this study as a "response" to ref 18: High Prevalence of Concomitant Oncogene Mutations in Prospectively Identified Patients with ROS1-Positive Metastatic Lung Cancer. Wiesweg et al. JTO 2017.

Reply 6: Thank you for your comment.
The reference to the cited studies was not correctly explained in the text. Lin JJ et al showed that the rare overlap of ROS1 rearrangement and other driver mutations is not confirmed by further diagnostic methods, effectively giving an answer to the study in Ref. 15.
We revised the text with these indications.

Comment 7: Refs 5,15,17,18 refer to the sentence "With regards to molecular setting, ROS1-positive NSCLCs are usually wild-type for EGFR and do not harbor ALK rearrangements, but in few cases of lung cancer alterations of ROS1, ALK and EGFR mutations may be coexistent".
Refs 5 and 15 are not appropriate in this space.

Reply 7: Thank you for your comment. I agree with ref number 5, not really relevant to the concept

developed, however ref number 15 relates to a cohort study about the rarity of overlap between ROS1 rearrangement and other driver mutations in NSCLC.

Comment 8: 2.2. ROS1 rearrangement diagnosis in FISH: Authors spend 10 lines on break-apart pattern and they do not describe atypical pattern.

Reply 8: thank you for your comment. We have corrected the paragraph.

Comment 9: When dealing with inhibitors, there are several references of trials in ALK-positive NSCLC and resistance in ALK-positive disease. These should be avoided as out of context.

Reply 9: Thank you for your comment. Available studies related to the efficacy of different inhibitors in advanced ROS1-positive NSCLC are mentioned and described; however we find appropriate reference to studies on translocated ALK disease, because many drugs (Crizotinib, Lorlatinib) have been first investigated, indicated and approved in this context.

Comment 10: Authors should clearly report the potential contribution of liquid biopsy, as they put some references on that.

Reply 10: Thank you for relevant observation. The topic of liquid biopsy is becoming relevant also in clinical practice, particularly in lung cancer where molecular characterisation have therapeutic and prognostic value; however currently is not applied as standard for molecular detection because NGS is not ever available and diagnosis confirmation on tissue samples is still relevant.

Comment 11: I am very skeptical about the role of lorlatinib against G2032R mutations, as both preclinical evidence and clinical trials (Shaw et Lancet Oncology 2019) sustain the opposite.

Reply 11: Thank you for your correct observation. We have revised text accurately on molecular targets of resistance.

Comment 12: Lorlatinib and repotrectinib are the most promising agents for ROS1. More space should be dedicated to them, with a clear reporting of activity, efficacy and toxicity.

Reply 12: Thank you for comment. Lorlatinib and Repotrectinib have promising efficacy data in ROS1-positive disease, however they are not yet approved and not available in clinical practice. Their efficacy and toxicity profile, which overlaps with other TKIs, has been developed in the manuscript, but we give more attention in this review to agents currently approved and available in clinical practice, especially in report of safety profile.

Comment 13: The title of the review is “Current state of the art on the diagnosis and treatment of ROS1-rearranged non-small cell lung cancer. A Narrative Review.”
Authors only deal with molecular diagnosis and TKI. They should address other current issues like how to treat ROS1-positive NSCLC patients besides TKI.

Reply13: Thank you for observation. Aim of this review should be to describing principal methods for ROS1-rearrangements detection. About treatment we focused on target therapy and on efficacy data and safety of main TKIs investigated, considering currently importance of molecular typing of advanced NSCLC and role and prognostic impact of target therapy.
We changed the title to indicate that the topic of treatment developed in this paper is target therapy.

Comment 14: "According to recently published data, Lorlatinib and Repotrectinib are highly effective ROS1 inhibitors, due to high activity on central nervous system (CNS) disease and also to the ability to overcome resistance mutations. While the use of Repotrectinib in clinical practice is still experimental, Lorlatinib currently represents the preferred second-line treatment for ROS1-positive, pretreated NSCLC, due to its ability to overcome acquired resistance [98,99]."
This paragraph is a repetition of what has been just stated above.

Reply 14: Thank you for your observation. We modified manuscript as to streamline and not repeat concepts.

Comment 15: "To date, despite the consolidated availability of targeted agents, many patients are still not tested for the four most frequent molecular targets, which include EGFR, ALK, BRAF and ROS1. Quite surprisingly, only approximately 50% of patients are being tested in Europe for all the aforementioned targets, in addition to PD-L1 expression. ROS1 is tested in approximately 70-75% of cases [100]." I agree on the concept that molecular diagnosis is crucial, but Authors should report references for their statements. Ref 100 is "Biomarker tissue journey among patients (pts) with untreated metastatic non-small cell lung cancer (mNSCLC) in the U.S. Oncology Network community practices.", is only an abstract and refers to US and not to Europe.

Reply 15: Thank you for your comment. We have correted and we added a reference about frequency of testing molecular alteration in Europe

Comment 16: Table 3 is not accurate, as for example entrectinib is a bad inhibitor of ROS1-positive, mutated tumors.

Reply 16: Thank you for your comment. Table 3 is a summary overview of TKIs and their molecular targets, including ROS1 regardless of high or low activity, which is specified in the text. However, we modified the table revising resistance mutations target.

Comment 17: Table 4 is not accurate, as for example the last evidence of lorlatinib in ROS1+ disease is from Shaw Lancet Oncol 2019.

Reply 17: Thank you for your observation, we revised table 4.

Comment 18: Authors should carefully review the content of both Table 3 and 4 for correctness and completeness, even more as their approach seems to be "systematic".

Reply 18: Thank you for your comment. Table 4 has no systematic literature review approach, but is a summary table of studies cited in the manuscript on respective TKIs and their efficacy results demonstrated in ROS1-positive NSCLC. About Table 3, aims to present TKIs and their molecular targets, including ROS1.