Peer Review File
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Reviewer A
Comment 1. lines 120-122 ("and surprisingly detection rate is influenced by the type of assay (lowest for polymerase chain reaction [PCR], intermediate for NGS and highest for fluorescence in situ hybridization [FISH] and immunohistochemistry [IHC]) -> please consider modifying this statement, because it is misleading. The cited reference (ref. 28) did not compare the three methods against each other, for example, the slightly higher positivity rate for FISH (3.0%) compared to NGS (2.1%) could be because FISH was used as a confirmatory assay for positive IHC results in some cases considered in this work. There are proper direct comparisons for the three methods (e.g. https://pubmed.ncbi.nlm.nih.gov/31027700/) which clearly show that FISH has a lower sensitivity than NGS, and IHC more false positives than NGS. I suggest that the authors modify the mentioned passage (lines 120-122) and other pertinent sections of the manuscript accordingly.

Reply 1: We have deleted the inaccurate sentence and included the new reference.

Comment 2. line 224 ("is the panel partner-agnostic?") -> Please consider rephrasing. Strictly speaking, this is not a property of the panel, but of the sequencing method / assay technology used. Hybrid-capture based NGS, anchored multiplex PCR, and the exon tiling assay (with 5/3-ratio) are three examples of methods to identify fusions with unknown partners.

Reply 2: We have used “sequencing method” instead of “panel”.
Changes in the text: “[1] is the sequencing method partner-agnostic?”

Comment 3. lines 232-233: EML4-ALK variant 3 is not only associated with more resistance mutations, but also with a more aggressive disease and shorter survival, which are clinically more important endpoints (e.g. https://pubmed.ncbi.nlm.nih.gov/29363116/)

Reply 3: We have modified the sentence accordingly and included the above reference.
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Changes in the text: “For example, *EML4-ALK* variant 3 is associated with aggressive disease, shorter survival and more resistance mutations.”

Comment 4. An important aspect is missing in this analysis: the fact that with the large number of genes to be screened for alterations in newly diagnosed patients with advanced NSCLC, NGS is the only practicable method. It is not possible to use non-NGS methods for all of the following genes from the same patient: ALK, ROS1, NTRK, RET, EGFR, BRAF, MET, HER2, KRAS, because, with several slides necessary for

each method, the tissue available from the small biopsies (which are standard in case of metastatic disease) will run out before testing can be completed. Therefore, parallel testing via NGS becomes necessary. Fortunately, NGS has also better performance characteristics than FISH and IHC for the testing of ALK (ase outlined in the point 2 above). The manuscript in the current form considers ALK testing alone, as though it were isolated from the need to test any other biomarker in advanced NSCLC, which is not true. There are also some recent and broader reviews of oncogenic fusion testing in NSCLC, which could be cited, e.g. https://pubmed.ncbi.nlm.nih.gov/34997651/.

Reply 4: We completely agree with the reviewer as we state throughout the manuscript:
"Although broad molecular profiling is usually the recommended testing option in most guidelines, NGS is not universally available."
"NGS should be performed using a clinically validated NGS panel, which ideally should include all guideline recommended biomarkers for patients with advanced NSCLC."
"Although most guidelines recommended broad molecular profiling for patients with advanced NSCLC, single-gene assays are still widely used across the globe. Therefore, the key factor for success (i.e., increase testing rates and avoid false positive and false negative results) is to develop sensible testing algorithms, that level the advantages and disadvantages of the different methodologies. This approach can be reconciled with the fact that RNA sequencing is now becoming the gold standard for fusion identification, because of its superior sensitivity."
To further emphasize the message, we have replaced "*ALK*" by "predictive biomarkers" in the last paragraph of the introduction and cited the suggested reference alongside the recently released IASLC atlas.
Changes in the text: "Results: In-depth knowledge of the different *ALK* testing methodologies can help clinical and molecular tumor boards implement and maintain sensible algorithms for a rapid and effective detection of predictive biomarkers in patients with NSCLC."
Changes in the text: "Therefore, we hypothesized that in-depth knowledge of the advantages and disadvantages of the different *ALK* testing methodologies can help clinical and molecular tumor boards implement and maintain sensible algorithms for a 3 rapid and effective detection of predictive biomarkers in patients with NSCLC, regardless of the histologic subtype."

Comment 5: Optional: one important aspect that could be added is that the field is moving towards the NGS testing of all metastatic NSCLC, regardless of histology (e.g. the ASCO recommendations from 2022, https://ascopubs.org/doi/full/10.1200/JCO.21.02767) in contrast to earlier recommendations to test only non-squamous tumors (https://www.annalsofoncology.org/article/S0923-7534(20)39971-3/fulltext)

Reply 5: We completely agree, and have now included this statement and the suggested reference.

Comment 6. Abstract: please consider adding a Results section, summarizing what was found, and formulating more concretely how ALK testing should be performed / could be improved for newly diagnosed NSCLC patients today (only vague statements are included in the abstract currently).

Reply 6: Following the reviewer´s suggestion, we have included a Results section in the abstract.
Changes in the text: "Results: In-depth knowledge of the different *ALK* testing methodologies can help clinical and molecular tumor boards implement and maintain sensible algorithms for a rapid and effective detection of predictive biomarkers in patients with NSCLC."

Reviewer B
Comment 1. In Main body, they mentioned the false positive and negative results. However, they did not mention the NGS. Please add to discuss it.

Reply 1: We have specified that when we mention PCR-based methods, NGS is also within that group. Accordingly, there is now a sentence summarizing the topic, both for false-negative and false positive results.
Changes in the text:
"When using PCR-based assays (real-time PCR or NGS), a precise knowledge of the breadth of the kit/panel and its real-world performance can help rule-out a false negative result."
"Regarding PCR-based methods (real-time PCR or NGS), when an *ALK* fusion is identified by expression imbalance or with a low number of reads, orthogonal testing is recommended to improve specificity."

Comment 2. Please describe with some information how IHC or FISH testing should be performed if the NGS is negative for the ALK fusion gene.

Reply 2: See comment below.

3. The author states multimodality testing contributes to more accurate testing for ALK fusion detection. Please mention recommended workflow for these testing in a clinical setting, based on some guidelines.

Reply 3: We have included a new sentence and a reference to a recently released multidisciplinary algorithm from the IASLC, for which the last author has contributed.
Changes in the text: "In such circumstances, reflex testing with IHC, FISH or a different/larger NGS panel is strongly recommended."

Comment 4
a. In page 6, line 137, the period is needed to be inserted after "...-46)".

b. In Figure 1 and 2, "Is" is recommended (change lowercase to uppercase).
c. Please delete the wavy underlines in Figure 1 and 2.
d. In page 10, line 256, period is not needed.
e. In page 11, line 263, they showed that FISH slides "than" only show the atypical pattern of positivity. Is it a typo for "that" not "than"?
f. In Figure 2, line 308, (F): "An" small cell lung carcinoma is a typo for "a" small cell lung carcinoma.

Reply 4: We have made all the suggested changes according to the minor comments.