Next-Generation Sequencing and Immunohistochemistry as Future Gold Standard of ALK Testing in Lung Cancer?

We read with much interest the study by Pekar-Zlotin et al. [1] concerning the use of fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), and next-generation sequencing (NGS) for the detection of EML4-ALK rearrangement in lung cancer. We would like to comment on some issues.

First, this study of 51 patients reported a high percentage of *ALK*-positive/rearranged tumors with a global rate upgraded from 7.8% with FISH alone to 13.7% with the combination of FISH, IHC, and NGS. No multistep oncogenic screening algorithm (i.e., *ALK* testing in only *KRAS* and *EGFR* wild-type tumors for example) leading to a higher *ALK* rearrangement frequency among the tested samples was reported in the methods. Furthermore, the *KRAS* and *EGFR* mutational status of these tumors was not mentioned. This frequency of 13.7% is surprisingly far superior to the 2%–7% reported in lung cancer and, although the authors assume that their cohort may not be representative of their lung cancer population, it remains impressively high [2, 3]. Second, many studies have pointed out a valuable substitution of *ALK* FISH testing by ALK IHC testing in lung cancer, although some other studies noted discrepancies not only between FISH and IHC but also in the clinical response to crizotinib in both $FISH^+IHC^-$ and $FISH^-IHC^+$ tumors [4, 5].

Clinical response to crizotinib therapy was reported in only two of the five discordant cases, that is, in an $IHC^+FISH^-NGS^+$ patient and in an $IHC^+FISH^-NGS^-$ patient, without data concerning a third patient who was $FISH^+IHC^-NGS^-$ [1]. In fact, replacing FISH with IHC would lead to misdiagnosis and inappropriate therapeutic strategy in $FISH^+IHC^-$ patients. Furthermore, the percentages of FISH *ALK*-rearranged cells were not mentioned, raising questions about true *ALK*-negative FISH tumors versus *ALK* FISH borderline tumors (i.e., with a percentage of rearranged cells around the cutoff of 15%).

The authors considered NGS the gold standard in *ALK* testing instead of the U.S. Food and Drug Administration-approved FISH test and reported a high rate of false negativity with FISH. Nevertheless, when looking at the few available data concerning the clinical response of $FISH^-NGS^+$ *ALK* patients to crizotinib, the superiority of NGS compared with FISH is not evident. Pekar-Zlotin et al. [1] reported a $IHC^+FISH^-NGS^-$ patient with partial clinical response to crizotinib. Two $FISH^-NGS^+$ *ALK* patients with no response to crizotinib were reported by Ali et al. [6]. These cases are examples of actual limitations to consider NGS as a new gold standard in *ALK* testing.

To conclude, we agree that NGS offers a great opportunity to obtain additional data concerning the molecular mechanisms of cancer and to target oncogenic pathways in lung cancer. Nevertheless, we think that it should be considered more as a supplementary test associated with FISH and IHC combined *ALK* testing rather than as the new gold standard method. Faced with technique drawbacks and challenging biopsy samples, this combination of the three methods appears a more effective screening tool in an intent-to-treat strategy.

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Disclosures

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