Validation of an IHC screening tool for ROS1 gene rearrangements

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Immunohistochemistry (IHC) is an effective tool that can be used for identifying proto-oncogene 1 receptor tyrosine kinase (ROS1) gene rearrangements and screening patients for the administration of the targeted therapy crizotinib, a small-molecule tyrosine kinase inhibitor.

Chromosomal rearrangements of the gene encoding ROS1 occur in approximately 1-2% of adenocarcinoma lung cancer. Flourescence in-situ hybridization (FISH) is currently the gold-standard screening method to identify ROS1 gene rearrangements in patients that can receive ROS1 targeted therapy with crizotinib. However, FISH is a laborious and expensive option for a screening setting. Using immunohistochemistry (IHC) as a screening tool for ROS1 gene rearrangements may be a faster, more cost-efficient approach that could be used as a first-line screening option.

Investigators from the United Kingdom recruited 170 patients to phase 1 of the Cancer Research UK-Stratified Medicine Project (CRUK-SMP). Patient tissue samples were screened for ROS1 gene rearrangements using a Dako EnVision IHC system with the ROS1 D4D6 antibody and by FISH analysis. IHC results were scored semi-quantitatively as negative, or weakly, moderately or strongly positive, along with the percentage of positive cells. An h-score was then generated with a score of >100 viewed as positive. FISH analysis was performed on samples without the knowledge of ROS1 IHC status.

The results published in the *Journal of Thoracic Oncology*, the official

journal of the International Association for the Study of Lung Cancer (IASLC), revealed that from the 170 patients, 103 were selected for further analysis based on negative testing for EGRF, KRAS, and/or BRAF mutations, as well as ALK translocations. Of the 103 cases, 39 were adenocarcinomas, 39 squamous cell carcinomas, 5 small cell carcinoma, 2 adenosquamous carcinoma, 3 pleomorphic carcinomas, 4 large cell carcinoma, 2 large cell neuroendocrine cell carcinoma, 3 non-small cell lung cancer (NSCLC), and 6 carcinoids. Of all the samples tested, 2 adenocarcinoma cases tested positive for ROS1 gene rearrangements using IHC and 1 of the 2 was positive for ROS1 by FISH analysis. Due to the low number of cases in the screening cohort, the results from the screen were combined with 4 cases from the diagnostic archive that tested positive for ROS1 gene rearrangement using FISH. The overall specificity of the validation study was 83% (95% CI 86-100) with a sensitivity of 100% (95% CI 48-100). Four of the patients that tested positive for ROS1 gene rearrangements using IHC showed partial response when treated with crizotinib.

The authors comment that, "The main limitation of the study is that the number of cases proving positive for the ROS1 gene rearrangement was low, necessitating enrichment from the diagnostic archive to support the high level of sensitivity. However, only one prior publication has more than 10 cases, highlighting the rarity of this gene rearrangement and the need for cost-efficient screening. With a high sensitivity rate and relatively high specificity rate, IHC screening to identify patients that might harbor ROS1 gene rearrangements is feasible and would be less expensive and time consuming than FISH testing, which could be reserved for a confirmatory second step."

More information: Patrizia Viola et al. A Validation Study for the Use of ROS1 Immunohistochemical Staining in Screening for ROS1 Translocations in Lung Cancer, *Journal of Thoracic Oncology* (2016). DOI: 10.1016/j.jtho.2016.03.019

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