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Interpretation of Anti-ALK Immunohistochemistry Results

In their report,[1] Conklin et al. compared five antianaplastic lymphoma kinase immunohistochemistry (ALK IHC) systems. I agree with their conclusion that IHC is reliable for detection of *ALK* rearrangement; however, I would like to comment on their interpretation of individual results.

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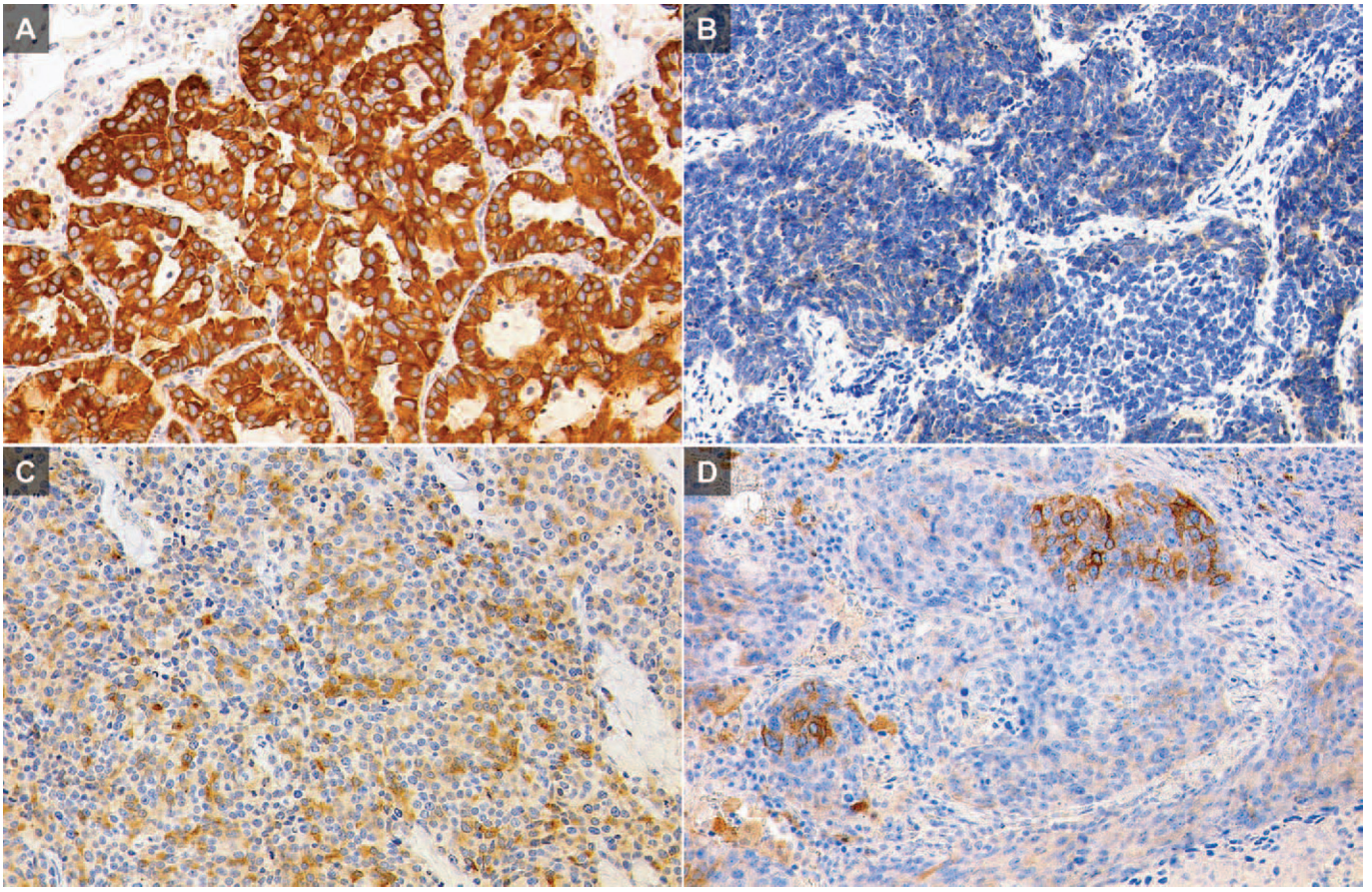

FIGURE 1. ALK iAEP IHC in lung cancers with or without ALK fusion genes. Expression of ALK fusion protein is dependent on the promoter-enhancer activity of ALK partner genes, including EML4, which are usually housekeeping genes. In ALK fusion-positive tumors, therefore, all tumor cells are immunostained for the ALK kinase domain in IHC using anti-ALK antibody directed to the kinase domain, when stained appropriately by using a highly sensitive method, such as iAEP. All the cancer cells in an EML4–ALK-positive lung cancer express EML4-ALK protein (A). Some lung cancer cases (<1%) may express full length ALK. In such a case, the staining intensity usually varies from cell to cell, showing staining heterogeneity. This heterogeneity is probably because ALK expression in these cases is physiological, as in normal nerve cells, and because the ALK promoter-enhancer activity varies among cells. ALK rearrangement-negative small-cell carcinoma (B), large-cell neuroendocrine carcinoma (C), and poorly differentiated carcinoma (squamous cell carcinoma in this case, D) of lung may sometimes express the full length ALK, and the staining pattern is usually heterogeneous. IHC, immunohistochemistry; ALK, anaplastic lymphoma kinase; iAEP, intercalated antibody-enhanced polymer.

They determined the sensitivity and specificity of five systems including "any ALK expression by IHC on tissue microarray (TMA) or whole section (WS)," using fluorescent in situ hybridization (FISH) on WS as the standard.[1] According to their criteria, both sensitivity and specificity of ALK1-ADVANCE should be 100%; however, these values are stated as 66% and 87.5%, respectively, in Table 3.[1]

A TMA specimen corresponds to a part of the WS. Therefore, if a TMA scores 2+, the corresponding WS should score 2+ focal (heterogeneity) or higher. If a WS scores 2+ diffuse, the corresponding TMA always scores 2+. Interestingly, for the 5A4-Histofine staining in cases 10 and 11, the TMA and WS results are different by two scores (2+ or 0) (Tables 1 and 2).[1] This score difference might be because of either serious staining errors, or accidental interchanging of the results because such high difference is unlikely unless heterogeneity exists in the WS, and actually, heterogeneity was not observed in the WS. Similarly, the score difference of ALK1-ADVANCE for case 3 is also unlikely. Therefore, I would like the authors to check these results and restain the sections.

For ALK1 staining, the TMA and WS scores show discordance (1+ or 0) for cases 3 and 11. Such discordance may occur because the observer struggled to determine whether the faint positivity of score 1+ was real positivity, unlike the readily detectable staining of score 2+ and 3+, as mentioned by the authors.[1] Given that, would it be appropriate to define score 1+ as positive while calculating sensitivity? From this point of view, in case 11, the best and practical sensitivity was obtained only with 5A4-Histofine staining—a readily detectable staining of score 2+—whereas with other stains, the scores were either 1+ or 0 (Table 2).[1]

In my published[2,3] and unpublished records for anti-ALK IHC of more than 4500 lung cancer cases by using the intercalated antibody-enhanced polymer (iAEP) method,[3] a highly-sensitive method on which the 5A4-Histofine staining is based, almost all cancer cells were stained in more than 300 *ALK*-rearranged cases. This staining homogeneity supports the view that all tumor cells of *ALK*-rearranged tumors harbor *ALK* rearrangement.[4] Wild-type ALK is weakly expressed physiologically in normal nerve cells.[5] Therefore, lung cancers without *ALK* rearrangement sometimes show positivity in highly sensitive anti-ALK IHC, such as the iAEP method, especially in cases with neuroendocrine differentiation (small-cell, large-cell neuroendocrine, and other carcinomas with focally neuroendocrine differentiation).[2] However, unlike in *ALK*-rearranged cases, the staining pattern in these cases is usually heterogeneous probably because the physiological expression status varies from cell to cell (Fig. 1). In highly sensitive anti-ALK IHC for detection of *ALK* rearrangement, therefore, a heterogeneous staining pattern should not be interpreted as *positive for ALK rearrangement*, but should be considered *probably negative for ALK rearrangement*, and then be confirmed through FISH. This anti-ALK IHC interpretation would have made the specificities of 5A4-ADVANCE and 5A4-Histofine 100% (Table 2).[1]

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