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**Title:** The art of detecting EML4-ALK gene rearrangements in NSCLC patients – Immediate implications for patient care

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**Body:** Background and aims: Patients with lung adenocarcinoma carrying the EML4-ALK rearrangement show dramatic response to crizotinib. There are several methods for identifying this rearrangement, the currently approved being fluorescence in situ hybridization (FISH). Other methods include: gene sequencing and immunohistochemistry (IHC).The purpose of this study is to examine IHC as an alternative method for detecting EML4-ALK translocation. Methods: All the slides were tested by FISH and IHC using D5F3 antibody. When the FISH and IHC results were not identical, the samples were sequenced by "FoundationOne" next-generation sequencing (NGS) based assay Results: 58 consecutive NSCLC adenocarcinoma samples referred for ALK FISH testing were included in the study. 52 had sufficient tissue for further ALK IHC study. Four (6.9%) were positive by FISH; Seven (12%) were positive by IHC while three of them were both FISH+ and IHC+. One sample was FISH+ and IHC-. NGS was done for the mismatched samples (IHC(+); FISH(-); N=4) showing positive ALK rearrangement. Two of the samples were found to harbor a unique novel ALK rearrangement at intron 19. One of the patients that harbor this unique rearrangement showed a complete respond. Conclusion: This study provides evidence to support change of practice when ALK rearrangement detection is required. We recommend ALK IHC as a first screener and NGS as a validated method when IHC is not diagnostic. Due to the significant clinical benefit for patients whose tumor harbor ALK rearrangement, it is extremely important to keep the clinical senses alert and to combine different methodologies for a better personalized medicine.

