

**Concordance between immunohistochemistry (clone 5A4), ALK testing (clone D5F3-Ventana®) and FISH (Vysis) in patients with advanced-stage lung adenocarcinoma without EGFR, KRAS or BRAF mutations.**

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**Background:** Anaplastic Lymphoma Kinase (ALK) translocations are a novel molecular target in a subpopulation of patients with Non Small Cell Lung Cancer (NSCLC). These alterations occur between 2-6% of Lung Adenocarcinomas. Fluorescent In Situ Hybridization (FISH) was established as the gold standard test for detecting ALK positive cases, but recently immunohistochemistry (IHC) was approved as the gold standard for screening.

**Hypothesis:** The objective of the study was to determine the prevalence of positive ALK in a population of patients with stage IIIB-IV lung adenocarcinoma (ADC) and wild type (wt) EGFR, KRAS, and BRAF status. A secondary objective was to correlate three different testing methodologies: immunohistochemistry (IHC) with clone 5A4, ALK IHC Testing with clone D5F3 (Ventana®) and FISH (Pretreatment Reagent Kit (Vysis® & LSI ALK Break Apart Rearrangement Probe Kit (Vysis®)). **Methods:** ALK was studied in 50 patients with stage IIIB/IV lung ADC and wt status for mutations in codons: G12V / D / S / A / C / R, G13D, V14X and G15X the KRAS exon 2; G469A in exon 11 of the BRAF gene; V600E, D594G and K601E of exon 15 of the BRAF gene; G719C / S / A, V689M, N700D, E709K / Q, and S720P exon 18 of the EGFR gene; 746-759 deletions and insertions in exon 19 of the EGFR gene; T790M, D700\_N771; V769L, S768I, V765A and T783A EGFR exon 20 and L858R, L884Q, G863D, N826S, A839T and K846R in the exon 21 of the EGFR gene. ALK positivity was defined as a 3+ score with IHC clone 5A4, presence with any percentage of strong granular cytoplasmic staining in tumor cells positive tumor cells for IHC ALK testing D5F3 and at least 50 cells counted and at least 15% of the counted cells demonstrating separated green and red signals by at least two signal diameter for FISH.

**Results:** The mean age was 64 years (range 31-89), all Caucasian population. 52% were men (M) and 42% were smokers (F). Three patients had ALK translocation (6%) in enriched wt sample. All three samples were positive by FISH and ALK Testing. With clone 5A4, 2 had a 3+ score and 1 had a 1+. Other 2/47 had a 1+ score with the 5A4 IHC being negative with other methods. In conclusion, in our wild type for EGFR, KRAS and BRAF mutations population, 6% were positive for ALK rearrangement with a 100% positivity between FISH (Vysis®) and ALK Testing D5F3 (Ventana®).