# Detection of *ALK* and *ROS1* Rearrangements Using Next Generation Sequencing in Lung Cancer: Comparison between FISH, IHC and NGS

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## BACKGROUND

- Detection of *ALK* and *ROS1* rearrangements in non-small cell lung cancer (NSCLC) is required for directing patient care.
- While fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC) have been established as gold standard methods, next generation sequencing (NGS) platforms are called to be at least equally successful, but also more compatible with multiplexing and diagnostic workflows.
- Our aim was to investigate the performance of NGS in the detection of rearranged cases.

## DESIGN

Forty-two NSCLC samples were selected retrospectively from our database (n= 3.360) based on previous *ALK* (n= 34) and *ROS1* (n= 8) FISH results (positive or inconclusive) and material availability (**Figure 1**).

Cases were tested by both FISH (Abbott Molecular) and IHC (Ventana) in paraffin blocks, and were reviewed centrally to determine the tumor area. DNA and RNA were manually extracted from paraffin sections. Ion Torrent sequencing technology with Oncomine™ Focus Assay (Thermo Fisher Scientific) were applied using 10ng DNA and 100ng RNA from each sample (**Figure 2**).

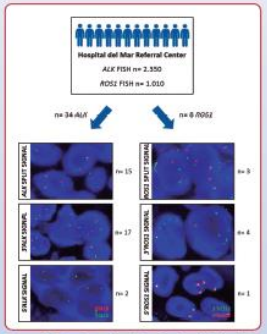


**Figure 1. Sample collection. Our institution has been testing NSCLC samples as a referral center since 2011. We screened all samples by FISH with break-apart probes.**

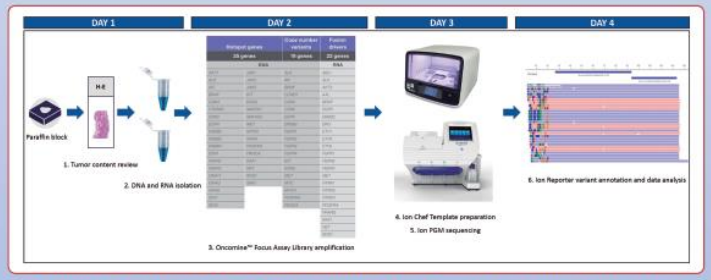


**Figure 2. Oncomine™ Focus Assay workflow. Paraffin blocks were re-evaluated to ensure the minimum tumor area and percentage of infiltration. DNA and RNA were isolated using RecoverAll Total Nucleic Acid Isolation Kit for FFPE and 10ng DNA and 100ng RNA were used to perform Oncomine™ Focus Assay (Thermo Fisher Scientific).**

## RESULTS

Patient's characteristics were median age 60 years, 52% males, and 83% diagnosed as adenocarcinoma (ADC).

Regarding FISH results, 18 cases had split signals, 21 had isolated 3' signals, and three had negative FISH pattern with isolated 5' signals (**Figure 1**). Testing with IHC, nine out of the 42 cases were negative: the three isolated 5' FISH negative and six isolated 3' FISH positive cases (discordance FISH vs. IHC) (**Figure 3**).

NGS technology detected positive *ALK* and *ROS1* fusions in 82% of the assessable samples (27/33), being *EML4*(13)-*ALK*(20) and *EZR*(10)-*ROS1*(34) the most prevalent (**Figure 4**). Nine cases (21%) were non-evaluable by NGS due to insufficient sequencing coverage (seven were small biopsies with low RNA input).

Regarding the six cases with negative NGS result three were the isolated 5' FISH negative cases in accordance with negative IHC, and the other three presented isolated 3' FISH positive pattern, negative by IHC (**Figure 5**).

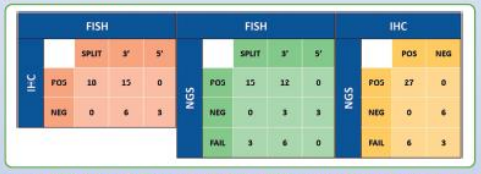


**Figure 3. Comparison between FISH, IHC and NGS. Cases with *ALK* or *ROS1* positive FISH split signals were all positive by IHC and NGS. Discordant cases were restricted to those considered FISH positive with 3' isolated signals.**

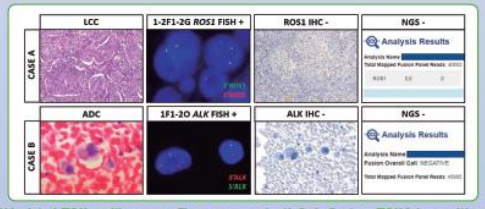


**Figure 5. Discordant 3' isolated FISH positive cases. These cases potentially indicate a FISH false-positive result caused by non-productive rearrangements. Both IHC and NGS are valid alternative tests to check these alterations.**

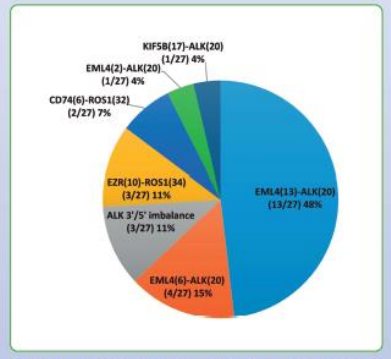


**Figure 4. Fusion gene frequency. In agreement with previous series, *EML4*(13)-*ALK*(20) and *EZR*(10)-*ROS1*(34) fusions were the most prevalent. Remarkably, there were three *ALK* rearranged cases without partner defined (imbalance 3'/5').**

## CONCLUSIONS

- NGS technology for detecting *ALK* and *ROS1* rearrangements in NSCLC could be considered as a screening test although the success rate is closely related to the correct evaluation of the initial amount of tumor tissue, particularly in small biopsies.
- The discordance observed in the isolated 3' FISH positive cases potentially indicates that this alteration could be a FISH false-positive result.
- NGS technology could be used as an additional molecular technique for cases with inconclusive or discordant FISH/IHC results.