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 prospectively from our database (n=3,380) 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 Assays (Thermo Fisher Scientific) were applied using 10ng DNA and 100ng RNA from each sample (Figure 2).

RESULTS
Patient characteristics were median age 60 years, 52% males, and 83% were diagnosed as adenocarcinomas (ADC). Regarding FISH results: 18 cases had split signals, 23 had isolated 3’ signals, and three had negative signals (Figure 1). Testing with IHC, nine out of the 42 cases were negative, the three isolated 3’ FISH negative and six isolated 3’ FISH positive cases (discordance FISH vs. IHC) (Figure 3). NGS technology detected positive ALK and ROS1 fusions in 62% of the assessable samples (27/33), being EML4-ALK (26%) and E2R-ROS1 (15%) the most prevalent (Figure 4). Nine cases (21%) were non-assessable by NGS due to insufficient sequencing coverage (seven were small biopsies with low RNA input).

Regarding the six cases with negative NGS results: three were isolated 3’ FISH negative cases in accordance with negative IHC, and the other three presented isolated 3’ FISH positive pattern, negative by IHC (Figure 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.