NSCLC Patients with an Isolated 5' Green Signal Pattern in ALK FISH Testing May Be Positive for Complex EML4-ALK Rearrangement with 3’ Deletion and May Benefit from Crizotinib Targeted Therapy

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Introduction
Non-small cell lung cancer (NSCLC) is a molecularly heterogeneous disease. 
Receptor tyrosine kinase coactivation networks in cancer is a recent focus that points to the importance of the ALK and EGFR gene family in cancer. 
ALK gene rearrangement is essential for selective crizotinib targeted therapy. 
Currently, the results reporting guide in the FDA approved companion diagnostic FISH test using breakapart (ba) probes consider only the separation and isolated 3 red signal patterns as positive.

Results
Isolated 5 green signals along with fusion signals were observed in ~1-5% of interphase cells (median 0.4%) by the ALK ba probe, and 197 cases were positive (95% CI: 0.5-1.5%) by the EML4 ALK probe. 
Four red EML4 ALK mutation tests done twice on two of them were positive for the USN2 with a 3' deletion and the 3' red probe deletion by next-generation sequencing.

Discussion and Conclusion
1. NSCLC is biologically and genetically heterogeneous. The less commonly observed isolated pattern of isolated 5 green signal may well represent a molecular subclass of NSCLC.
2. A combination of ALK and EGFR fusion signal patterns by the ALK ba probe and the 3' red probe deletion detected by an isolated 5 green signal pattern confirmed the EML4-ALK rearrangement in our study. 
3. The complex chromosomal alterations and the higher dual mutation rate with EGFR/ALK-suggest socal benign or regional chromosomal rearrangement. We suggest that further verification by RT-PCR should be performed as a reflex test. 
4. Isolated 5 green signal pattern or other variant patterns may be indicative of genomic complexity, chromothripsis and/or intra-tumor heterogeneity with the presence of multiple rearrangements or complex chromosomal translocations. 

References

Materials and Methods
FISH was tested on FFPE tumor tissue using ALK ba probe kit (Abbott Molecular). A confirmatory ALK FISH or translocation FISH was performed using a probe set by Kreatech (Figure 1) with a 360kb 3' EML4 green probe and a 350kb 5' EML4 green probe. Two cases with isolated 5 green signal pattern were included in this study. In addition, 3 cases with diminished signal patterns were presented to back our discussion/conclusion. 

Results
Isolated 5 green signals were considered as positive. 

Discussion and Conclusion
1. NSCLC is biologically and genetically heterogeneous. The less commonly observed isolated pattern of isolated 5 green signal may well represent a molecular subclass of NSCLC.
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References

Figure 1
Genomic Positions of ALK Gene and LSI ALK Break Apart Rearrangement Probe

Table 1
Result Comparison using ALK ba (ABBOTT) and EML4-ALK (KREATECH) Probes

Figure 2
The EML4-ALK Inversion/translocation probe signal patterns and the underlying chromosomal rearrangement (courtesy of Kreatech, Inc., Durham, NC)

Figure 3
Negative control: Case 17 shows multiple copy of ALK with no rearrangement and a normal EML4-ALK pattern. Positive control: Case 16 shows a separation of red and green signals and an abnormal fusion positive pattern for the EML4-ALK probe.

Figure 4
Positive control: Case 14 shows an atypical pattern with fusion and red signals and an abnormal fusion positive pattern for the EML4-ALK probe.

Figure 5
Cases 11 and 12 show abnormal diminished signal patterns due to a partial deletion of the 5 green signal probe, and the concurrent EML4-ALK probe tests are fusion positive. Case 13 shows a 3 red deleted (partially deleted) signal pattern, and the concurrent EML4-ALK probe test is fusion positive.