Concordance between a novel RT-PCR assay and FISH for the detection of *EML4-ALK* fusion genes in FFPE specimens

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Background

Echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (*EML4-ALK*) is a fusion-type protein tyrosine kinase identified recently in a subset of human lung carcinomas. The recent licensing approval of Crizotinib (PF0234-1066, Pfizer) in NSCLC patients harboring the *EML4-ALK* fusion will intensify screening efforts. Due to unknown differences in treatment outcomes between the fusion variants, methods other than FISH will be required.

The scope of the study was to design a robust RT-PCR assay that permits sensitive detection of all published *EML4-ALK* variants.

Methods

The study included FFPE specimens from NSCLC patients without *EGFR* or *K-RAS* mutations. Detection of all *EML4-ALK* fusions was achieved using a reverse transcription-PCR (RT-PCR). For this reason specific primers that selectively enhanced *EML4-ALK* transcripts 1, 2, 3a, 3b, 4, 5a, 5b, 6, 7, "4" and "5" were designed (figure 1). Synthetic DNA fragments for each variant were cloned using the pCR2.1 cloning vector and used as positive controls (figure 2). DNA sequencing analysis was performed to confirm the specificity of the obtained PCR products (figure 3).

FISH analysis was performed using the FDA approved kit «Vysis ALK Break Apart FISH Probe Kit» (Abbot)

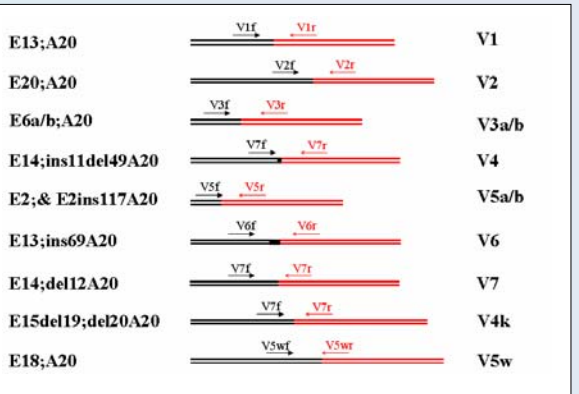

Figure 1: Schematic representation of *EML4-ALK* variants in NSCLC

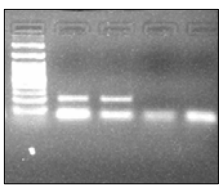

Figure 2: Positive control for the *EML4-ALK* fusion

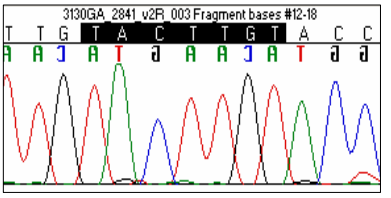

Figure 3: Sequencing analysis for the V2 *EML4-ALK* variant

Additional variants reported and identified by our assay 3c, d, e, f, g, h, 6b, 8a, b

Results

- Sensitivity of the test: 22 copies of the translocation could be detected per µg of RNA.
- All *EML4-ALK* FISH positive samples (n=7) were positively subtyped using RT-PCR and sequencing.
- None of the 123 FFPE specimens, obtained from Greek NSCLC patients, were positive for the *EML4-ALK* fusion.

Discussion

- For the detection of *EML4-ALK* transcripts a variety of methods have been used, including immunohisto-chemistry, fluorescent in situ hybridization, and reverse transcriptase polymerase chain reaction (RT-PCR).
- Pathological review was obtained for all samples and macro-dissection was used to ensure a tumor cell content (%TCC) of >75% in all possible cases (figure 4). A high concordance rate was observed in our series (figure 5) between FISH and RT-PCR.
- Limited data exists regarding the incidence and spectrum of *EML4-ALK* variants, RT-PCR will be required for such classification. Here we demonstrate that RT-PCR is a method of high sensitivity and specificity for the detection of *EML4-ALK* variants and their classification. Once standardized this may offer an alternative analytical technique to FISH, in the diagnostic setting.

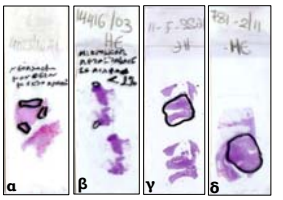

Figure 4. Use of macrodissection for the selection of cancer tissue.

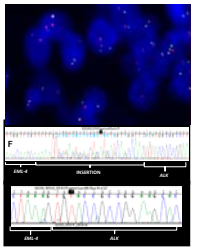

Figure 5: Sequencing analysis for the V3 *EML4-ALK* variant and corresponding FISH.

Conclusions

- We have designed a robust RT-PCR assay that permits the sensitive detection of all published *EML4-MLK* variants and it is feasible for commonly available materials such as FFPE specimens, cytological specimens and other aspirates.
- Concordance between the results obtained by RT-PCR and FISH analysis is warranted in a larger series.

References

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2. Takahashi T. et al. Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. Ann Surg Oncol. 2010 Mar;17(3):889-97.
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