Development of a novel RT-PCR assay for the detection of *EML4-ALK* fusion genes in FFPE specimens.

Eirini Papadopoulou[1], Evangelia Razis[2], Paris Kosmidis[3], Evangelos Briasoulis[4], Samuel Murray[1], George Nasioulas[1].

[1]GeneKOR S.A. Glyka Nera, 15354, Athens, Greece - [2]2nd Department of Oncology, DTCA HYGEIA, 15123, Athens, Greece - [3]2nd Pathology-Oncology Clinic, DTCA HYGEIA, Athens, Greece
[4]Department of Medical Oncology, Medical School, University of Ioannina, Greece

Background

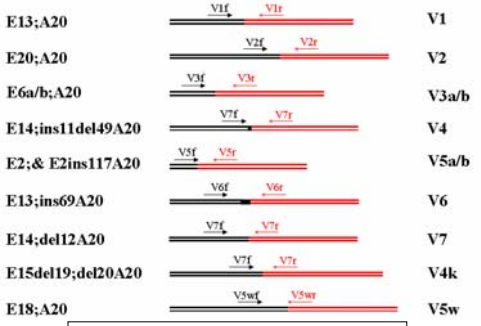
Echinoderm microtubule-associated protein-like 4–anaplastic lymphoma kinase (*EML4-ALK*) is a fusion-type protein tyrosine kinase identified recently in a subset of human lung carcinomas. The recent licensing approval of Crizotinib (PF0234-1066, Pfizer) in NSCLC patients harboring the *EML4–ALK* fusion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

The scope of the study was to design a robust multiplex 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 multiplex 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).
The sensitivity of the method was calculated by adding to 1μg RNA, serially diluted synthetic DNA fragments.

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



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

Figure2: Positive control for the *EML4-ALK* fusion

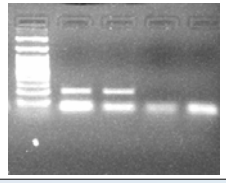
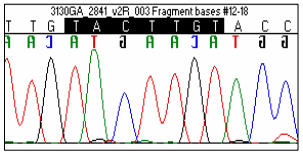


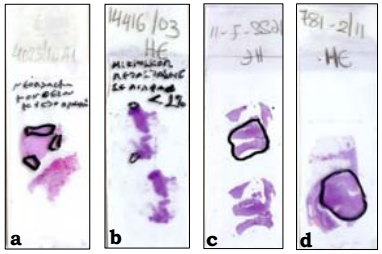
Figure 3: Sequencing analysis for the V2 *EML4-ALK* variant



Results

Sensitivity of the test: 22 copies of the translocation could be detected per μg of RNA
To date none of the 79 FFPE tissues tested was positive for an *EML4-ALK* fusion.

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



Discussion

For the detection of *EML4-ALK* transcripts a variety of methods have been used, including immunohistochemistry, 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).
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.
We are currently increasing our sample size of Greek patients and are in collaboration with other centres to further understand the clinical impact of the variant spectrum.

Conclusions

We have designed a robust RT-PCR assay that permits the sensitive detection of all published *EML4-ALK* variants. It is suitable for use with commonly available materials such as FFPE specimens and sputum samples.

References
1. Soda M. et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007 Aug 2;448(7153):561-6.
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.
3. Shaw AT. et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol. 2009 Sep 10;27(26):4247-53.
4. Horn L. Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. J Clin Oncol. 2009 Sep 10;27(26):4232-5. Epub 2009 Aug 10