Detecting fusion genes in NSCLC tumors using a targeted NGS approach

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Detecting fusion genes in NSCLC tumors using a targeted NGS approach

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Introduction

Fusion genes can act as **drivers of malignant transformation** and **progression** in many human cancers like **Non-Small Cell Lung Cancer** (Mitelam et al. 2007). In NSCLC the rate of therapy-relevant gene fusions sums up to **5-10%** in Caucasian population.
The most common fusion genes are **ALK** (3-7%; Pillai & Ramalingam 2012), **ROS1** (2%; Bergethon et al. 2012) and **RET** (1%; Kohno et al. 2012; Takeuchi et al. 2012). Fusion genes are traditionally detected through **FISH/CISH** or **RT-PCR**. **High throughput sequencing** is emerging as a new alternative to detect and identify fusions in a very sensitive way (Annala et al. 2013).

Methods

Material: 12 FFPE tumor tissues (Städtisches Klinikum, Dessau; Universitätsklinikum, Göttingen)
Initial fusion detection: FISH/CISH analyses **(Figure 1)**
Purification: AllPrep DNA/RNA FFPE (Qiagen)
ReliaPrep™ FFPE Total RNA Miniprep System (PROMEGA), including modifications from Archer
Kit: Archer® FusionPlex® Lung Focus Kit combined with TruSight Tumor 15 (Illumina, data not shown)
Sequencer: Miseq (Illumina)
Analysis: Archer Analysis software

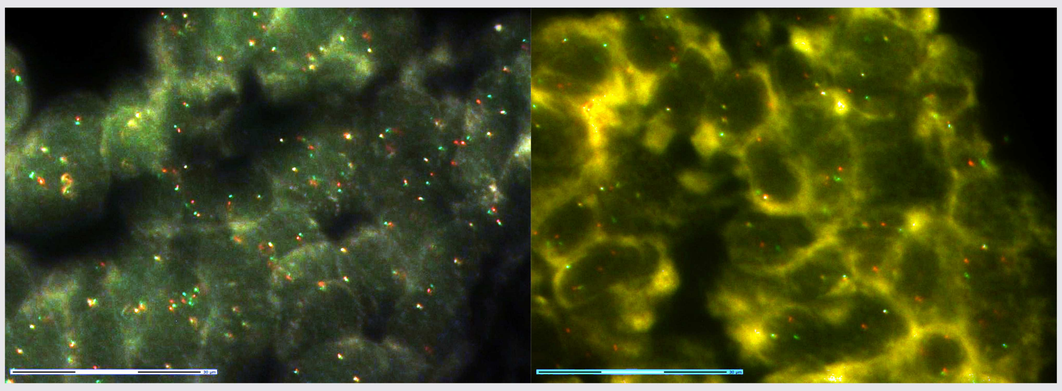

Figure 1. FISH analyses of 2 NSCLC tumor samples: RET fusion (left) and ALK fusion (right).

Results

11 of the 12 samples passed the Quality Control (QC) recommendations for the Archer® FusionPlex® Lung Focus Kit. In one case (sample 1) we were not able to create a sufficient amount of unique RNA fragments (Sample 1, **Table 1**). From the 11 samples which passed the QC, 5 samples were negative by FISH/CISH (e.g. **Figure 1**), 5 samples were tested positive by FISH/CISH (4x ALK, 1x RET). 1 sample harboured a MET intron 13 variation of unknown significance (Class 3). We detected 3/4 ALK fusions, 1/1 RET fusion. In complement, we correctly identified 4/5 negative controls.

sample ID	RNA Input [ng]	Total # reads (pairs)	Fusions expected	Previous method for fusion detection	Fusions detected	Run
1	248	632,425	ALK positiv (22%)	FISH/CISH	failed	1
2	246	2,584,225	ALK positiv (48%)	FISH/CISH	EML4->ALK	1
3	246	3,500,000	ALK positiv (60%)	FISH/CISH	EML4->ALK	1
4	252	1,061,554	ALK positiv (59%)	FISH/CISH	EML4->ALK	1
5	244	3,500,000	MET Exon 14 skipping? (cMET: c.2887+70G>A)	SANGER	no E14 skipping	1
6	248	3,500,000	ALK/ROS1 negativ	FISH/CISH	SMG7->ALK	1
7	245	3,500,000	ALK/ROS1 negativ	FISH/CISH	no fusion detected	2
8	244	2,011,720	RET positiv	FISH/CISH	KIF5B->RET	2
9	242	354,827	ROS1 negative	FISH/CISH	no fusion detected	2
10	242	3,136,370	ALK positiv (22%)	FISH/CISH	no fusion detected	2
11	244	2,304,195	ALK/ROS1 negativ	FISH/CISH	no fusion detected	2
12	249	1,038,907	ALK/ROS1 negativ	FISH/CISH	no fusion detected	2

Table 1. Fusion detection results for 12 FFPE samples from NSCLC tumors

Discussion

Our NGS based fusion detection results do not totally agree with previosly obtained FISH/CISH results.
1 negative control revealed a novel ALK fusion (SMG7>ALK) applying the Archer fusion panel. 1 FISH-positive ALK-sample could not be confirmed by NGS. Since the discrepancy cannot be explained by low RNA quality, we cannot exclude a potentially false-positive FISH result. In this case further investigations e.g. ALK-IHC are required to identify any causes.
1 sample with a putative MET Exon 14 skipping mutation proved negative in the Archer fusion assay. In this case we proved this particular MET-Intron 13 mutation as benign.

In general, the automated workflow from Archer provided a **ready-to-use solution** and **comparable framework** for the detection of ALK-/RET-Fusions. Obvious discrepancies between the presumed gold standard FISH and new NGS methods highlights the urgent need for **harmonisation studies** among currently applied molecular gene fusion-tests in lung cancer.