Detection of a novel RET gene fusion in a non-small cell lung cancer patient using AMP chemistry

Peng Fang, Zhenyu Yan, Weihua Liu, Jennifer Biroscak, Paul Labrousse, Jennifer Wright, Cindy Spittle, Chad Galdieri, and Jin Li, MolecularMD Corp., Cambridge, MA and Portland, OR

Introduction
In lung cancer, several driver Receptor Tyrosine Kinases (RTKs) have been identified in gene fusions, including ALK, RET, ROS1, NTRK1, NTRK2, and NTRK3. These oncogenic gene fusions have become either direct targets or biomarkers for molecular therapies for patients with lung cancer. Screening patients for the presence of these gene fusions would be critical for oncologists to select appropriate therapeutic intervention or for drug developers to design novel therapeutics and conduct clinical trials. However, detection of fusions, especially unknown fusions, from FFPE samples is challenging for many diagnostic techniques.

ArchER™ FusionPlex™ Lung Thyroid Panel is built on Anchored Multiples PCR (AMP™) Chemistry (Figure 1), allowing detection of both novel and known fusions. The panel targets fusions involving the driver genes ALK, FGFR3, MET, NTRK1, NTRK2, NTRK3, BRAF, RET, and ROS1 (Table 1).

Here we report the results from our study using the ArchER Lung Thyroid Panel. We identified and verified the presence of a novel fusion between MethylPhosphonate-Retrotranscribing Protein (MPRIP) and RET oncogene, in an FFPE sample from a non-small cell lung cancer (NSCLC) patient.

Results

Materials and Methods
FFPE samples from 20 NSCLC patients (InTouch, GmbH) were analyzed in the study. One RNA sample containing ROS1 fusion was used as the positive control, and total RNA extract from FFPE normal lung tissue was used as the negative control (Table 1). In addition, F/FEQ controls containing TPMD-NTRK1, ROS1/RET, CCDC6/RET were also used to evaluate the ability of the panel to detect fusions (Table 3). Total nucleic acid (TNA) was extracted from the FFPE sections using the Agencourt® FORMAPURE® Kit (EPA/Clinical), 200–250 ng of TNA from each sample was used for library preparation. The barcoded libraries from 13-16 samples were pooled and sequenced on an Illumina MiSeq using a 212 flow cell. The FASTA files from the MiSeq were then analyzed by the ArchER data analysis pipeline (v3.3.0) to determine the presence of gene fusions with driver genes including ALK, FGFR3, MET, NTRK1, NTRK2, NTRK3, BRAF, RET, and ROS1. The detected fusions in clinical samples were further confirmed by larger sequence and FISH.

Table 1. Novel Targets of AnchorER™ FusionPlex™ Lung Thyroid Panel

Table 2. NoSCC FFPE clinical sample data

Table 3. Summary of TNA extraction and DNA QC

Table 4. Summary of TNA extraction and DNA QC

Results continued

Fusion detection in NSCLC FFPE samples

Figure 1. Total Reads and Read-on-Target (ROT) per Sample

Figure 2. Unique start sites per GSP2 and RNA mean read Length

Table 5. Summary of TNA extraction and DNA QC

Average Unique RNA Start Site Per GSP2 Control

Figure 3. MPRIP-exon12-RS1-exon12 in-frame fusion detected in MMD-2-203

Figure 4. Confirmation of MPRIP-RF fusion by Sanger

Figure 5. Confirmation of MPRIP-RF fusion by Sanger

Figure 6. Structure of RET fusion proteins identified (adapted partially from Ref. 2)

Conclusions

1. Detection of a novelRET gene fusion in a non-small cell lung cancer patient using AMP chemistry

References


For Further Information

www.molecularmd.com or visit www.molecularmd.com for further information on ArchER FusionPlex Lung Thyroid Panel and other ArchER products.