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MOLECULAR ANALYSIS FOR PERSONALISED THERAPY

# Detection of *ALK*, *RET*, *ROS1*, *NTRK1* and *MET* rearrangements and actionable mutations using Next Generation Sequencing in patients with Non-Small Cell Lung Cancer

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## Background & objective of the study

Non-Small Cell Lung Cancer (NSCLC) is the leading cause of death by cancer worldwide. Targeted therapies have been developed this last decade and considerably improved progression-free and overall survivals of patients with NSCLC. The development of the theragnostic for the management of NSCLC required reliable and affordable molecular pathology assays. Next-Generation Sequencing (NGS) is commonly used for the detection of actionable mutations in a panel of genes and FISH or IHC are the gold-standard assay for the detection of rearrangements of *ALK*, *RET*, *ROS1*, *NTRK1* and *MET*. The aim of this study is to evaluate the suitability and reliability of NGS assay for the detection of rearrangements compared to the results obtained using FISH and IHC.

## Methods

A total of 86 FFPE samples, primary tumors or micro biopsies of patients with NSCLC, were analyzed in 7 European labs for this study. All samples were qualified by a senior pathologist prior to analysis. DNA and RNA were extracted from FFPE samples using Qiagen AllPrep kit (Qiagen, Hilden, Germany) or Maxwell 16 FFPE plus LEV DNA purification kit and Maxwell 16 LEV RNA FFPE kit (Promega, Madison, USA). TruSight™ Tumor 15 and INCa panel (illumina, San Diego, CA, USA) kits were used for DNA library preparation and Archer® FusionPlex® panel kit (ArcherDX, Boulder, CO, USA) for RNA library preparation. Both libraries were finally pooled and analyzed using illumina MiSeq sequencing system. Data were finally analyzed using Variant Studio software (illumina) or Biomedical Genomics Workbench and Ingenuity Variant Analysis (Qiagen) for SNV or indel and Archer Analysis software (ArcherDx) for rearrangements. SNV, indel and rearrangements found for each sample were finally compared to FISH and IHC results and previous DNA sequencing data when available in the labs (figures 1 & 2).

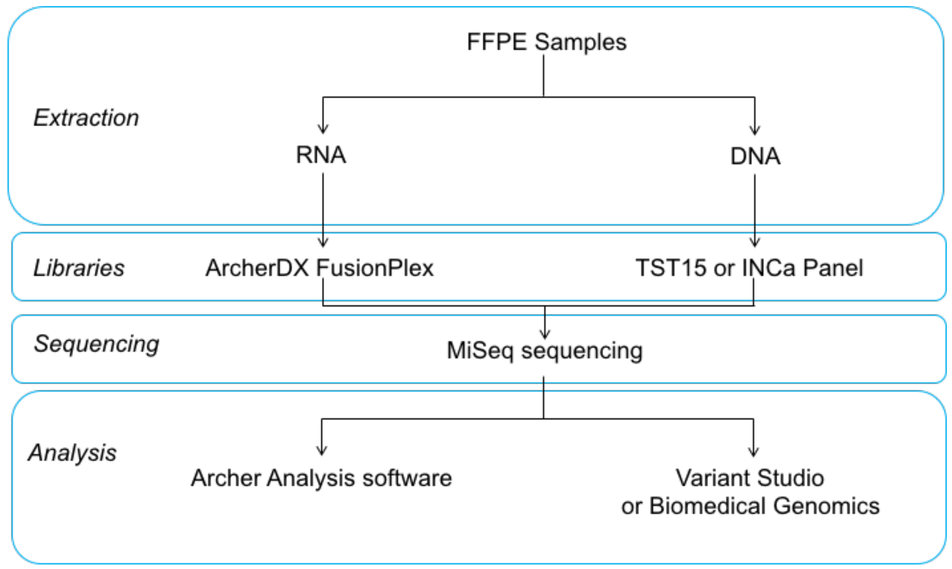


Figure 1. Experimental workflow

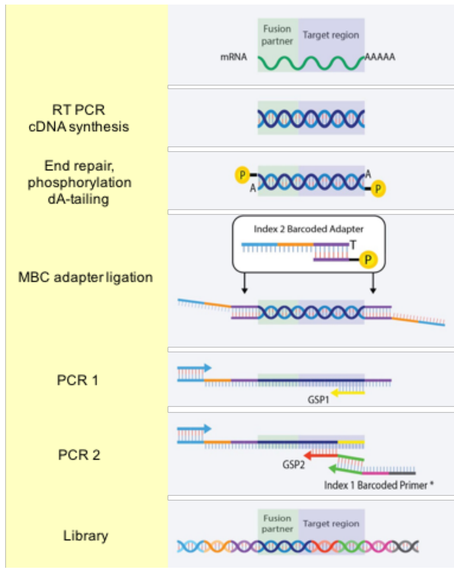


Figure 2. Preparation of the sequencing library from RNA

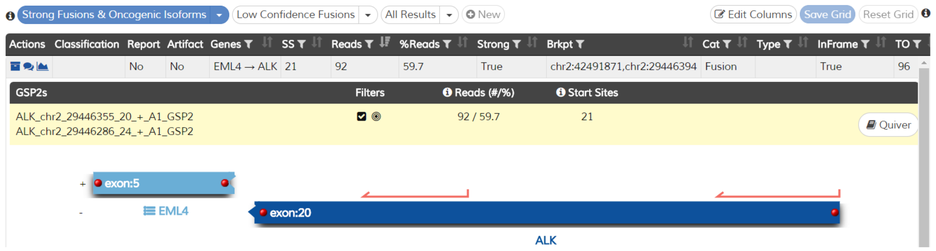


Figure 3. Example of results for a sample with am EML4-ALK rearrangement (Breakpoints: EML4 exon 5 chr2:42491871 and ALK exon 20 chr2:29446394)

| | ALK + | ROS1 + | RET + |
|---|---|---|---|
| NGS | 22 | 5 | 1 |
| FISH / IHC | 19 | 5 | 1 |

Table I. Rearrangements results with NGS and FISH or IHC. Investigations showed that the 3 ALK fusions were missed by FISH and are not NGS false positive.

## Results

A total of 86 DNA and RNA libraries were sequenced. Among 86 samples 8 failed fusion quality control, in majority of cases due to too low amount or too degraded input RNA and no FISH and IHC results were available for 12 samples. Rearrangements were found for 28 samples, 22, 5 and 1 with an *ALK*, *ROS1* and *RET* rearrangements respectively. Among 74 samples with previously known rearrangements, 71 samples showed full concordance with previous methods. Three samples were found discrepant. In 3 samples NGS detected a new fusion which wasn't detected by FISH. No *NTRK1* rearrangement and no MET exon 14 skipping were found in these samples (figure 3 and table I).

## Conclusions

RNA based NGS is a suitable and reliable alternative to FISH and/or IHC for the detection of rearrangements of *ALK*, *RET*, *ROS1*, *NTRK1* and *MET* genes for the theragnostic management of patients with NSCLC. Further investigations are needed to determine if an "all NGS strategy" is more likely cost-effective and faster than a "FISH and NGS strategy".

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