ID: 00720
Type: COMUNICACIÓN ORAL
Topic: 4. Research and diagnostic platforms

Single-molecule quantification of DNA and RNA by nCounter 3D™ Technology in advanced NSCLC patients

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Introduction: Currently, assessment of several tumor drivers is critical for individualized treatment of non-small cell lung cancer (NSCLC). Tools for molecular profiling are based on DNA, RNA and protein (PCR, NGS, FISH, IHC). However, these tests have several disadvantages including hands-on-time and tissue requirements. Nanostring digital barcoding technology enables simultaneous assay of different analytes, DNA and RNA from a single sample in 3D Biology Technology.

Objective: To simultaneously detect several relevant lung cancer gene alterations including fusion transcripts, single nucleotide variants (SNVs), multinucleotide variants (MNVs), and insertion/deletion variants (InDels) from RNA and DNA extracted from FFPE tissue samples.

Methods: The nCounter Vantage 3D SNV:Fusions Lung Assay was used to analyzed a total of 36 formalin-fixed paraffin embedded (FFPE) samples from advanced NSCLC patients. Samples were known to harbor mutations (*EGFR, KRAS, NRAS, PIK3CA, BRAF, P53)* or gene-fusion rearrangements (*ALK, ROS1, RET*, *NTRK1*) as verified by sequencing (Ion Torrent, Gene Reader), nCounter Elements, IHC and/or FISH. Probes were designed to target 25 genes for SNVs (104 different point and InDel mutations) as well as four fusion transcripts (*ALK, ROS1, RET*, *NTRK1*) including 33 specific variants. The 3D workflow requires pre-amplification of gDNA, whereas RNA does not require any enzymatic reaction. After hybridization, all analytes (DNA/RNA) are united for simultaneous, single-lane, digital counting in total turnaround of 3 days. A total amount of 5 ng DNA and 150 ng RNA from two-4 micron FFPE-sections was used for the assay without microdissection.

Results: A total of 72 analyses (DNA/RNA) were performed with an evaluation pass of 97.2% (70/72 analyses yielded results) and 89% concordant results (64/72). Sensitivity of the technique was 92.1%. Among 41 SNVs interrogated in this study 34 were successfully detected (two not evaluable). Four new mutations were found involving NRAS, FBXW7, GNA11 and FGFR2 genes. Of those, only two were considered false positives as they were not confirmed by alternative sequencing and/or PCR. The remaining two were not assessable for test confirmation. For gene fusion analysis, 13 known positive samples were tested. All fusion transcripts were detected for ALK (n=5) RET (n=2) and NTRK1 (n=1). For ROS1 (n=5) there were two false negatives only detected by nCounter Elements target-specific assay.

Conclusions: We have shown that the SNV detection chemistry can be successfully combined with fusion gene expression analysis by using the nCounter 3D™ single-workflow. Nanostring nCounter Vantage 3D SNV:Fusions Lung Assay is highly efficient in detecting hotspot mutations as well as gene rearrangements.
The assay is simple, features a brief hands-on time and requires low amounts of genomic material, supporting minimal use of precious samples.