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Simultaneous detection of gene fusions, METex14 mutations and immune genes in advanced NSCLC patients by nCounter Technology

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Introduction: Assessment of several immune-genes and tumor drivers is critical for individualized treatment of non-small cell lung cancer (NSCLC). We have previously demonstrated the ability of the transcript-based nCounter Technology for the detection of *ALK*, *ROS1* and *RET* gene fusions, using a customized codeset (Reguart *et al*. Clinical Chemistry 2017). Here, we present the first results of the prospective validation in advanced NSCLC samples of a new CodeSet designed to simultaneously characterize clinically relevant gene fusions, *MET* alterations and the expression of immune genes.

Objective: To simultaneously detect gene fusions, MET exon 14 skipping mutations and immune genes expression in advanced NSCLC by nCounter Technology from RNA.

Methods: We have designed an in-house custom set to detect driver fusions involving four genes (*ALK*, *ROS1, RET, NTRK1*), *MET* exon 14 skipping mutation (*MET*ex14), *MET* overexpression and immune genes (*PD1, PD-L1, CD4, CD8, FOXP3, GZMM, IFNG*). A panel of *ALK-ROS-RET-NTRK* positive cell lines (H2228, H3122, SU-DHL-1, HCC78, BaF3 pBABE, LC2/ad and a *NTRK*-positive cell line), Hs746T (*MET*ex14), EBC-1 (overexpressing *MET*) and a negative cell line (PC9) were used for the initial validation of the panel. Subsequently, 58 FFPE samples from advanced NSCLC patients, previously characterized by FISH, RT-PCR and IHC, have been analyzed. Total amount of 150 ng RNA was used for detection. Workflow includes RNA isolation, hybridization and digital counting with for a total turnaround of three days. Raw counts were normalized using positive controls, negative controls and house-keeping genes.

Results: Results obtained with the cell lines positive for *ALK, ROS1, RET* and *NTRK1* fusion genes were exactly coincident with their genotypes, with fusion transcripts successfully detected in all cases by 3'/5' imbalance and direct fusion probes. In addition, *MET*ex14 splicing transcripts were detected in the Hs746T cells at significant levels, higher than those of wt *MET* mRNA. In contrast, *MET*ex14 mRNA counts were almost undetectable in the rest of cell lines. Regarding FFPE samples from advanced patients, 56 could be successfully analyzed by nCounter. Among 13 patients positive for *ALK* and *ROS1* fusions, 12 were confirmed by nCounter. Regarding the *MET*ex14 splicing variant, 5 out of 6 patients previously detected by RT-PCR were also positive by nCounter.

Conclusions: Our data suggest that multiplex detection of several drivers can be successfully analyzed by using nCounter Technology. The assay is simple, requires short hands-on-time, needs low input RNA and is highly efficient in detecting gene rearrangements and *MET*ex14 splicing variants. Results will be prospectively validated in a larger cohort of advanced NSCLC patients and we will determine if clusters of different inmune-phenotypes exist among oncogenic-driven NSCLC tumors.