

# New Approaches for Successful Identification of Several Gene Fusion Oncogenes in Paraffin-Embedded Tissue Samples from Advanced Non-Small-Cell Lung Cancer Patients

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Lung cancer is the leading cause of cancer worldwide. Most patients with non-small-cell lung cancer (NSCLC) are diagnosed in advanced stage and their prognosis is extremely poor [1]. Although for years the approach to treat advanced disease has been based on the systemic treatment with chemotherapy, the comprehensive characterization of the tumor genome has allowed identifying several oncogenic drivers facilitating the advent of directed targeted therapies which have transformed the outcomes of these patients [2]. About 5%-7% of NSCLC rely on fusion oncogenes for growth and survival such as the anaplastic lymphoma kinase (ALK), ROS proto-oncogene 1 (ROS1) and RET proto-oncogene (RET) [3,4]. Detection of these fusion genes, generally identified by standard fluorescence in situ hybridization (FISH) or immunohistochemistry (IHC) techniques, is thus very important in guiding specific treatment decisions and selecting the most appropriate targeted therapy.

Genetic testing in advanced NSCLC is endorsed by clinical guidelines at diagnose [5,6]. In clinical practice, paraffin-embedded tissue samples (FFPE) of advanced NSCLC are used to detect the three most common genetic alterations in this pathology: mutations in the epidermal growth factor receptor (EGFR) mutations, KRAS proto-oncogene, GTPase (KRAS) mutations and rearrangements of ALK, whereas other less frequent but also relevant oncogenes such as ROS1 and RET are analyzed more exceptionally. There are several conditions in this disease that challenge clinicians to succeed in performing an appropriate and complete genetic testing; on the one side the inherent degree of subjectivity by the evaluator with traditional techniques such as FISH and IHC tests and on the other hand, the usual shortage of tissue and the difficulty of getting tissue from the lung. Thus, the sequential one-by-one gene testing approach initially used in the clinic to identify just a few genes, is no longer optimal as it would involve high tissue demands to provide results for all the oncogenes of interest. Is this why, new multiplexing assays with potential diagnostic for several genes at a time are entering with strength into the field of lung cancer molecular testing.

The nCounter platform allows multiplexed tests of fusion transcripts (mRNA) using direct digital profiles or counting technology [7]. This technology uses probes of 50 nucleotides and, in contrast to the new platforms of sequencing (next generation sequencing, NGS), is based on the direct hybridization without synthesis of complementary DNA or amplification by PCR. In the study recently published in by Reguart et al. [8] we have validated this platform in the clinical setting, demonstrating that it allows effective detection of ALK, ROS1 and RET fusion genes from FFPE samples and identifies a greater number of positive cases that could benefit from targeted therapies. To do so, our group analyzed, using nCounter technology, RNA extracted from FFPE samples from a retrospective large cohort of 108 patients with advanced NSCLC. The results were compared with those obtained using standard techniques (FISH and IHC) and clinical information was collected from a subgroup of patients. The cohort was enriched with ALK and ROS1 positive patients detected by standard FISH or IHC techniques, as well as EGFR-KRAS wild type (WT) samples.

Our work [8] and the work of others [9] demonstrate that nCounter platform needs a reduced amount of genetic material for optimum analysis. Specifically, a thickness of four  $\mu\text{m}$  with a tumor area of 1.1  $\text{mm}^2$  and 10% tumor content with a total amount of 25-200 ng RNA are sufficient to obtain satisfactory results [8].

In the final set of samples evaluated by nCounter (n=98), a total of 55 were identified with fusion transcripts: 32 for ALK, 21 for ROS1 and two for RET. The positivity for ALK, ROS1 and RET in our patient population was mutually exclusive with other oncogenic drivers. NCounter showed excellent agreement with the ALK-IHC (98.5%, CI 91.8-99.7, Cohen  $\kappa$  0.97) and a substantial concordance with ALK-FISH (87.5%, CI 79.0-92.9, Cohen  $\kappa$  0.71). Of key interest, in our study nCounter allowed to identify 10 ALK-positive cases for fusion genes that were qualified as negative by FISH [8]. In this respect, there is growing evidence that new molecular platforms with NGS techniques are more sensitive than FISH in detecting ALK rearrangements [8,10,11].

When it comes to ROS1 fusions, we obtained 86% (CI=76.5-91.9, Cohen  $\kappa$  0.63) and 87% (CI=78.0-92.9, Cohen  $\kappa$  0.7) concordance between nCounter and traditional ROS1-IHC and -FISH, respectively, with a significant number of positive samples identified only by one or two techniques. In our study, among 21 ROS1 positive patients per nCounter, two (10%) and seven (33%) were negative per FISH and IHC, respectively [8]. Taken together, and unlike ALK rearrangements, our results would not endorse the use of IHC as the standard technique for ROS1 fusions screening.

In our study, we could provide retrospective information on the outcomes in a subset of 29 patients treated with targeted therapies. Of the 25 patients who achieved clinical benefit (defined as partial response or stable disease for more than 6 months), 24 were positive for nCounter while only 22 were positive by FISH. All patients who benefited from an ALK inhibitor (n=18) were nCounter positive, while three were negative or non-evaluable by the standard FISH technique. One of the FISH-negative and nCounter-positive patients had complete clinical follow-up, being on partial response to crizotinib for more than three years [8]. These results, along with the recent published cases of

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responses in ALK FISH-negative/IHC-positive patients treated with ALK inhibitors [12,13] questions FISH as the technique of choice for screening and illustrates the clinical importance of identifying ALK gene expression rather than the chromosome alteration itself, which indeed, is one of the advantages of nCounter-based transcript technology.

Likewise, response data was available from nine patients identified as ROS1-positive and who were treated with crizotinib. All patients (n=7) who obtained clinical benefit were FISH-positive and six were also positive by nCounter. Re-examination of the remaining sample revealed very low tumor infiltration (5%). The two patients who did not obtain clinical benefit to crizotinib were positive for FISH, while one of them was negative for nCounter [8].

To sum up, our results demonstrate that nCounter may be more useful than standard FISH and IHC techniques for gene fusion detection in patients with advanced NSCLC since, unlike previous, it allows the analysis of multiple molecular drivers, from small FFPE samples with minimum requirements of genetic material. Our results pave the way for the implementation of the nCounter technology at the care level for the screening of ALK, ROS1 and RET fusion genes in advanced NSCLC patients.

## References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. (2015) Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 136: 359-86.
2. Reck M, Rabe KF (2017) Precision Diagnosis and Treatment for Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* 377: 849-861.
3. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, et al. (2007) Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 448: 561-566.
4. Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, et al. (2012) RET, ROS1 and ALK fusions in lung cancer. *Nat Med* 18: 378-381.
5. Novello S, Barlesi F, Califano R, Cufer T, Ekman S, et al. (2016) Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 27: v1-1v27.
6. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman J, et al. (2017) Non-Small Cell Lung Cancer, Version 5.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 15: 504-35.
7. Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, et al. (2008) Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol* 26: 317-325.
8. Reguart N, Teixidó C, Giménez-Capitán A, Paré L, Galván P, et al. (2017) Identification of ALK, ROS1, and RET fusions by a multiplexed mRNA-based assay in formalin-fixed, paraffin-embedded samples from advanced non-small-cell lung cancer patients. *Clin Chem* 63: 751-60.
9. Evangelista AF, Zanon MF, Cruvinel Carloni A, de Paula FE, Andozia Morini M, et al. (2017) Detection of ALK fusion transcripts in FFPE lung cancer samples by NanoString technology. *BMC Pulm Med* 17:86
10. Rogers TM, Arnau GM, Ryland GL, Huang S, Lira ME, et al. (2017) Multiplexed transcriptome analysis to detect ALK, ROS1 and RET rearrangements in lung cancer. *Sci Rep* 7: 42259.
11. Ali SM, Hensing T, Schrock AB, Allen J, Sanford E, et al. (2016) Comprehensive genomic profiling identifies a subset of crizotinib-responsive ALK-rearranged non-small cell lung cancer not detected by fluorescence *in situ* hybridization. *Oncologist*. 21: 762-770.
12. Marchetti A, Di Lorito A, Pace MV, Iezzi M, Felicioni L, et al. (2016) ALK protein analysis by IHC staining after recent regulatory changes: A comparison of two widely used approaches, revision of the literature and a new testing algorithm. *J Thorac Oncol* 11: 487-495.
13. Rosoux A, Pauwels P, Duplaquet F, D'Haene N, Weynand B, et al. (2016) Effectiveness of crizotinib in a patient with ALK IHC-positive/FISH-negative metastatic lung adenocarcinoma. *Lung Cancer* 98: 118-121.