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# Towards a more comprehensive molecular characterization of non-squamous and squamous non-small cell lung cancer using DNA and RNA-based targeted NGS

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## Introduction

Next-generation sequencing (NGS) has revolutionized the field of cancer genomics. NGS reveals predictive biomarkers at large scale and affordable costs, and is becoming an essential tool for guiding treatment decisions. Currently, the use of NGS-based molecular profiling in non-small cell lung cancer (NSCLC) depends on the histological subtype. NGS testing has become routine before first-line treatment in non-squamous NSCLC due to the known treatable oncogenic alterations that are significantly impacting clinical decisions. In contrast, molecular profiling of squamous NSCLC is not yet routinely recommended, despite the fact that recently-developed comprehensive NGS panels may also identify valuable predictive biomarkers in this histology subtype.

## Objective

In this study we evaluated the performance, feasibility and potential impact on clinical decision-making of DNA and RNA-based targeted NGS testing in both non-squamous and squamous NSCLC.

## Methodology

A total of 96 resectable formalin-fixed, paraffin-embedded (FFPE) NSCLC tissues were collected between 2000 and 2017. The NSCLC subtypes included 48 adenocarcinomas (ADC), 38 squamous cell carcinomas (SCC), and 10 large cell carcinomas (LCC). Fourteen patients died from lung cancer during follow-up. Median follow-up was 28.5 months. Oncomine Comprehensive Assay v3C (OCA; ThermoFisher Scientific) was used for DNA and RNA-based targeted molecular profiling of the 96 FFPE samples. OCA analyzes mutational hotspots in 86 oncogenes, the complete coding sequence of 48 tumor suppressor genes, copy number alterations (CNVs) in 47 genes and structural rearrangements in 51 genes. Of note, 12 cases (4 ADCs and 8 SCCs) were included in which no biomarker had been previously identified with the use of the Oncomine Focus Assay (OFA, ThermoFisher Scientific), which interrogates hotspots of 35 cancer genes, CNVs in 19 genes and structural rearrangements in 23 genes. Multiplexed PCR-based DNA (for point mutations/indels and CNVs) and RNA (for gene fusions) libraries were sequenced on an Ion S5 System (8 samples per Ion 540 chip). Genetic alterations identified by the Torrent Suite Software (ThermoFisher Scientific) were correlated with clinico-pathological characteristics of the patients.

## Results

DNA-based targeted enrichment sequencing was successful in all cases; while RNA sequencing rendered reliable results in 80 samples. All the samples for which the RNA quality was not adequate had been archived for more than 7 years.

At least one mutation was found in 86% of the cases (90% of ADCs, 82% of SCCs, and 90% of LCCs), with a median number of mutations per case of 2 for ADC, 3 for SCC and 3 for LCC. The number of mutations did not correlate with age, gender, smocking history, stage or neo-adjuvant chemotherapy. However, the percentage of patients who died during the follow-up period differed between those with or without identified mutations (22% versus 8%, respectively).

As expected, we observed distinct patterns of somatic genomic alterations in non-squamous and squamous NSCLC. In ADC, *KRAS* point mutations were the most frequent oncogenic alterations (37%), followed by point mutations in *EGFR* and *BRAF* (10% and 6%, respectively), and CNVs in *TERT* (8%), *MDM2* (6%), *RICTOR* (6%), *CDK4* (6%), and *MYC* (4%). The *EML4-ALK* fusion was detected in two patients (4%). On the other hand, the most recurrent oncogenic alterations found in SCC were point mutations in *PIK3CA* (32%) and *NFE2L2* (13%), and CNVs in *FGFR1* (13%) and *MYC* (13%). Of note, two SCC tumors showed 102 and 42 copies of *EGFR*, a genetic feature associated with sensitivity to the anti-EGFR antibody necitumumab. Two other SCC tumors had amplification of NTRK genes (*NTRK1* and *NTRK2*). Targeting gene fusions involving *NTRK* genes has demonstrated histology-agnostic efficacy in patients, and preclinical data suggest that *NTRK* amplifications may also be oncogenic. Unexpectedly, *KRAS* codon 12 mutations were identified in two SCC cases. Immunohistochemical characterization allowed us to reclassify one of these cases as a tumor with areas of ADC differentiation (positive for TTF1 and napsin A, and negative for P40 and P63). In LCC, two cases showed biallelic inactivation of *TP53* and *RB1*, a genetic hallmark suggested to have a predictive value for chemotherapy response.

Finally, a higher diagnostic yield of OCA *versus* OFA was observed. OCA identified relevant mutations in regions not interrogated by OFA and, more notably, detected alterations, such as amplifications in *PIK3CA*, *MET* or *EGFR*, which went unnoticed by OFA. These results may have had clinical relevance in the context of patients requiring systemic treatment, since responses to crizotinib or necitumumab have been reported in NSCLC patients with *MET* or *EGFR* amplification, respectively.

## Conclusions

Actionable mutations were found in both non-squamous and squamous NSCLC tumor samples using a comprehensive DNA and RNA-based targeted NGS molecular profiling approach. Interestingly, a tumor was histologically reclassified based on the NGS results. Our study supports the establishment of a histology independent DNA and RNA-based NGS molecular profiling approach as part of the diagnostic framework for NSCLC.

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