Clinical Cancer Research

# Broad, Hybrid Capture–Based Next-Generation Sequencing Identifies Actionable Genomic Alterations in Lung Adenocarcinomas Otherwise Negative for Such Alterations by Other Genomic Testing Approaches

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## Abstract

**Purpose:** Broad, hybrid capture–based next-generation sequencing (NGS), as a clinical test, uses less tissue to identify more clinically relevant genomic alterations compared with profiling with multiple non-NGS tests. We set out to determine the frequency of such genomic alterations via this approach in tumors in which previous extensive non-NGS testing had not yielded a targetable driver alteration.

**Experimental Design:** We enrolled patients with lung adenocarcinoma with a $\leq$15 pack-year smoking history whose tumors previously tested "negative" for alterations in 11 genes (mutations in *EGFR*, *ERBB2*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1*, *PIK3CA*, and *AKT1* and fusions involving *ALK*, *ROS1*, and *RET*) via multiple non-NGS methods. We performed hybridization capture of the coding exons of 287 cancer-related genes and 47 introns of 19 frequently rearranged genes and sequenced these to deep, uniform coverage.

**Results:** Actionable genomic alterations with a targeted agent based on NCCN guidelines were identified in 26% [8 of 31: *EGFR* G719A, *BRAF* V600E, *SOCS5-ALK*, *HIP1-ALK*, *CD74-ROS1*, *KIF5B-RET* ($n$ = 2), *CCDC6-RET*]. Seven of these patients either received or are candidates for targeted therapy. Comprehensive genomic profiling using this method also identified a genomic alteration with a targeted agent available on a clinical trial in an additional 39% (12 of 31).

**Conclusions:** Broad, hybrid capture–based NGS identified actionable genomic alterations in 65% [95% confidence interval (CI), 48%–82%] of tumors from never or light smokers with lung cancers deemed without targetable genomic alterations by earlier extensive non-NGS testing. These findings support first-line profiling of lung adenocarcinomas using this approach as a more comprehensive and efficient strategy compared with non-NGS testing. *Clin Cancer Res; 21(16); 3631–9. ©2015 AACR.*

*See related commentary by McCutcheon and Giaccone, p. 3584*

## Introduction

Therapeutic approaches to lung cancers have quickly shifted toward an emphasis on molecularly targeted therapy in genotypic subsets of patients. The discovery of activating mutations in the EGF receptor (*EGFR*) gene in 2003 (1–3) and rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene in 2007 (4) ushered in an era where the identification of key oncogenic alterations emerged as the critical determinant of benefit to targeted therapy. Phase III trials have demonstrated that response rate and progression-free survival are improved with targeted therapy in comparison to chemotherapy (5–7). In the United States, the EGFR tyrosine kinase inhibitors (TKI) erlotinib and afatinib and the ALK TKIs crizotinib and ceritinib are approved for patients with *EGFR*-mutant and *ALK*-rearranged advanced lung cancers, respectively (8, 9).

Recent data from The Cancer Genome Atlas (TCGA) revealed that lung cancers rank among the most genomically complex of tumors among the 12 cancer types studied by the TCGA Pan-Cancer effort (10, 11). This genomic complexity allows the opportunity to exploit the presence of other molecular alterations as therapeutic targets in patients. Over the last decade alone, the number of lung cancer drivers for which active targeted therapeutics have been identified has steadily risen. In lung adenocarcinomas, these include, beyond *EGFR* mutations and *ALK* fusions, mutations in *ERBB2* (*HER2*; ref. 12), *BRAF* (13), *PIK3CA* (14), and *AKT1* (15), recurrent gene fusions involving *ROS1* (16), and *RET* (17), and *MET* amplification (18), with an ever-growing list of other potential candidates. Lung cancers from patients with a never smoking history have a unique molecular profile in comparison to lung cancers from smokers. Tumors from never smokers are characterized by lower overall mutation frequencies and are enriched for targetable

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

Selecting the most optimal platform for molecular diagnostic profiling represents a crucial step in the management of patients with advanced lung adenocarcinomas. The use of broad, hybrid capture–based next-generation sequencing (NGS) resulted in the identification of actionable genomic alterations in close to two thirds of lung adenocarcinomas that previously tested "negative" for known alterations by multiple conventional non-NGS tests, including multiplex mass spectrometry, sizing assays, and FISH. Upon driver identification via NGS, several patients whose tumors would have otherwise been deemed "driver-negative" via non-NGS testing subsequently received and responded to targeted therapy. These results underscore the important role of broad, hybrid capture–based NGS in the clinic as a single test that interrogates a wide range of genomic alterations using potentially less tissue than standard non-NGS testing.

drivers such as *EGFR* mutations and *ALK*, *ROS1*, and *RET* fusions (19).

The evolution of molecular diagnostic platforms that permit rapid identification of oncogenic alterations has played a central role in allowing continued expansion of this approach. In the face of few targetable oncogenes, molecular testing previously followed a one-driver-one-test approach, with the use of Sanger sequencing to detect *EGFR* mutations and break apart FISH to detect *ALK* fusions. With an ever-expanding number of drivers of interest, multiplex PCR-based platforms such as Sequenom (Sequenom) and SNaPShot (Applied Biosystems) were developed to simultaneously interrogate mutation hotspots in multiple oncogenes (20). In several larger centers, the pre-NGS approach to diagnostic testing commonly involved one of the latter methodologies in addition to multiplex sizing assays, FISH tests for recurrent gene fusions, and immunohistochemistry to determine overexpression or protein loss. However, from the perspective of the clinician, the clinical laboratories, and the patient, the amount of tissue, effort, and time required to complete such as an algorithm has become less and less feasible.

Next-generation sequencing (NGS) or massively parallel DNA sequencing represents an important technologic advance in the evolution of molecular diagnostic tools. NGS allows for the simultaneous detection of multiple alterations in relevant cancer genes in a single test (21), but this ability can depend on the type of target enrichment used, namely hybrid capture or PCR. Most PCR capture–based NGS assays are designed as "hot spot" tests, sequencing predefined areas of oncogenes with known associations with resistance or sensitivity to approved agents. These tests commonly detect base substitutions with high sensitivity but small insertions and deletions with lower sensitivity. Although providing a substantial advance over single gene or marker analysis, PCR-based NGS assays do not routinely detect copy number changes (amplification or homozygous deletions) or gene fusions.

In contrast, hybrid capture–based NGS assays not only allow the identification of hotspot mutations but also interrogate the entire coding sequence of oncogenes and tumor suppressor genes and the introns of selected genes involved in gene fusions and allow assessment of copy number alterations, all from a single formalin-fixed, paraffin-embedded (FFPE) specimen (22).

With this in mind, we set out to perform a broad, hybrid capture–based NGS assay (FoundationOne) on tumor specimens from patients with lung adenocarcinomas who tested negative for a panel of 11 known drivers via a standard molecular diagnostic algorithm previously used at our institution. Our intent was to define the incremental potential benefit of such an approach to detect previously undiagnosed genomic alterations amenable to targeted therapy in this defined patient population.

## Materials and Methods

### Patient identification and selection

Patients with lung adenocarcinomas harboring no evidence of a genomic alteration based on a focused panel of non-NGS testing who were treated at the Memorial Sloan Kettering Cancer Center (MSK; New York, NY) between 2006 and 2013 were identified. Testing for these alterations was performed under a separate, ongoing, prospective program (the MSK Lung Cancer Mutational Analysis Program or LC-MAP) in patients with pathologically confirmed lung adenocarcinomas. Tissue and clinical data were collected under a protocol approved by the institutional review board or waiver of authorization.

Non-NGS testing was composed of a number of tests for known lung cancer alterations in 11 genes (*EGFR*, *ERBB2*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1*, *PIK3CA*, *AKT1*, *ALK*, *ROS1*, and *RET*; ref. 14). A multiplex mass spectrometry–based system (Sequenom) was used to study 91 point mutations in *EGFR*, *ERBB2*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1*, *PIK3CA*, and *AKT1* (Supplementary Table S1). Multiplex sizing assays tested for insertions or deletions in *EGFR* exons 19 and 20 and *ERBB2* exon 20. Three FISH break-apart assays were used to screen for gene rearrangements involving *ALK*, *ROS1*, and *RET*.

Patients whose tumors tested negative for the above alterations were eligible if they were never smokers or smoked ≤15 pack-years of cigarettes, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, and stage IIIB/IV disease or early-stage disease with radiographic findings suspicious for recurrence not amenable to local therapy. Sufficient tumor tissue for broad, hybrid capture–based NGS was required. Our intent was to identify patients who were candidates for targeted therapy should an actionable genomic alteration be identified.

### Broad, hybrid capture–based NGS assay

Tumor samples were prescreened at MSK for adequacy as defined by an initial requirement of 10 to 15 unstained slides of FFPE tissue. Tissue was sent to a Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory (Foundation Medicine) for NGS. Additional pathologic review of submitted specimens was performed to determine tissue adequacy (defined as ≥20% tumor nuclei and ≥50 ng of DNA) before testing. Macrodissection to enrich specimens of ≤20% tumor content was performed as warranted. DNA was extracted from unstained FFPE sections and quantified by a Picogreen fluorescence assay.

In samples deemed adequate, 50 to 100 ng of DNA was used for whole-genome shotgun library construction. Hybridization capture of 4,557 exons of 287 cancer-related genes and 47 introns of 19 genes frequently rearranged in solid tumors was performed. Hybrid capture libraries were then sequenced to >500× average unique coverage with >100× at >99% of exons using Illumina HiSeq2000 or 2500 sequencers (Supplementary Figs. S1 and S2).

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Sequencing data were processed using a customized analysis pipeline designed to detect multiple classes of genomic alterations, including base substitutions, short insertions and deletions, copy number alterations, and genomic rearrangements. Once available, results were released to the treating physician to allow identification of appropriate targeted therapeutics for patients whose tumors harbored an actionable genomic alteration.

## Results

### Patients

We identified 47 patients with lung adenocarcinomas who harbored no evidence of a genomic alteration via focused non-NGS testing. Of these patients, non-NGS testing with multiple assays resulted in tissue exhaustion in 34% ($n = 16$ of 47) of cases and a repeat biopsy was either not feasible or declined by the patient.

Testing was successfully performed on tumor from the remaining 31 cases. Clinical and pathologic features are summarized in Table 1. The median age at diagnosis was 60 years (range, 29–78 years), and the majority of patients was never smokers (71%, $n = 22$ of 31). In 71% of patients ($n = 22$ of 31), tumor tested was obtained from the same procedure as tumor used for non-NGS testing.

Most patients (84%, $n = 26$ of 31) required $\geq$2 tumor biopsies (median, 3; range, 2–6) to complete testing. Of these patients, 69% ($n = 18$ of 26) underwent multiple biopsies to complete non-NGS testing alone and sufficient tissue remained for the NGS assay. In 31% of cases ($n = 8$ of 26), non-NGS testing resulted in tissue exhaustion and an additional biopsy was required to complete NGS testing. For each new biopsy, an attempt to run all non-NGS tests was made, including tests that were previously performed on prior tissue. Only 29% ($n = 5$ of 31) of samples were derived from the initial procedure performed to diagnose lung cancer. Most samples (71%, $n = 22$ of 31) were obtained from a surgical procedure such as a lobectomy, wedge resection, VATS pleural biopsy, or excision of a metastatic focus.

**Table 1.** Clinicopathologic features

| Clinicopathologic features ($n = 31$) | |
|---|---|
| Age at diagnosis, y | Median, 60 (range, 29–78) |
| Sex | |
| M | 42% ($n = 13$) |
| F | 58% ($n = 18$) |
| Cigarette smoking history | |
| Never | 71% ($n = 22$) |
| $\leq$15 pack-years | 29% ($n = 9$) |
| Pathology | |
| Lung adenocarcinomas | 100% ($n = 31$) |
| Tumor sample source | |
| Primary | 52% ($n = 16$) |
| Metastatic | 48% ($n = 15$) |
| Pleura | 16% ($n = 5$) |
| Lymph node | 16% ($n = 5$) |
| Other | 16% ($n = 5$) |
| Procedure to obtain tumor | |
| Core needle | 29% ($n = 9$) |
| Surgical | 71% ($n = 22$) |
| Lobectomy | 19% ($n = 6$) |
| Wedge | 19% ($n = 6$) |
| VATS pleural biopsy | 16% ($n = 5$) |
| Excision of metastatic focus | 16% ($n = 5$) |
| Number of procedures to complete both non-NGS and NGS testing | |
| 1 | 16% ($n = 5$) |
| 2 | 36% ($n = 11$) |
| $\geq$3 | 48% ($n = 15$) |

NOTE: The clinicopathologic profile of 31 patients whose tumors successfully underwent broad, hybrid capture–based NGS is shown. These tumors previously tested "negative" for alterations in 11 genes (mutations in *EGFR*, *ERBB2*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1*, *PIK3CA*, and *AKT1*, and fusions involving *ALK*, *ROS1*, and *RET*) via non-NGS methods.

### Genomic alterations

One or more genomic alterations were uncovered by hybrid capture–based NGS in tumors from 94% ($n = 29$ of 31) of patients. Across 31 patient samples tested, a total of 96 individual genomic alterations were found, with a median of 3 alterations (range, 0–7) per sample. These alterations are summarized by patient sample in Table 2 (including median exon coverage, mutation allele frequency, and copy number) and most commonly involved *TP53* (14%, $n = 13$ of 96), *EGFR* (7%, $n = 7$ of 96), *MDM2* (5%, $n = 5$ of 96), *KRAS* (4%, $n = 4$ of 96), *CDK4* (4%, $n = 4$ of 96), and *SETD2* (4%, $n = 4$ of 96).

Small mutations comprised 55% of the detected abnormalities (Fig. 1): 36% ($n = 34$ of 96) were nonsynonymous base substitutions, 16% ($n = 15$ of 96) insertion or deletion and 3% ($n = 3$ of 96) splice site mutation. For nonsynonymous point mutations, *TP53* was the most commonly mutated gene (24%, $n = 8$ of 34), followed by *EGFR* (9%, $n = 3$ of 34) and *KRAS* (6%, $n = 2$ of 34). Insertion or deletion most commonly involved *EGFR*, *TP53*, and *SETD2* (20%, $n = 3$ of 15 for each). The majority of splice site mutations involved *TP53* (67%, $n = 2$ of 3).

Gene amplification comprised 31% ($n = 30$ of 96) of genomic alterations. *MDM2* was the most frequently amplified gene (17%, $n = 5$ of 30). Gene loss comprised 9% ($n = 9$ of 96) of all genomic alterations and was most commonly observed with *CDKN2A* (40%, $n = 2$ of 5). Fusion genes were found in 9% ($n = 9$ of 96). These most commonly involved *RET* (33%, $n = 3$ of 9) and *ALK* (22%, $n = 2$ of 9).

### Clinically relevant genomic alterations

A genomic alteration with a corresponding targeted therapeutic based on the National Comprehensive Cancer Network (NCCN) guidelines for non–small cell lung cancer (NSCLC) was identified in 26% ($n = 8$ of 31) of patients. The drivers identified in tumors from these 8 patients are as follows: *EGFR* G719A, *BRAF* V600E, *SOCS5-ALK*, *HIP1-ALK*, *CD74-ROS1*, *KIF5B-RET* ($n = 2$), and *CCDC6-RET* (Fig. 2).

In these 8 patients, mass spectrometry genotyping (Sequenom) and break-apart FISH testing had not detected these alterations, for a variety of possible reasons detailed in Table 2. Of note, in all but 1 of 8 patients, tumor samples used for non-NGS and NGS testing were obtained from the same biopsy or procedure.

In an additional 39% ($n = 12$ of 31) of patients, an actionable genomic alteration was discovered for which targeted therapy was available either on an ongoing trial at the institution or off-protocol. These include the following alterations and the corresponding therapy available at detection: *CDKN2A* loss (CDK4/6 inhibitor, NCT01237236), *EGFR* L747P (erlotinib, afatinib), *EGFR* exon 18 deletion ($n = 2$, pan-ERBB inhibitor, NCT01858389), *EGFR* exon 20 insertion (pan-ERBB inhibitor, NCT01858389), *ERBB2* L755F (ERBB1/2/3 inhibitor, NCT01953926), *FGFR1* T141R (FGFR inhibitor, NCT01948297),

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**Table 2.** NGS results

| Stage at diagnosis | Age, y/sex | Smoking history (pack-years) | Number of genomic alterations detected | Med exon coverage | Mutation (allele frequency) | Fusion | Amplification (copy number) | Loss | Genomic alteration uncovered by NGS that prior non-NGS testing was poised to detect: Alteration | Potential reason not detected by non-NGS testing |
|---|---|---|---|---|---|---|---|---|---|---|
| IV | 59 F | 0 | 7 | 754 | *BRCA1* E1011K (5%)<br>*DNMT3A* R882H (2%)<br>*ATR* H2277fs*38 (7%)<br>*SETD2* R1598* (7%)<br>NF2 splice (8%) | None | *NFKBIA* (10)<br>*NKX2-1* (10) | None | None | N/A |
| IIIA | 57 F | 4 | 0 | 652 | None | None | None | None | None | N/A |
| IV | 61 M | 0 | 3 | 815 | None | *SOCS5-ALK* | *CDK4* (>20)<br>*MDM2* (>20) | None | *SOCS5-ALK* | *ALK* FISH clearly negative, possible complex rearrangement |
| IV | 48 M | 0 | 2 | 695 | *SETD2* K1486*fs (29%) | *CCDC6-RET* | None | None | *CCDC6-RET* | *RET* FISH failure, after fusion detected by NGS, alternate FISH protocol performed and *RET* fusion was confirmed |
| IV | 53 M | 0 | 3 | 1,077 | *TP53* V197G (10%) | None | *NFKBIA* (10)<br>*NKX2-1* (10) | None | None | N/A |
| IV | 51 M | 0 | 1 | 600 | *KRAS* Q61H (13%) | None | None | None | *KRAS* Q61H | Potentially secondary to lower sensitivity of Sequenom |
| IB | 34 F | 0 | 3 | 860 | None | *SHC1-ERBB2* | *MCL1* (9) | None | None | N/A |
| IIB | 57 F | 0 | 1 | 962 | *LRP1B* R790* (50%) | None | None | None | None | N/A |
| IA | 60 M | 0 | 3 | 917 | *TP53* Y220C (33%)<br>*EGFR* exon20 ins V769_D770insGGTR (33%) | None | *MYC* (8) | None | *EGFR* exon20 ins V769_D770 insGGTR | Potentially secondary to lower sensitivity of *EGFR* sizing assay |
| IIIA | 52 F | 0 | 6 | 809 | None | *BRCA2* rearrangement | *MDM2* (11)<br>*MDM4* (8)<br>*AKT3* (8)<br>*IKBKE* (8) | *CDKN2A* | None | N/A |
| IIA | 66 F | 2 | 6 | 802 | *BCORL1* P380L (59%)<br>*CTNNB1* T41A (1%)<br>*FGFR1* T141R (52%)<br>*TP53* E198* (15%) | None | *NFKBIA* (8)<br>*NKX2-1* (8) | None | None | N/A |
| IV | 48 F | 0 | 4 | 1,017 | *APC* E1309fs*4 (46%)<br>*EGFR* L747P (50%) | None | None | *PTEN*<br>*SMAD4* | None | Note: *EGFR* L747P was a double mutation (c.2239_2240TT>CC) that was not interrogated by Sequenom |
| IV | 78 F | 0 | 1 | 842 | *ARID1A* S303fs*50 (10%) | None | None | None | None | N/A |
| IV | 63 F | 10 | 1 | 616 | *TP53* P64fs*84 (5%) | None | None | None | None | N/A |
| IIIA | 64 F | 0 | 1 | 672 | *EGFR* exon 18 E709_T710>D (17%) | None | None | None | None | N/A |
| IB | 77 F | 0 | 1 | 760 | None | *CD74-ROS1* | None | None | *CD74-ROS1* | Negative via *ROS1* FISH, poor hybridization noted in some areas |
| IIIA | 45 F | 4 | 0 | 488 | None | None | None | None | None | N/A |
| IV | 75 M | 0 | 1 | 562 | *BRAF* V600E (1%) | None | None | None | *BRAF* V600E | Potentially secondary to lower sensitivity of Sequenom: a low frequency of the alteration was detected via NGS |
| IIA | 55 M | 2 | 4 | 729 | *TP53* S127F (7%)<br>*TBX3* S615A (47%)<br>*RB1* Y321* (9%) | *KIF5B-RET* | None | None | *KIF5B-RET* | Negative via *RET* FISH at MSK and outside institution, initially identified as complex *RET* fusion by NGS and later determined to be *KIF5B-RET* after RNAseq testing |
| IV | 70 M | 0 | 6 | 580 | *PMS2* V415M (41%) | *ADAM2-PRKDC* | *CDK4* (8)<br>*MDM2* (16)<br>*IGF1R* (10)<br>*EGFR* (16) | None | None | N/A |

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**Table 2.** NGS results (Cont'd)

| Stage at diagnosis | Age, y/sex | Smoking history (pack-years) | Number of genomic alterations detected | Med exon coverage | Mutation (allele frequency) | Fusion | Amplification (copy number) | Loss | Genomic alteration uncovered by NGS that prior non-NGS testing was poised to detect: Alteration | Genomic alteration uncovered by NGS that prior non-NGS testing was poised to detect: Potential reason not detected by non-NGS testing |
|---|---|---|---|---|---|---|---|---|---|---|
| IV | 43 M | 2 | 2 | 659 | *CSF1R* V32G (53%)<br>*FAT3* R3910L (2%) | None | None | None | None | N/A |
| IV | 76 M | 0 | 5 | 638 | *NF1* Q83fs*2 (7%)<br>*SETD2* S2117FS*10 (23%)<br>*SETD2* S918fs*1 (18%) | None | *KRAS* (6)<br>*MDM2* (7) | None | None | N/A |
| IV | 73 F | 0 | 5 | 685 | *EP300* P925T (62%)<br>*TP53* K132N (20%)<br>*FLT3* E765* (7%) | None | *EPHA3* (7)<br>*MYC* (7) | None | None | N/A |
| IB | 29 F | 0 | 6 | 312 | *CDKN2A* R80* (39%)<br>*ARID2* R80fs*10 (12%) | *HIP1-ALK* | *CCND1* (>20)<br>*FGF19* (>20)<br>*FGF4* (>20) | None | *HIP1-ALK* | ALK FISH negative on review |
| IIIA | 62 F | 0 | 3 | 914 | *KIT* R956Q (46%)<br>*TP53* A159fs*19 (9%) | *KIF5B-RET* | None | None | *KIF5B-RET* | *RET* FISH clearly negative; however, performed on tumor sample from a separate biopsy from that subjected to NGS testing |
| IIA | 71 F | 0 | 4 | 536 | *EGFR exon 18* E709_T710>D (31%)<br>*TP53* P219fs*10 (13%) | None | *KRAS* (6)<br>*CCNE1* (14) | None | none | N/A |
| IV | 41 F | 2 | 6 | 851 | *EGFR* G719A (36%)<br>*EGFR* L707W (35%)<br>*PTCH1* T1064M (51%)<br>*TP53* splice (25%) | None | None | *CDKN2A*<br>*CDKN2B* | *EGFR* G719A | Found on the same allele as concurrent *EGFR* L707W mutation, likely interfering with hybridization of Sequenom extension primer |
| IV | 44 F | 12 | 3 | 706 | *STK11* H168R (10%)<br>*TP53* Y234C (9%)<br>*TP53* splice (6%) | None | None | None | None | N/A |
| IV | 62 M | 0 | 2 | 747 | None | None | *CDK4* (14)<br>*MDM2* (>20) | None | None | N/A |
| IV | 78 M | 15 | 3 | 745 | *KRAS* G12C (22%)<br>*TP53* G245R (15%)<br>*ATRX* P2478fs*2 (18%) | None | None | None | *KRAS* G12C | Potentially secondary to lower sensitivity of Sequenom |
| IV | 75 M | 0 | 4 | 778 | *ERBB2* L755F<br>*EZH2* R213H<br>*TP53* R248Q | None | *CDK4* | None | None | N/A |

NOTE: The results of hybrid capture–based clinical NGS assay in tumors from 31 patients are presented. In cases where the NGS assay identified a genomic alteration that previous non-NGS testing interrogated but did not detect, the potential reasons for nondetection are detailed.

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| Genomic alteration ($n$ = 96) | Genes most commonly involved | |
|---|---|---|
| Base substitution ($n$ = 34) | *TP53* | 24% ($n$ = 8) |
| | *EGFR* | 9% ($n$ = 3) |
| | *KRAS* | 6% ($n$ = 2) |
| Amplification ($n$ = 30) | *MDM2* | 17% ($n$ = 5) |
| | *CDK4* | 13% ($n$ = 4) |
| | *NFKBIA* | 10% ($n$ = 3) |
| | *NKX2-1* | 10% ($n$ = 3) |
| Insertion or deletion ($n$ = 15) | *EGFR* | 20% ($n$ = 3) |
| | *TP53* | 20% ($n$ = 3) |
| | *SETD2* | 20% ($n$ = 3) |
| Rearrangement ($n$ = 9) | *RET* | 33% ($n$ = 3) |
| | *ALK* | 22% ($n$ = 2) |
| Homozygous loss ($n$ = 5) | *CDKN2A* | 40% ($n$ = 2) |
| Splice-site mutation ($n$ = 3) | *TP53* | 67% ($n$ = 2) |

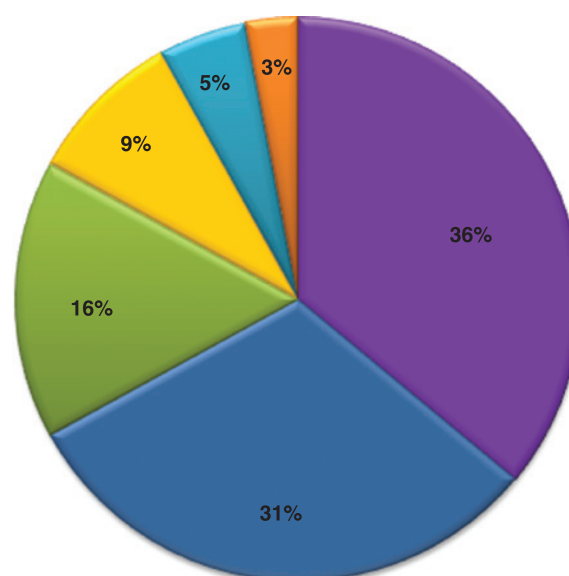


**Figure 1.**
Frequency of genomic alterations (GA) identified via NGS. A total of 96 GAs were identified in 31 lung adenocarcinomas. GAs were divided into 6 categories: base substitution, amplification, insertion/deletion, rearrangement, homozygous loss, and splice site mutation. The frequency of each of these categories is depicted in the pie chart on the right. The frequency of each of the most commonly involved genes under each category is detailed on the left.

*KRAS* G12C (ERK inhibitor, NCT01781429), *KRAS* Q61H (ERK inhibitor, NCT01781429), and *MDM2* amplification ($n$ = 3, MDM2 inhibitor, NCT01877382).

**Outcomes of targeted therapy use**

Of the 8 patients in whose tumors an alteration with a corresponding targeted agent based on the NCCN NSCLC guidelines was identified by the NGS assay, 6 (with tumors harboring *HIP1-ALK*, *SOCS5-ALK*, *CD74-ROS1*, *KIF5B-RET*, *KIF5B-RET*, *EGFR* G719A) went on to receive targeted therapy.

Two patients demonstrated a partial response (RECIST v1.1) to targeted therapy: *HIP1-ALK* with crizotinib and *KIF5B-RET* with cabozantinib (NCT01639508). Both patients remain on therapy and are progression-free at 5 and 7 months, respectively. Disease shrinkage < 30% (stable disease by RECIST v1.1) and a clinical response to therapy were noted in 2 others: *SOCS5-ALK* with crizotinib and *KIF5B-RET* with cabozantinib (NCT01639508). The former patient died from disease progression, whereas the latter remains progression-free on cabozantinib at 3 months. Two additional patients have begun targeted therapy but are pending a response evaluation: erlotinib for *EGFR* G719A and crizotinib for *CD74-ROS1*. Unless otherwise specified, targeted therapeutic agents were prescribed as standard of care and were acquired commercially.

One of the remaining 2 cases, the patient whose tumor harbors a *CCDC6-RET* fusion is currently receiving chemotherapy and will receive cabozantinib on disease progression. The patient whose tumor harbored a *BRAF* V600E mutation died before targeted therapy could be considered.

Of the 12 additional patients in whose tumors an actionable genomic alteration was discovered for which targeted therapy was available either on an ongoing trial at the institution or off-protocol, 11 remain on alternate systemic therapy and are candidates for targeted therapy on progression. The patient whose tumor harbors *EGFR* L747P recently started erlotinib and a response evaluation is pending.

## Discussion

While some composite algorithms that use multiple non-NGS tests, such as previously used at our center, identify a driver in most patients with lung adenocarcinomas, more comprehensive hybrid capture–based NGS assays afford the possibility of driver alteration detection in patients where no such genomic alteration was found initially on tumor analysis (23). In a report from the Lung Cancer Mutation Consortium (LCMC), comprehensive non-NGS genomic profiling of 1,007 lung adenocarcinoma specimens across multiple institutions was performed. A driver was identified in 64% of tumors, resulting in the use of targeted therapy in 28% of patients (24). However, largely because of limited tissue resources, only 70% of cases could have the full complement of LCMC-mandated testing. Despite this, patients whose genomic alteration was matched to a targeted therapy appeared to live substantially longer than those with a similar driver who received nontargeted therapies.

In this series, we demonstrate that, despite extensive prior conventional non-NGS testing, 26% of apparently "driver-negative" never or light smokers with lung adenocarcinoma may have tumors that harbor a genomic alteration uncovered only by a more comprehensive NGS approach. Of the 8 patients for whom such an alteration was detected in this study, 6 went on to receive targeted therapy. All 6 of these patients derived clinical benefit from targeted therapy initiation, and a partial response or evidence of disease shrinkage was noted in all 4 patients who have undergone a radiographic evaluation for disease response. Interestingly, the majority of these alterations were recurrent gene rearrangements involving *ALK*, *ROS1*, and *RET*.

Reasons for nondetection of these genomic alterations via non-NGS testing are varied. These include lower sensitivity, complex

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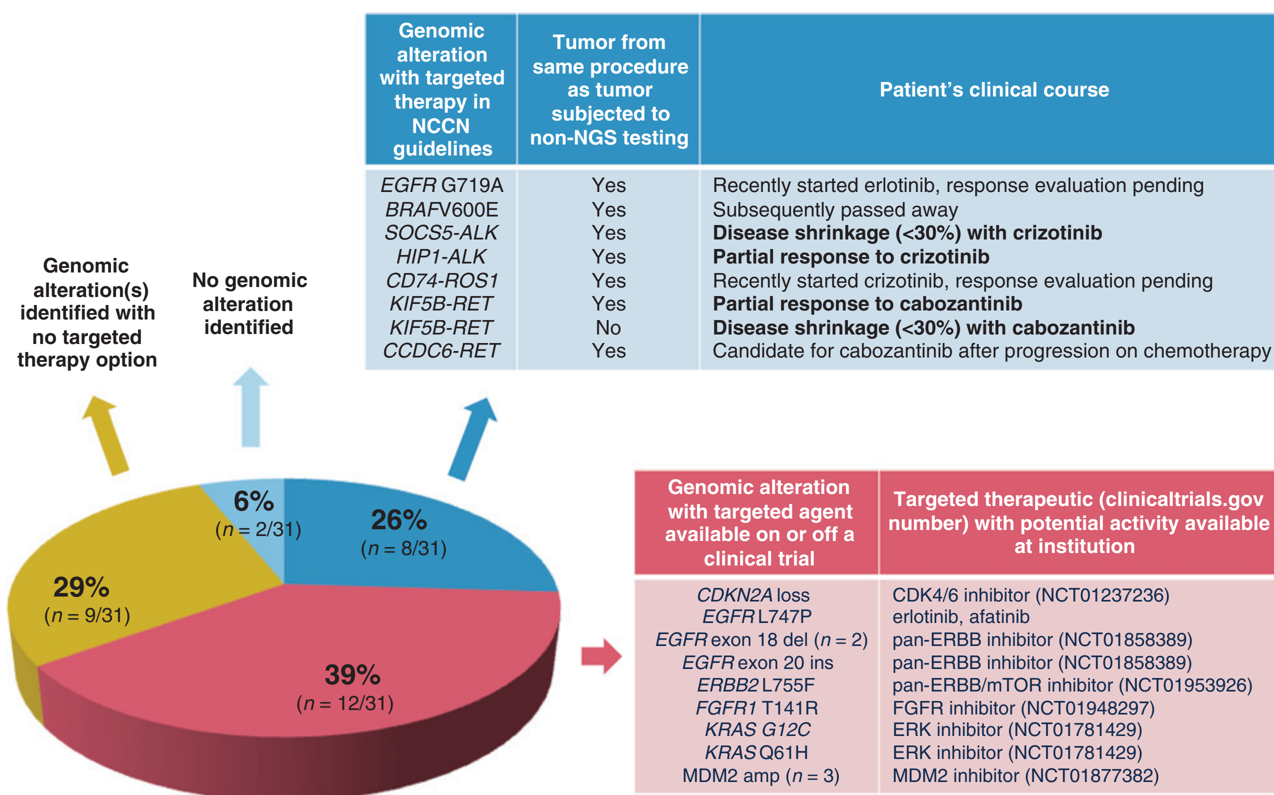


| Genomic alteration with targeted therapy in NCCN guidelines | Tumor from same procedure as tumor subjected to non-NGS testing | Patient's clinical course |
|---|---|---|
| *EGFR* G719A | Yes | Recently started erlotinib, response evaluation pending |
| *BRAF* V600E | Yes | Subsequently passed away |
| *SOCS5-ALK* | Yes | **Disease shrinkage (<30%) with crizotinib** |
| *HIP1-ALK* | Yes | **Partial response to crizotinib** |
| *CD74-ROS1* | Yes | Recently started crizotinib, response evaluation pending |
| *KIF5B-RET* | Yes | **Partial response to cabozantinib** |
| *KIF5B-RET* | No | **Disease shrinkage (<30%) with cabozantinib** |
| *CCDC6-RET* | Yes | Candidate for cabozantinib after progression on chemotherapy |

| Genomic alteration with targeted agent available on or off a clinical trial | Targeted therapeutic (clinicaltrials.gov number) with potential activity available at institution |
|---|---|
| *CDKN2A* loss | CDK4/6 inhibitor (NCT01237236) |
| *EGFR* L747P | erlotinib, afatinib |
| *EGFR* exon 18 del (*n* = 2) | pan-ERBB inhibitor (NCT01858389) |
| *EGFR* exon 20 ins | pan-ERBB inhibitor (NCT01858389) |
| *ERBB2* L755F | pan-ERBB/mTOR inhibitor (NCT01953926) |
| *FGFR1* T141R | FGFR inhibitor (NCT01948297) |
| *KRAS G12C* | ERK inhibitor (NCT01781429) |
| *KRAS* Q61H | ERK inhibitor (NCT01781429) |
| MDM2 amp (*n* = 3) | MDM2 inhibitor (NCT01877382) |

**Figure 2.**
Clinical NGS and targeted therapy use. The results of NGS of lung adenocarcinomas that harbored no genomic alterations (GA) in 11 genes (*EGFR*, *ERBB2*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1*, *PIK3CA*, *AKT1*, *ALK*, *ROS1*, and *RET*) via a focused panel of non-NGS testing in never or ≤15 pack-year smokers are shown. The percentage of patients with results that fall into 1 of 4 categories is depicted in the pie chart.

rearrangements undetectable by standard FISH, and, possibly, heterogeneity between different tumor biopsies or sites. Clinical samples sometimes contain biologically relevant genomic alterations at low allele frequencies due to excess nonneoplastic cells, leading to false-negative results on some forms of non-NGS testing. For such samples, deep and uniform unique coverage (median > 500×) is often necessary for a thorough analysis due to low tumor purity common in many metastatic tumor types (21). Regarding recurrent gene rearrangements, at least one large series has previously demonstrated the capability of this type of NGS assay to detect *ALK* fusions in patients whose tumors previously tested negative for an *ALK* rearrangement via FISH (25).

It is important to point out that this effort focused on a select population of patients with pathologically confirmed adenocarcinomas who were never smokers or smoked cigarettes ≤15 pack-years. This strategy was undertaken in an attempt to enrich the diagnostic yield of NGS for potential drivers. However, while many clinically actionable lung cancer drivers are more commonly found in tumors of never smokers, these drivers have been identified in tumors from smokers as well, and patients treated with the associated therapy appear to fare as well as the never or light former smoker population (26, 27). In addition, other actionable drivers such as some *BRAF* (13, 28) and *KRAS* mutations (29, 30) are enriched in tumors from patients with a significant history of smoking. No clinical characteristics can be used to select patients with NSCLC whose tumors should be tested, and current guidelines recommend routine *ALK* and *EGFR* testing of tumors from all patients (preferably as part of a multiplex panel) with adenocarcinomas, large cell carcinomas, NSCLC NOS (not otherwise specified), and squamous lung cancers from never smokers and small diagnostic biopsies (31).

Molecular diagnostic algorithms that use multiple non-NGS tests are becoming less tenable due to their relatively large tissue requirements. The majority of patients in this series required one or more procedures to complete testing. Specifically, more than two thirds of these patients required multiple biopsies to complete non-NGS testing alone. In addition, those who had sufficient tissue for analysis were largely patients who had undergone a surgical procedure for diagnosis and/or molecular testing. The type of NGS assay used in the present study offers the advantage of more comprehensive genomic characterization using as low as 50 ng of DNA, which can be obtained from lung cancer specimens with sufficient tumor content using core biopsy needles on average or a carefully prepared fine needle aspiration (32, 33).

Finally, the use of broad, hybrid capture–based NGS for more comprehensive molecular genotyping expands the scope molecular alterations interrogated and permits new driver alteration discovery. Highly specialized types of massively parallel sequencing, after rigorous analytic validation such as that described here, allow for the simultaneous interrogation of a broader spectrum of genes, including mutations in all exons of oncogenes and tumor suppressor genes, copy number gains and losses, and recurrent gene rearrangements (34). Partly to address the types of testing concerns revealed in the present study, our center (MSKCC) has

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recently implemented a similar broad, hybrid capture–based NGS assay (35).

With the advent of this approach, the process of matching the range of genomic alterations discovered with potential targeted therapeutics will undoubtedly represent an enormous challenge and an unprecedented opportunity for clinicians. If functional data are available that should help in discriminating strong potential drivers from passenger alterations. In the absence of such data, however, for patients with limited systemic therapy choices, targeted therapy that inhibits the oncogenic protein of interest or a downstream pathway remains a valid option (36).

## Conclusions

Broad, hybrid capture–based NGS assays have the potential to uncover clinically actionable genomic alterations in never smokers or ≤15 pack-year smokers whose lung adenocarcinomas do not harbor a potential driver via non-NGS testing. This approach provides a comprehensive and rapid interrogation of the cancer genome using potentially less tumor tissue than standard algorithms. In this series, the majority of patients either received or became eligible for targeted therapy due to the discovery of a clinically actionable genomic alteration via NGS. When available, we recommend the use of such NGS-based assays as the optimal molecular diagnostic platform for patients with lung cancers. Our center (MSKCC) has since adopted this type of NGS assay as our primary testing method of choice for patients with advanced lung cancers and other tumor types (35).

### Disclosure of Potential Conflicts of Interest

L. Wang is a consultant/advisory board member for Exelixis. S. Balasubramanian, P. Stephens, D. Lipson, V.A. Miller, and M. Ladanyi have ownership interest (including patents) in Foundation Medicine. J.S. Ross reports receiving commercial research grants from, and holds ownership interest (including patents) in Foundation Medicine. M.G. Kris is a consultant/advisory board member for AstraZeneca, Clovis, and Genentech/Roche. No potential conflicts of interest were disclosed by the other authors.

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