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# Changing the Therapeutic Landscape in Non-small Cell Lung Cancers: the Evolution of Comprehensive Molecular Profiling Improves Access to Therapy

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## Abstract

Targeting genomic alterations has led to a paradigm shift in the treatment of patients with lung cancer. In an effort to better identify potentially actionable alterations that may predict response to FDA-approved and or investigational therapies, many centers have migrated towards performing targeted exome sequencing in patients with stage IV disease. The implementation of next-generation sequencing (NGS) in the evaluation of tumor tissue from patients with NSCLC has led to the discovery of targetable alterations in tumors that previously had no known actionable targets by less comprehensive profiling. An improved understanding of the molecular pathways that drive oncogenesis in NSCLC and a revolution in the technological advances in NGS have led to the development of new therapies through biomarker-driven clinical trials. This review will focus on the advances in molecular profiling that continue to fuel the revolution of precision medicine, identifying targets such as *MET* exon 14 skipping alterations and select recurrent gene alterations with increasing frequency.

### Keywords

Non-small cell lung cancer (NSCLC); Precision medicine; Targeted therapy; Molecular diagnostics; Next-generation sequencing (NGS); RNA sequencing; MET exon 14 skipping alteration; NTRK fusion

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Compliance with Ethical Standards

**Conflict of Interest** Joshua K. Sabari declares that he has no conflict of interest.
Fernando Santini declares that he has no conflict of interest.
Isabella Bergagnini declares that she has no conflict of interest.
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**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## Introduction

Despite broad advances in diagnostics and therapy, lung cancer remains the leading cause of cancer related mortality in the USA [1].The 5-year overall survival for patients with stage IV non-small cell lung cancers (NSCLCs) is 1–5% and has not changed over the past 25 years [2]. Prior to the advent of targeted therapy, systemic therapy for lung cancers was thought to be a “one-size-fits-all approach,” with the selection of cytotoxic chemotherapy based largely on histologic features. While cytotoxic chemotherapy remains a critical tool in the oncologist’s armamentarium, our treatment paradigms have moved toward the inclusion of strategies that involve more precisely selecting biologic therapies in molecular subgroups of patients. Precision medicine, defined by the National Institute of Health as an “approach to treatment based on individual differences in a patients genome” is now a reality for patients with NSCLC [3]. We now understand that NSCLC is not a one-size-fits-all disease; rather, there is a diverse landscape of genomic alterations that drive oncogenesis.

It is now standard of care to test for *EGFR* (epidermal growth factor receptor) mutations, and for rearrangements involving *ALK* (anaplastic lymphoma kinase) and *ROS1*, given that targeted therapy results in substantial benefits in terms of response and progression-free survival [4]. Sensitizing mutations in *EGFR* such as deletions in exon 19 and a single-point mutation in exon 21 (L858R), predict response to the *EGFR* tyrosine kinase inhibitors (TKIs) erlotinib, gefitinib, afatinib, and osimertinib [5], whereas most *EGFR* exon 20 insertions predict resistance to EGFR TKIs [6]. *KRAS* mutations are associated with intrinsic EGFR TKI resistance [7]. Patients harboring fusions involving the *ALK* gene, most commonly *EML4-ALK*, predict response to the ALK inhibitors crizotinib, alectinib, and ceritinib [8]. *ROS1* has a high degree of homology with *ALK*, and rearrangements involving *ROS1* predict response to ROS1 tyrosine kinase inhibition with crizotinib [9].

## The Evolution of Molecular Profiling

There are multiple laboratory techniques that can be used to screen for clinically actionable alterations in non-small cell lung cancers. Over the last 12 years, testing strategies have evolved from a “one-gene, one-test” approach, to intermediate multiplex testing using several tests, to more comprehensive massively parallel sequencing with or without complementary plasma-based genomic profiling.

Real-time polymerase chain reaction (PCR) and Sanger sequencing were viewed as the gold standard for the detection of *EGFR* mutations, whereas fluorescence in situ hybridization (FISH) can be used to detect *ALK* and *ROS1* rearrangements. Both PCR and FISH require a priori knowledge of the genomic target alteration of interest in order to build specific DNA primers (PCR) or fluorescent-labeled DNA probes (FISH). While reflex testing for *EGFR*, *ALK*, and *ROS1* alterations using PCR and FISH have become standard of care in the workup of patients with advanced lung cancer, these are single tests that look at sensitizing events in single genes. As an intermediate step, the field moved toward incorporating multiplex assays such as Sequenom (Sequenom) and SNaPshot assays (Applied Biosystems) as a means of interrogating mutational hotspots in a panel of different genes.

In more recent years, testing algorithms have moved towards the adoption of next-generation sequencing (NGS) technology that allowed for the detection of common alterations, in addition to less common or previously unknown genomic alterations. Sequencing of the entire gene is a comprehensive method for mutation testing. Whole genome sequencing is useful when the target abnormality is not well defined, but this process is both time-consuming and costly, and often unable to detect the genomic alteration when present at low levels. Advances in next-generation massively parallel sequencing allows for the quantitative analysis of rare alleles. This technology is now cost effective and can be performed in real time.

The implementation of next NGS in the evaluation of a patient with stage IV NSCLC has led to the discovery of targetable alterations in patients who previously had no known actionable targets. An improved understanding of the molecular pathways that drive oncogenesis in NSCLC and a revolution in the technological advances in NGS has led to the development of new therapies that target these specific genomic alterations; in essence, the pursuit of personalized medicine.

## Single-Gene Testing

### Sanger Sequencing

Developed in the late 1970s, Sanger sequencing was one of the earliest methods to detect mutations in lung cancer such as *EGFR* and *KRAS* [10]. Sanger sequencing, also referred to as chain termination sequencing, is the process of determining the sequence of nucleotides in a fragment of DNA. This process requires a DNA template of interest, the DNA polymerase enzyme, four deoxynucleotides (dNTPs: dATP, dTTP, dCTP, and dGTP), and four dideoxynucleotides (ddNTPs, chain-terminating versions of the nucleotides that are color labeled). Using PCR technology, DNA is amplified by heating the template DNA strand leading to denaturation. Once the DNA is cooled, the DNA primer binds to the single-stranded DNA template. The suspension is again heated to allow for DNA polymerase to synthesize new DNA using the available dNTPs. Once a ddNTP or chain-terminating nucleotide is added at random, the reaction is terminated and no further nucleotides can be added. This process is repeated over multiple cycles, allowing dNTPs to be added at every single position of the target DNA. This, in turn, produces differing lengths of DNA chains that are then separated on a single lane capillary gel. The resulting bands are read by an imaging system and subsequent computational analysis is performed. Sanger sequencing can provide high-quality DNA sequencing for up to 900 base pairs with depth coverage of 10–100×. This process is expensive, time-consuming, and inefficient at sequencing whole genes or cancer genomes. As an example, using Sanger sequencing methodology, the Human Genome Project completed in 2003 took 10 years to complete at a cost of nearly $3 billion dollars [11].

### Reverse-Transcriptase Polymerase Chain Reaction

Reverse-transcriptase polymerase chain reaction (RT-PCR) allows for the detection of predefined gene fusions [12, 13]. RNA is first extracted from the patient's tumor sample. Primers are designed to hybridize with chimeric transcripts and the RNA sequence is reverse

transcribed to DNA. The DNA transcripts are then amplified using PCR technology. This technology is efficient and requires a low volume of tumor cells. The caveats to RT-PCR are that it is highly specific for the predefined fusion and cannot detect alternate fusion partners. RT-PCR also requires a high level of technical skill and high-quality RNA that is often difficult to obtain in paraffin-embedded specimens.

### Fluorescence In Situ hybridization

Fluorescence in situ hybridization (FISH) is a unique technology that utilizes break-apart probes that label the fusion breakpoint. The 3′ telomeric end and the 5′ centromeric end of a gene of interest (such as *ALK* or *ROS1*) is labeled with a different fluorochrome. When the fusion is not present, these predefined probes lie close to one another on the chromosome, and the two fluorescence signals appear fused. In the presence of a rearrangement, chromosomal inversion or translocation, the fluorescence signals appear to be split or isolated to the 3′ or 5′ ends [14]. In the clinic, FISH remains a widely used assay to detect fusions in lung cancer. There is an FDA-approved companion diagnostic test for the detection of *ALK* fusions for crizotinib use in *ALK*-rearranged lung cancer (Vysis LSI ALK Break Apart Rearrangement Probe Kit; Abbott Molecular). FISH is also used in the detection of *ROS1* and *RET* rearranged lung cancers and there are ongoing validation studies for their companion diagnostic testing. FISH, unlike RT-PCR, affords the ability to identify fusions with variant partners, granted that the fusion event is identified without specifically identifying the upstream gene partner. Newer multicolor and multiprobe assays can now interrogate more than one gene rearrangement for specific upstream partners. FISH testing is also a commonly used modality to detect copy number alterations such as *MET* and *FGFR1*-amplififed lung cancers. Challenges of FISH testing include the technical complexity to perform the assay, and more importantly, to interpret the result.

## Early Multiplex Testing

As mentioned previously, molecular diagnostics such as immunohistochemistry (IHC), RT-PCR, and FISH are all characterized by a one-gene one-test strategy, representing a piecemeal analysis of a patient's tumor. As the list of actionable driver alterations continues to grow, mostly through the development of targeted therapies, some may argue that single-gene testing may not be feasible or cost effective. There is also growing concern that there is often insufficient tissue, from small-volume biopsies, to perform multiple individual molecular tests. Here we discuss the move towards multiplex testing through mutational hotspot assays [15••].

### Multiplex Hotspot Mutational Testing

Multiplex PCR is the simultaneous amplification of at least two DNA or cDNA targets in a single reaction. The currently FDA-approved compendium diagnostic test for erlotinib, cobas EGFR Mutation Test (Roche), is a real-time PCR based molecular diagnostic test that identifies 41 mutations across exons 18, 19, 20, and 21 of the *EGFR* gene. This assay is performed on formalin-fixed paraffin embedded tissue. In June of 2016, the FDA approved the first "liquid biopsy," cobas EGFR Mutation Test v2, a multiplex real-time PCR assay which is performed on plasma [16]. In contrast, Sequenom assays (Sequenom) and

SNaPshot assay (Applied Biosystems) can sequence a large number of mutations in several recurrently mutated regions or hotspots. These platforms sequence through a multiplex-PCR system, followed by individual base extension reactions. The Sequenom platform tests for over 200 somatic mutations across multiple genes whereas the SNaPshot assay can detect upwards of 50 mutations in multiple cancer genes [17]. These panels can be tailored to include a specific set of driver mutations, for example in lung adenocarcinoma: *EGFR*, *ERBB2*, *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *PTEN*, and *AKT*.

## Next-Generation Sequencing

Next-generation sequencing (NGS), also known as high-throughput sequencing, refers collectively to the recent advances in DNA sequencing. This term encompasses targeted exome, whole exome, genome, transcriptome, and epigenome analyses [18]. NGS has the potential to sequence the entire target gene for changes that might occur at both hotspot and non-hotspot regions. Most alterations are located in the hotspot coding regions; however, interrogating non-hotspot areas remains relevant in assessing alterations in tumor suppressor genes and oncogenes that can occur across the length of a gene. NGS also has the ability to uncover gene rearrangements or fusions by tiling introns from recurrently rearranged genes whose breakpoints are relatively well conserved. Gene amplifications or loss can also be detected by referencing a standard for that particular assay. Of the various types of NGS, the most clinically relevant assay to date remains targeting exome sequencing which can interrogate hundreds of potentially actionable alterations in a single test. By intentionally targeting specific genomic locations, targeted exome sequencing can provide higher sequencing depth of coverage and can more accurately sequence variants at these loci [19]. Depth of coverage is the average number of sequencing reads that align to each base within the sample DNA. Higher depth of coverage provides more certainty that a true base change will be detected. Whole exome and whole genome sequencing remain critical research tools in that they provide a broader scope of coverage, however the cost and time to perform these analyses prohibits this testing from routine use in the clinic.

The methodology by which NGS is performed is not universal. Some assays require both tumor DNA and matched normal peripheral blood (to rule out germline alterations), whereas other assays only test DNA in the tumor. DNA is extracted from the tumor sample and a DNA library is prepared. It is critical to have sufficient tissue to perform this analysis, and most NGS testing requires at least 50–100 ng of DNA (Illumina). Once the DNA is extracted from the tissue, it is then amplified using PCR technology as described above. NGS is different from Sanger sequencing in that the templates are sequenced in massively parallel fashion in a single run [20]. Highly paralleled sequencing allows large scale sequencing reactions to take place simultaneously, as well as shorter reads ranging from 50 to 700 nucleotides, with a depth coverage of 500–1000×.

### Next-Generation Sequencing in Lung Cancer

In 2012, Imielinski et al. published the first large report of whole exome and genome sequences of 183 lung adenocarcinoma tumor and matched normal DNA pairs [21]. In 2014, The Cancer Genome Atlas (TCGA) Research Network published the molecular profile of

230 resected lung adenocarcinomas and matched normal DNA pairs [22••]. Whole exome sequencing of tumor and germline DNA with a mean coverage depth of ~100× was performed in patients with previously untreated disease. High rates of somatic mutations were seen with a mean of 12.0 and 8.9 mutations per megabase, in each study respectively [21, 22••]. Commonly altered genes included *TP53* (46%), *KRAS* (33%), *KEAP1* (17%), *STK11* (17%), *EGFR* (14%), *NF1* (11%), *BRAF* (10%), *PIK3CA* (7%), and *MET* (7%). The TCGA and other studies provocatively showed that mutations such as *EGFR* and *KRAS* were mutually exclusive. There was also a significant difference in the genomic alterations of smokers and non-smokers (defined as less than 100 cigarettes lifetime). Patients with a history of smoking had a tenfold increase in mutation burden when compared to non-smokers [23]. In 2012, the TCGA Research Network also published the molecular profile of 178 resected squamous cell lung cancers and matched normal DNA pairs, showing a mutation rate of 8.1 mutations per megabase [24]. Almost all lung squamous cell carcinomas (SQCC) displayed a somatic mutation of *TP53*, with frequent alterations in *CDKN2A*/*RB1*, *NFE2L2*/*KEAP1*/*CUL3*, *PI3K*/*AKT*, and *SOX2/TP63/NOTCH1* pathways. *EGFR* and *KRAS* mutations were rarely seen in SQCC; one sample had a *KRAS* mutation, 7% of cases had an amplification in *EGFR*; however, there were no exon 19 or exon 21 (L858R) activating mutations. Interestingly, there were two instances of L861Q mutations which confer sensitivity to an EGFR TKI.

Highly parallel, micro scaled, shorter length DNA sequencing has made sequencing more efficient and cost effective. In combination with advances in micro-scaled technology, the raw cost of sequencing a genome using NGS is now around $US1000 [11, 25, 26]. NGS allows for rapid, efficient, and cost effective sequencing. NGS in contrast to Sanger sequencing makes large-scale whole genome sequencing accessible to the patient [27]. The National Comprehensive Cancer Network (NCCN) now recommends mutation testing for *EGFR*, *BRAF*, *ERBB2*, *MET*; rearrangements in *ALK*, *ROS1* and *RET*; and *MET*amplification in all patients diagnosed with metastatic NSCLC [4]. NGS platform offers the patient and the clinician a single test that is able to capture point mutations (base substitutions), insertions and or deletions, gene rearrangements, and amplification or loss in hundreds of cancer-related genes [28]. Table 1 is a selection of currently available molecular diagnostic platforms in relation to the genomic alterations these tests are poised to target. Recent genomic studies in lung adenocarcinoma have identified actionable oncogenic alterations involving the RTK/RAS/RAF/PI3K axis such as *EGFR*, *KRAS*, *HER2*, *BRAF*, *ARAF*, *CRAF*, *PIK3CA*, *MET*, RITi1, *MAP2K1*, *NRAS*, *HRAS* mutations and *ALK*, *ROS1*, *RET*, *ERBB4*, *NTRK*, *NRG1* and *BRAF* rearrangements [22••, 29]. For multiplex platforms and NGS, the genomic alterations that are interrogated by these assays often can be customized based on histology and clinical need. NGS as a whole, outperforms each individual test, and is therefore a critical tool in the effective diagnosis and treatment of patients with stage IV lung cancer.

## Blood-Based Biomarker Testing

More recently plasma genotyping of circulating cell-free tumor DNA has been explored both in the clinic and within clinical trials. Droplet digital PCR (ddPCR) represents a sensitive method for the detection of actionable alterations such as hotspot mutations in the plasma. A

recent study showed that ddPCR had a sensitivity of 100% and specificity of 69–80% for the detection of *EGFR*-sensitizing mutations with a rapid turnaround time of 3 business days [30]. Avariety of other plasma-based assays are available, some of which are now available commercially. Clinical trials for third-generation EGFR TKIs have demonstrated that patients whose plasma is positive for *EGFR* T790M have responded to targeted therapy. Of note, profiling of tumor DNA in urine has also recently been explored as a means of biomarker testing.

While molecular profiling of plasma has increased the rapidity at which actionable alterations are detected in the clinic, thereby decreasing the time to targeted therapy initiation [30], these tests are not without their limitations. First, these tests are unable to detect histology and histologic changes within tumors that direct therapy selection. As an example, acquired resistance to EGFR TKI therapy in *EGFR*-mutant lung cancers can be mediated in select cases by small cell transformation, thus warranting the consideration of small-cell-directed cytotoxic chemotherapy. Second, the breadth of coverage of plasma assays in terms of the number of alterations detected has yet to approach the complexity that we are able to detect via comprehensive NGS assays. In addition, the ability to detect all clinically-relevant recurrent gene rearrangements remains questionable. In summary, while blood-based testing is an extremely useful adjunct test in the clinic, it is unlikely to replace tumor biopsies in the near future.

## Advantages of Next-Generation Sequencing

Broad, hybrid-capture NGS is able to detect actionable genomic alterations that have not been previously identified by other testing modalities such as FISH, PCR, and other multiplex assays. A 2015 retrospective review of never or light smokers with stage IV lung adenocarcinoma whose tumors did not harbor actionable alterations after being subjected to a prior non-NGS testing algorithms (Sanger sequencing, Sequenom, and several FISH assays) revealed that with NGS, 26% of patients had tumors that harbored actionable genomic alterations with targeted therapy outlined in the NCCN guidelines. Furthermore, an additional 39% of patients had tumors with genomic alterations that made them potentially eligible for additional targeted therapy on or off a clinical trial. In total, 65% of patients who had previously tested negative for alterations via multiple non-NGS methods had potentially actionable alterations when interrogated with broad, hybrid-capture-based NGS [31••]. These findings, as well as others, underscored the importance of first-line NGS profiling of patients with stage IV lung cancer [32, 33••].

Two newly actionable genomic alterations, *MET* exon 14 skipping alterations and *NTRK* fusions, provide evidence that NGS sequencing can expand the number of actionable alterations that are detected in the clinic. The use of NGS has led to expanded therapeutic options for patients through enrollment in biomarker-driven clinical trials. *MET* exon 14 skipping represents 3–4% of all lung adenocarcinoma and *NTRK* fusions likely represent a smaller proportion of lung adenocarcinomas [22••, 34••, 35••]. These two alterations both have therapies that are currently under investigation in clinical trials.

### *MET* Exon 14 Skipping in NSCLC

While *MET* alterations in lung cancer were discovered over 10 years ago [36], it is only recently with the advent of improved NGS technologies that the routine detection of actionable drivers such as *MET* exon 14 skipping mutations has become more feasible. Mesenchymal-epithelial transition (*MET*) is a high-affinity tyrosine kinase that upon activation drives a broad array of pathways involved in cell proliferation, survival, and metastasis [37]. Gain-of-function alterations in *MET* drive oncogenesis, and specific subsets can predict poor prognosis [38]. *MET* exon 14 skipping results in the deletion of the juxtamembrane domain of MET, leading to enhanced signaling through the *MET* receptor pathway. These mutations have been shown to be tumorigenic in vitro and in vivo [36]. Over 100 mutations in *MET* result in exon 14 skipping and they occur in both the presence and absence of concurrent *MET* amplification [34••, 39••, 40••, 41]. As described in the initial TCGA paper in 2014, *MET* splice site mutations results in the absence of recognition of splice sites that flank exon 14, leading to skipping of the exon and high levels of MET protein expression due to decreased binding of the E3 ubiquitin ligase CBL [22••].

Diagnostic testing strategies for MET have similarly evolved over the last few years. Earlier clinical trial strategies focused on MET overexpression detected by IHC, leading largely to negative results. This is not surprising given that protein overexpression alone can be observed in various settings and is not a clear driver of tumor biology on its own. Of note, many *MET* exon 14-altered lung cancers highly overexpress MET, but the converse is not true. Overexpression of MET does not, in all cases, signify the presence of underlying *MET* exon 14 skipping. In addition to IHC, FISH has been used to detect amplification of *MET* that has been shown to potentially drive tumor growth. A report from an expansion cohort of a phase I trial of crizotinib showed responses to crizotinib in patients with *MET*-amplified lung cancers [42]. Beyond FISH, however, NGS has the ability to detect both copy number changes, and specifically interrogate mutations in intronic and exon regions that lead to *MET* exon 14 skipping.

*MET* exon 14 skipping represents a unique target responsive to MET inhibition with agents such as crizotinib and a variety of other *MET* inhibitors [40••, 41, 43, 44]. There are multiple ongoing clinical trials assessing the efficacy of *MET* inhibition in this unique subset of NSCLC with TKI inhibitors including crizotinib, cabozantinib, capmatinib, merestinib, savolitinib and tepotinib. The efficacy and safety of crizotinib in 18 patients in the ongoing phase 1 PROFILE 1001 study (NCT00585195) was recently reported, showing antitumor activity via RECIST criteria in 10 out of the 15 evaluable patients (response rate of 44%). In addition, there were observed responses in patients with advanced sarcomatoid carcinoma, a histology traditionally thought of as refractory to cytotoxic chemotherapy [45].

### *NTRK* Fusions in NSCLC

The tropomyosin receptor kinase (TRK) receptor family is comprised of three transmembrane receptors: TRKA, TRKB, and TRKC. These receptors are encoded by the genes *NTRK1*, *NTRK2*, and *NTRK3*, respectively. These receptor tyrosine kinases are expressed in normal neuronal tissue and are activated by neutrophins leading to the development and function of the nervous system [46]. The binding of the ligand or

neutrophin to the receptor leads to the activation of downstream signal transduction pathways responsible for cell proliferation, differentiation, and survival. Fusions involving *NTRK1* result in a constitutively active chimeric protein leading to oncogenic activation of the receptor tyrosine kinase and represent a potential therapeutic opportunity [47]. *NTRK1* rearrangements have been identified in a broad range of malignancies including colon cancer, soft tissue sarcoma, papillary thyroid carcinoma, glioblastoma, NSCLC, and others [48].

The frequency of *NTRK1* fusions in patients with lung adenocarcinoma is reported as 3.3%; however, the true incidence is likely lower [35••]. *NTRK1–3* fusions can be detected with a variety of methods. Unlike with *ALK* and *ROS1* rearrangements, *NTRK* FISH was not developed as a standard of care test for patients with lung cancers. It was largely through advances in DNA-based broad hybrid-capture NGS, with RNA-based anchored multiplex PCR as an adjunct, that fusions involving *NTRK1–3* are now increasingly detected. Entrectinib and LOXO-101 are pan-TRK inhibitors that are currently under investigation in phase I/II trials [49, 50]. A brisk and durable response to TRK inhibition has already been described in a patient with an advanced NSCLC. This same patient had a complete response intracranially and a substantial improvement in quality of life. This dramatic response highlights the need to check for rare genomic alterations using a comprehensive platform [51].

## Conclusions

NSCLC is a diverse disease with multiple oncogenic drivers and each individual patient is truly unique. Advances in technology have improved our ability to detect these drivers in the clinic. When available, clinicians should perform comprehensive molecular testing such as broad hybrid-capture NGS on a validated platform in an attempt to identify an oncogenic driver in patients with advanced lung cancers. Identifying a clinically actionable target will provide the patient with additional systemic therapy options including both FDA-approved targeted therapies or investigational agents being explored under clinical trials. Given its breadth and depth, NGS holds immense promise for the future of diagnostic testing in patients with lung cancer. NGS in conjunction with complementary plasma-based molecular profiling is quickly becoming the standard of care testing modality to uncover actionable genomic alterations.

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**Table 1**

Selections of currently available molecular diagnostic platforms are shown in relation to the genomic alterations these tests are poised to target

| Selected therapeutically relevant genomic alterations in NSCLC | Sanger sequencing | Immunohistochemistry | Fluorescence in situ hybridization | Multiplex hotspot mutation testing | Multiplex sizing assays | Anchored multiplex PCR RNA sequencing | Next-generation DNA sequencing |
|---|---|---|---|---|---|---|---|
| Point mutations | | | | | | | |
| *EGFR* | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| *KRAS* | | (EGFR L858R) | | | | | |
| *ERBB2 (HER2)* | | | | | | | |
| *MAP2K1 (MEK)* | | | | | | | |
| *BRAF* | | | | | | | |
| *PIK3CA* | | | | | | | |
| *AKT* | | | | | | | |
| Insertions or deletions | | | | | | | |
| *EGFR* | ✓ | ✓ | | | ✓ | ✓ | ✓ |
| *ERBB2 (HER2)* | | (EGFR exon 19 deletion) | | | | | |
| Rearrangements | | | | | | | |
| *ALK* | | ✓ | ✓ | | | ✓ | ✓ |
| *ROS1* | | (ALK and ROS1 require FISFI confirmation) | | | | (Novel fusion detection) | |
| *RET* | | | | | | | |
| *NTRK* | | | | | | | |
| Amplification/loss | | | | | | | |
| *MET* | | ✓ | ✓ | | | ✓ | ✓ |
| *PTEN* | | (MET amplification requires FISFI confirmation) | | | | | |
| Non-recurrent genomic alterations | | | | | | | |
| Other potentially relevant oncogenes and tumor suppressor genes | | | | | | | ✓ |

For multiplex platforms and next-generation sequencing, the genomic alterations that are interrogated by these assays often can be customized based on histology and clinical need