The Emerging Role of Molecular Testing in Non–Small Cell Lung Cancer

PATRICK J. KIEL,[1,2] PharmD, BCPS, BCOP, LIANG CHENG,[1] MD, ANNA LIVERS-ERTEL,[3] ANP-BC, MSN, OCN®, and GREGORY DURM,[2] MD

From [1]Indiana University School of Medicine, Indianapolis, Indiana; [2]Indiana University Simon Cancer Center—IU Health, Indianapolis, Indiana; [3]Franciscan Health Cancer Center, Indianapolis, Indiana

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Correspondence to: Patrick J. Kiel, PharmD, BCPS, BCOP, Indiana University Simon Cancer Center—IU Health, 535 Barnhill Drive, Indianapolis IN 46202. E-mail: pkiel@iuhealth.org

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Abstract

In recent years, the role of molecular testing in non–small cell lung cancer (NSCLC) has rapidly grown. The US Food and Drug Administration (FDA) has approved several new medications to treat patients with genetic alterations over the past 10 years, and the development of improved technology has made sequencing more affordable, efficient, and convenient. With these advances, the popularity of genomic sequencing will continue to rise rapidly, further affecting routine clinical practice and treatment recommendations. Therefore, it is increasingly important for advanced practitioners treating patients with NSCLC to understand how these genomic markers are used in practice, comprehend the updated treatment guidelines to be able to identify which patients to test with which type of test and at what point in their treatment, and have a firm grasp of where the world of molecular testing is headed.

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The era of precision medicine has been a deluge in the field of clinical medicine, and unprecedented progress has been observed in the prognostic and predictive genomic analysis of lung cancer. Major advances in molecular profiling to assess somatic mutations in non–small cell lung cancer (NSCLC) have led to the development of small molecules to target genomic aberrations. Combined with the advances in immunotherapy, the armamentarium of treatment options has greatly expanded in the past decade. This supplement will discuss genomic markers utilized in clinical practice, including targeting aberrations such as epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), and *ROS1* rearrangements with oral tyrosine kinase inhibitors (TKIs).

Lung cancer is the leading cause of cancer-related death in the United States, with an estimated 222,500 new cases diagnosed in 2017 and 155,870 attributable deaths (Siegel, Miller, & Jemal, 2017). Approximately 85% to 90% of lung cancers are the result of cigarette smoking either as an active smoker or through secondhand smoke (Centers for Disease Control and Prevention, 2004). It is

estimated that 18.1% of patients with lung cancer are alive 5 years after diagnosis.

The World Health Organization divides lung cancer into two major classes based on its biology, therapy, and prognosis: NSCLC (this supplement will focus on advanced NSCLC) and small cell lung cancer. Non–small cell lung cancer accounts for more than 80% of all lung cancer and includes the major subtypes of squamous cell carcinoma and nonsquamous carcinoma, such as adenocarcinoma, large cell carcinoma, and other types (Howlader et al., 2017). All patients with adenocarcinoma should be tested for *EGFR* mutations, *ALK* gene rearrangement, *ROS1* rearrangements, and programmed cell death ligand 1 (PD-L1) expression levels. Additional testing for rare somatic mutations such as *BRAF* V600E may also be considered. Good prognostic factors include early-stage disease, good performance status (Eastern Cooperative Oncology Group [ECOG] 0, 1, or 2) no significant weight loss, and female gender (Finkelstein, Ettinger, & Ruckdeschel, 1986).

BIOMARKERS IN NSCLC

In the past decade, several biomarkers have emerged as prognostic and predictive markers for NSCLC (Figure). A prognostic biomarker indicates patient survival independent of the treatment administered and is more an indicator of tumor aggressiveness. An example of a predictive biomarker is the *KRAS* G12D DNA mutation, which is an independent prognostic marker of poor survival and renders EGFR TKI therapy ineffective. A predictive biomarker indicates therapeutic efficacy because of a known interaction of the therapy and the biomarker. Predictive biomarkers supported by the National Comprehensive Cancer Network (NCCN) with high-grade evidence include the *ALK* fusion oncogene, *ROS1* gene rearrangements, sensitizing *EGFR* mutations, and PD-L1 expression (NCCN, 2017). Emerging biomarkers include *BRAF* V600E mutation, *RET* gene rearrangements, and *MET* exon 14 skipping mutations (NCCN, 2017). Testing for *ALK* gene rearrangements and *EGFR*-sensitizing mutations are NCCN Category 1 recommendations for patients with nonsquamous NSCLC or NSCLC not otherwise specified, whereas *ROS1* is considered a Category 2A recommendation (NCCN, 2017; Table 1).

EGFR mutations are the most common type of predictive biomarker observed in NSCLC, with an observed incidence of 10% and 50% in the Caucasian and Asian populations, respectively (Cheng et al., 2012; NCCN, 2017). The most common type of aberrations include deletions in exon 19 and exon 21 mutations (L858R), which account for 45% and 40% of *EGFR* mutations, respectively (Cheng et al., 2012; Ettinger et al., 2017). Both aberrations result in an activation of the tyrosine kinase domain and are associated with sensitivity to TKI small molecules such as erlotinib, gefitinib, and afatinib. Primary resistance to EGFR TKIs is associated with *KRAS* mutations, *ALK*, or *ROS1* gene rearrangements. An example of an acquired resistant mutation is T790M, an exon 20 mutation of an *EGFR* T790M DNA. The NCCN recommends that T790M mutations, identified after resistance to EGFR TKIs, should be treated with the third-generation EGFR TKI inhibitor osimertinib (NCCN, 2017).

ALK gene rearrangements occur in 2% to 7% of patients with NSCLC and are not commonly found in patients with squamous cell histology (Blackhall et al., 2014). The US Food and Drug Administration (FDA) has approved a molecular diagnostic test for detecting *ALK* rearrangements as a prerequisite for crizotinib treatment (Cheng et al., 2012). Crizotinib is also a potent inhibitor of *ROS1* and some *MET* tyrosine kinases, such as *MET* exon 14 skipping mutation (Jorge et al., 2015). In patients with *ALK* gene rearrangements, prospective randomized trials in the first and relapsed settings have resulted in improved progression-free survival (PFS) compared with standard chemotherapy. Second-generation TKIs ceritinib, alectinib, and brigatinib are potential treatment options for patients who experience disease progression on crizotinib (NCCN, 2017).

METHODS AND APPLICATIONS OF TECHNOLOGY

Molecular detection of sensitizing mutations in *EGFR* or *ALK* and *ROS1* rearrangements provide the unique opportunity for patients with molecularly selected lung cancer to receive targeted treatments. And along with these new molecular targets come several testing technologies to uncover them, as well as various clinical applications (Table 2).

EGFR Mutation Testing

The incidence of *EGFR* mutations in unselected NSCLC tumors ranges from 10% to 50%, depending on the ethnic makeup of the patient population and the detection methods used (Cheng et al., 2012; Jamal-Hanjani et al., 2017; Sharma, Bell, Settleman, & Haber, 2007). Detection of actionable driver and drug-resistant mutations has greatly expanded the therapeutic options for patients with advanced lung cancer (Hirsch et al., 2017; Jamal-Hanjani et al., 2017). Historically, direct sequencing of DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples was the gold standard for identifying *EGFR* mutations in patients with NSCLC (Hitij et al., 2017). A number of sequencing platforms for mutation testing have been developed and used in recent years, including Sanger sequencing, pyrosequencing, and next-generation sequencing (NGS).

Sanger Sequencing: Sanger sequencing is the classic method for detecting genomic mutations and is still widely employed to detect novel mutations. This method uses chain termination to stop DNA extension. Typically, it requires tumor DNA from tested samples comprising ≥ 25% of the total DNA to ensure consistent mutation detection (Angulo et al., 2012). A systemic comparison of 136 patients with NSCLC showed that Sanger sequencing was able to detect a mutation in a 1% dilution of the total DNA in 50% of positive cases (Angulo et al., 2012). The method detected all of the mutant cases when the DNA was diluted to 5% of total DNA. When the mutant DNA represented 30% of the total DNA, sequencing was able to detect mutations in 12 of 19 cases (63%).

Pyrosequencing: EGFR mutations analyzed by the pyrosequencing method are well correlated with clinicopathologic parameters (Paez et al., 2004). Pyrosequencing is based on the "sequencing by synthesis" principle, which detects the pyrophosphate release on nucleotide incorporation. Pyrosequencing was shown to detect *EGFR* mutation in 54 of 202 patients (26.7%) in one study (Kim et al., 2013). Activating *EGFR* mutation and *EGFR* wild-type patients had a response rate to EGFR TKI therapy of 82.4% and 5.9%, respectively.

Both Sanger and pyrosequencing can be used to detect genomic alterations, such as base substitutions, insertions, or deletion.

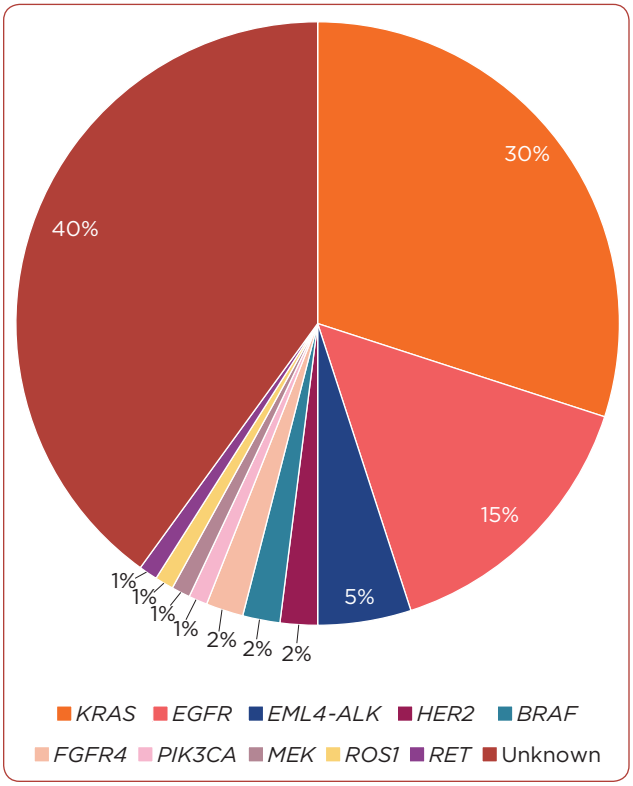

Figure. Genomic aberrations in lung cancer. EGFR = epidermal growth factor receptor; EML4-ALK = echinoderm microtubule associated protein–like 4–anaplastic lymphoma kinase; HER2 = human epidermal growth factor receptor 2; FGFR4 = fibroblast growth factor receptor 4; PIK3CA = phosphoinositide-3-kinase, catalytic, alpha polypeptide. Adapted from Dearden, Stevens, Wu, & Blowers (2013).

Mass Spectrometry DNA Sequencing: Mass spectrometry DNA sequencing has high resolution and fast operation, and it eliminates compressions associated with gel-based systems (Edwards, Itagaki, & Ju, 2001; Edwards, Ruparel, & Ju, 2005). Mass spectrometry sequencing unambiguously identifies frameshift and heterozygous mutations, making it an ideal method for resequencing. One study showed through mass spectrometry sequencing that the *EGFR* mutation status in plasma DNA corresponds to 5 of 14 *EGFR* exon 19 deletions and 3 of 4 *EGFR* L858R mutations, previously diagnosed in the matched FFPE tumors (Brevet, Johnson, Azzoli, & Ladanyi, 2011). Two cases were found positive in plasma DNA but negative in primary tumor tissue, which may reflect tumor clonal evolution during disease progression.

Table 1. Molecular Etiologies in NSCLC

Gene	Genetic alteration	Frequency	Major clinicopathologic correlates	Potential therapeutic agent
EGFR	Mutation	10%–35%	Asian, female, never smoker, adenocarcinoma	Erlotinib, gefitinib, afatinib, osimertinib
HER2	Mutation	2%–4%	Never smoker, female, adenocarcinoma	Afatinib, lapatinib
PI3K	Mutation	1%–3%	Squamous cell	Clinical trial
KRAS	Mutation	15%–25%	Smoker	None
MEK	Mutation	1%	Adenocarcinoma	Trametinib
BRAF	Mutation	2%–3%	Smoker	Vemurafenib
ALK	Translocation	3%–7%	Younger, never smoker, adenocarcinoma	Crizotinib, ceritinib, brigatinib, alectinib
ROS1	Translocation	1%	Younger, never smoker, adenocarcinoma	Crizotinib
MET	Amplification	3%	EGFR-mutant tumors	Crizotinib
FGFR	Amplification	2%	Squamous cell	Clinical trial

Note. EGFR = epidermal growth factor receptor; HER2 = human epidermal growth factor receptor 2; PI3K = phosphoinositide 3-kinase; ALK = anaplastic lymphoma kinase; FGFR = fibroblast growth factor receptor. Information from Thomas et al. (2013).

Digital Polymerase Chain Reaction: The droplet digital PCR (ddPCR) was recently developed and has been used for many clinical applications (Olmedillas-Lopez, Garcia-Arranz, & Garcia-Olmo, 2017). ddPCR amplifies DNA in a water-in-oil droplet. The advantage of this method is that it is not sensitive to nontumor DNA contamination since the result is based on the frequency counts.

Wang et al. (2010), using a digital PCR platform, tested 16 cell lines and 20 samples of genomic DNA from resected tumors. They found the digital PCR detected and quantified gefitinib/erlotinib-sensitizing *EGFR* mutations with 0.02% to 9.26% abundance. Takahama et al. (2016) evaluated *EGFR* T790M mutation in 260 patients with NSCLC using ddPCR. *EGFR* TKI–sensitizing and T790M mutations were detected in 120 (46.2%) and 75 (28.8%) patients, respectively. A study tested 41 paired samples before and after the acquisition of EGFR TKI resistance and found 65.9% of postresistance samples had T790M mutation (Takahama et al., 2016).

EGFR T790M mutation was analyzed from circulating cell-free DNA (cfDNA) in plasma in 59 plasma samples from 24 patients with NSCLC with *EGFR* mutations (Suzawa et al., 2017). T790M mutations were detected by ddPCR and compared with the T790M status, which were determined thorough rebiopsies. The plasma *EGFR* T790M detection using ddPCR had a sensitivity of 42.8% and a specificity of 97.3%, respectively.

Amplification Refractory Mutation System (ARMS): This is a PCR-based testing system using mutation-specific primers. This method is more sensitive and robust than direct sequencing for the assessment of *EGFR* mutations in FFPE tissue (Ellison et al., 2010). The ARMS detects any mutation involving single base changes or small deletions. Li et al. (2017) investigated *EGFR* mutations in 201 patients with advanced NSCLC using ARMS. The abundance of *EGFR*-activating mutation detected by ARMS was significantly associated with objective response to EGFR TKIs (Li et al., 2017). Qin, Zhong, Zhang, Li, and Wang (2011) compared the *EGFR* mutation test sensitivities among three platforms. Direct gene sequencing had the lowest sensitivity (6.9%), whereas Scorpion ARMS showed the highest mutation detecting capability (38.4%).

Next-Generation Sequencing: A major advantage of NGS is that it can sequence multiple mutations simultaneously. Next-generation sequencing sequences DNA samples in a catchall or targeted fashion. It has been tremendously successful in efficiently acquiring comprehensive cancer genomic information and has demon-

Table 2. Methods and Applications of Molecular Testing

Technique	Sensitivity (% mutant DNA)	Mutations identified	Detection of comutations	Applications
Direct sequencing	10%–25%	Known and new	No	Tissue
Pyrosequencing	5%–10%	Known	No	Tissue
Multiplex PCR	5%	Known	Yes (hotspots)	Tissue
Cobas	3%–5%	Known	No	Tissue, plasma
Mass spectrometry based	1%–10%	Known	Yes (hotspots)	Tissue, plasma
High-depth NGS	1%–10%	Known and new	Yes	Tissue, plasma
Real-time PCR (Therascreen)	1%–5%	Known	No	Tissue, plasma
Locked nucleic acid clamp	1%	Known	No	Tissue, plasma
Digital droplet PCR	< 0.1%	Known	No	Tissue, plasma

Note. PCR = polymerase chain reaction; NGS = next-generation sequencing. Adapted from Tan et al. (2016).

strated clinical utilities in identifying actionable genomic aberrations in numerous studies (Illei et al., 2017). In an analysis of 1,006 patients with NSCLC, the authors found *EGFR* mutations, including 8 mutations within the extracellular domain, in 19% of patients (Illei et al., 2017). Double mutations were observed in 29 of 187 *EGFR*-mutated tumors (16%).

The secondary *EGFR* T790M mutation is one of the major resistant mechanisms. Jin et al. (2016) investigated a cohort of 83 patients with NSCLC with TKI-sensitizing *EGFR* mutations at diagnosis and who acquired resistance to 3 different first-generation EGFR TKIs using targeted NGS. Thirty-six percent of patients acquired *EGFR* T790M. Using the Ion Torrent sequencing platform, Cai et al. (2014) sequenced 76 NSCLC samples, and *EGFR* mutations were detected in 32 patients (42.1%).

RNA sequencing (RNA-Seq) has emerged as a promising platform for *EGFR* mutation detection. RNA-Seq can detect alternative gene-spliced transcripts, post-transcriptional modifications, gene fusion, mutations/single nucleotide polymorphisms, and changes in gene expression over time or differences in gene expression in different groups or treatments. Yatabe et al. (2006) investigated *EGFR* mutations from a series of 195 NSCLC cases using RNA-Seq and detected 5% cancer cells in a background of normal cells. The practical application of this assay to 29 cases treated with gefitinib resulted in a high prediction rate (10 of 11). In addition, a mutation at codon 790, conferring gefitinib resistance, was successfully analyzed in a similar manner.

Immunohistochemistry (IHC): Using mutation-specific antibodies, IHC could potentially be used to screen patients who may be candidates for EGFR-targeted therapy. Immunohistochemistry using two mutation-specific antibodies has been tested as a screening tool for patients eligible for EGFR TKI therapy (Hitij et al., 2017). The current commercially available antibodies recognize two of the most common *EGFR* mutations: delE746_A750 and L858R. Yu et al. (2009) successfully detected *EGFR* alterations in 51 of 217 adenocarcinomas and in 1 of 217 squamous carcinomas using IHC. These findings were confirmed by DNA sequencing.

In light of currently available data, these two mutation-specific antibodies may be most useful for initial screening. However, there are concerns about the limited mutation types the antibodies recognize. A practical cutoff point for a positive or negative test has yet to be established (Cheng et al., 2012). A clinical trial, including 79 *EGFR* mutation–positive and 29 *EGFR* mutation–negative NSCLC cases, showed that the overall sensitivity and specificity of the IHC-based method were 84.8% and 100%, respectively. Immunohistochemistry showed a homogeneous staining pattern and correlated well with *EGFR* mutation status in 89% of cases (137 of 154; Kim et al., 2015). Overall sensitivity, specificity, positive predictive value, and negative predictive value for IHC were 75.6%, 94.5%, 85%, and 90.4%, respectively.

ALK and *ROS1* Rearrangement Test

Rearrangements in *ALK* are the most common fusions identified in NSCLC, with an incidence of 4% to 6% (Soda et al., 2007). *ROS1* rearrangement is less common, with an incidence of 2% (Bergethon et al., 2012; Janne & Meyerson, 2012). The *ALK* gene encodes a receptor tyrosine kinase found in a number of fusion proteins consisting of the intracellular kinase domain of *ALK* and the amino terminal portions of different genes. Echinoderm microtubule associated protein-like 4 (*EML4*)-*ALK* fusion is formed as the result of a small inversion within the short arm of chromosome 2 that joins intron 13 of *EML4* to intron 19 of *ALK* [inv(2)(p21;p23)], generating an oncogenic fusion encoding a constitutively activated protein tyrosine kinase (Soda et al., 2007). *ROS1* rearrangements allow for the retention of the *ROS1* kinase domain, constitutive kinase activity, and inferred transforming potential (Takeuchi et al., 2012). *ROS1* translocation leads to the formation of a fusion oncogene in NSCLC (Stumpfova & Janne, 2012). Methods for detecting the *ALK* and *ROS1* rearrangements include fluorescence in situ hybridization (FISH), reverse transcription (RT)-PCR, RNA-Seq, and IHC. However, IHC is only available for *ALK* rearrangement and not for *ROS1* detection.

Fluorescence in Situ Hybridization: A fusion of *ALK* with *EML4* results in constitutive activation of the ALK kinase (Cheng et al., 2012; Shaw & Engelman, 2013). *ALK* fusions have been reported in NSCLC at a frequency of 4% to 5%. The common FISH test for *ALK* rearrangement uses dual color–labeled probes covering the *ALK* gene and 3' flanking region of *ALK* with a split-apart design. In 2013, the FDA approved crizotinib, and its companion FISH detection kit, *ALK* FISH probe kit, highlighting the critical role of FISH triage for guiding ALK-targeted therapy (Solomon et al., 2014; Shen et al., 2017). The FDA-approved Vysis ALK Break Apart FISH Probe Kit is recommended by the College of American Pathologists (CAP). In general, a sample is considered positive if more than 15% of cells are positive for *ALK* separation of the green and orange signals (Cheng, Zhang, Wang, MacLennan, & Davidson, 2017).

ROS1 is a receptor tyrosine kinase of the insulin receptor family. The *ROS1* rearrangements lead to a constitutively activated fusion kinase and are detected in 1.2% to 2% of lung adenocarcinoma cases (Bergethon et al., 2012; Morton et al., 2007; Uguen & De Braekeleer, 2016). *ROS1* translocation–positive cancers tend to be adenocarcinoma and higher grade. A dual-probe break-apart meth-

Case Study 1: NSCLC With *ROS1* Mutation

A 52-year-old Caucasian female, nonsmoker, presented to her primary care physician with hemoptysis and cough for 6 months. She underwent workup including chest x-ray and chest CT, which showed a suspicious lung mass. Positron-emission tomography imaging showed hypermetabolic areas including a right lung mass, left lung nodules, and mediastinal lymphadenopathy. A brain MRI was negative. Pulmonology was consulted, and she underwent bronchoscopy. Pathology stained positive for CK7, CK20 negative, TTF-1 positive, consistent with primary lung adenocarcinoma. Molecular testing on tissue showed *EGFR* not detected, *ALK*–negative, *ROS1*–positive, and PD-L1–negative disease. The advanced stage of disease and overall prognosis was explained to the patient in detail.

According to 2017 NCCN Guidelines, crizotinib is recommended as first-line targeted therapy in these patients until progression. The prevalence of ROS1 rearrangement in NSCLC is rare, occurring in approximately 2% of patients (NCCN, 2017). In a phase I study by Shaw et al. (2014), patients with ROS1–rearranged NSCLC were treated with a standard oral dose of crizotinib and experienced an ORR of 72%, 3 complete responses, and 33 partial responses. The median duration of response was 17.6 months, median PFS was 19.2 months, and 25 patients were still in follow-up for progression. In conclusion, the study showed that crizotinib has marked antitumor activity with patients with advanced ROS1–rearranged NSCLC.

Options for treatment in this situation include systemic chemotherapy, immunotherapy, or TKI therapy with crizotinib. It was decided to proceed with targeted therapy in the form of crizotinib. She is currently tolerating it well and being monitored for side effects including thrombocytopenia, hepatotoxicity, respiratory symptoms, and electrocardiogram monitoring for QTc prolongation.

od is used to detect *ROS1* rearrangement, and criteria similar to those used for *ALK* rearrangement screening are used to evaluate the *ROS1* FISH test.

Reverse Transcription—Polymerase Chain Reaction: Amplification of hybrid messenger RNA is widely used in the detection of fusion genes. Because *ALK* rearrangement frequently involves intrachromosomal inversion, the subtle changes may sometimes be difficult to interpret by FISH analysis and have led to false-negative results (Cheng et al., 2017; Rodig et al., 2009). Due to multiple variants for *ALK* and *ROS1* rearrangements, RT-PCR must use many primer pairs to cover each targeted variant, which limits its clinical application.

Primer pairs were also designed to amplify different *ALK* hybrid subtypes. The test found one patient with *ALK* FISH–negative disease due to too few *ALK*-rearranged tumor cells.

A few studies have demonstrated that RT-PCR is sensitive and specific enough to determine *ROS1* rearrangement (Reguart et al., 2017).

RNA Sequencing: Next-generation sequencing platforms can detect multiple genetic alterations in a single assay. There are currently insufficient data on the sensitivity, specificity, and clinical validity of these platforms in a clinical setting. However, recent developments in high-throughput transcriptome-based methods may provide a suitable alternative to FISH, as they are compatible with multiplexing and diagnostic workflows (Moskalev et al., 2014; Shukla et al., 2017; Walther et al., 2015). Rogers et al. (2017) compared the results from 3 transcriptome-based platforms on 51 clinical specimens. The overall agreement with FISH ranged from 86% to 96%. Next-generation sequencing discovered minor fusions that were not detectable by FISH. The results demonstrated that transcriptome-based analyses are sensitive and robust methods for detecting actionable gene fusions in lung cancer and could be used as an alternative to the FISH test in the clinical setting. Next-generation sequencing was the most sensitive and accurate test, with sensitivity and specificity of 42.9% and 97.7%, respectively (Pekar-Zlotin et al., 2015). Non–in situ hybridization approaches could become stand-alone or complementary tests to FISH in discovering fusion genes.

Immunohistochemistry: The FDA recently approved VENTANA anti-ALK assay for selecting patients eligible to receive ALK TKI treatment. Different monoclonal antibodies for the detection of ALK protein expression are commercially available. The clones, 5A4 and D5F3, are the most widely used antibodies (Marchetti et al., 2016).

In a large multicenter study, 1+ tumors (low positive) were found to be positive by FISH analysis in 4% of cases, and 2+ tumors (moderately positive) were found in 60% of cases (Blackhall et al., 2014). Therefore, 1+ or 2+ samples should be considered equivocal and should be validated by FISH. A study tested 373 lung adenocarcinomas for *ALK* rearrangement by IHC and FISH (To et al., 2013). Multiplex RT-PCR was also performed to confirm the fusion variants. Of 373 lung adenocarcinomas, 22 (5.9%) were positive for *ALK* immunoreactivity. *ALK*-positive tumor cells demonstrated strong and diffused granular staining in the cytoplasm. All the *ALK* IHC–positive cases were confirmed to harbor *ALK* rearrangement, either by FISH or RT-PCR. Two cases with positive ALK protein expression, but negative for break-apart FISH signal, were shown to harbor *EML4-ALK* variant 1 by RT-PCR. None of the *ALK* IHC–negative cases were FISH-positive (To et al., 2013).

Pekar-Zlotin et al. (2015) systematically studied the detection of *EML4-ALK* rearrangement in 51 patients with lung adenocarcinoma. Fluorescence in situ hybridization detected *ALK* rearrangement in 4 of 51 (7.8%) patients; in contrast, IHC detected *ALK* positivity in 15.7% of patients. The results suggest that the FISH-based method may miss a significant number of patients who could benefit from targeted ALK therapy. Screening for *EML4-ALK* rearrangement by IHC for FISH-negative patients may benefit more patients.

TISSUE PROCUREMENT AND TISSUE QUALITY ISSUES

The specimens used for molecular testing include biopsy, surgical resection, cytology preparations, fine-needle aspiration (FNA), body fluids, and plasma (or "liquid biopsy"). Most clinically available samples are small FFPE biopsies from patients with advanced-stage lung cancer.

There is a long list of factors that affect the quality of tissue specimens. The most critical factors

Case Study 2: NSCLC With *ALK* Rearrangement

A 61-year-old Caucasian male with an extensive 40-pack-year smoking history originally presented to his primary care physician's office in May 2016 after failing to respond to multiple rounds of antibiotics for presumed pneumonia. After an extensive workup, including chest CT, PET scan, and brain MRI, he was found to have stage IB NSCLC, with adenocarcinoma histology. He underwent a left lower lobe lobectomy and recovered well. No adjuvant therapy was given based on NCCN guidelines. He never followed up as directed because of his heavy work schedule.

In February 2017, he presented to the emergency department with complaints of left rib pain, shortness of breath, and hemoptysis. A chest CT showed a large left pleural effusion and concern for disease recurrence. He underwent a left thoracentesis. Cytology was sent, which showed malignant cells consistent with adenocarcinoma. His pathology was reviewed from initial diagnosis in May 2016, which stained positive for TTF-1, positive for CK7, and negative CK 20, consistent with primary lung adenocarcinoma. Molecular testing on his tissue was positive for *ALK* rearrangement, negative for *ROS1*, *EGFR* not detected, and 60% positive for PD-L1.

According to the 2017 NCCN Guidelines and based on the fact that he was initially *ALK*-positive at the time of diagnosis, targeted therapy is recommended as first-line treatment. The overall incidence of *ALK*-gene rearrangements in NSCLC is approximately 4% and tends to occur independent of *EGFR* mutations (Solomon et al., 2014). According to one study, crizotinib showed superiority to standard first-line pemetrexed (Alimta)/carboplatin in patients with untreated, advanced, *ALK*-positive NSCLC, with 10.9 months vs. 7 months PFS, median overall survival (OS) not reached, and 84% probability of 1-year survival with crizotinib vs. 79% with chemotherapy. In conclusion, crizotinib was superior to standard first-line pemetrexed/carboplatin in patients with previously untreated, advanced, *ALK*-positive NSCLC.

He was started on crizotinib and tolerated it well, with no acute toxicities. In 3 months, he developed a worsening cough and recurrent left pleural effusion. Repeat imaging showed evidence of further disease progression. Second-line TKIs of ALK were considered, including ceritinib and alectinib. Based on the aggressive nature of his disease, his failure to have any response to initial treatment with ALK-targeted therapy, the rapid disease progression, and his symptomatology, he was started on systemic chemotherapy with pemetrexed/carboplatin. Repeat imaging after two cycles showed response to systemic chemotherapy. It was decided to proceed with the same treatment for the next two cycles, with further follow-up imaging and consideration of next-line TKIs after demonstrating disease stability.

include ischemic time, specimen size, storage conditions, time of storage, time of fixation, and post-fixation conditions (Auer et al., 2014; Neumeister, 2014). If formalin-fixation leads to extensive fragmentation of nucleic acids and the destruction or masking of antigens, the test should be adjusted accordingly. Good-quality DNA/RNA depends on tissue preservation, which is particularly affected by factors in the collection process. The minimum requirements for the molecular test samples should be augmented. Internal quality control, regular internal audit of the whole testing process, laboratory accreditation, and continual participation in external quality assessment schemes are prerequisites for delivery of a reliable test.

Tissue samples used for molecular analysis are subjected to conditions that cause degradation of the specimen before they can be appropriately processed. For example, time of vascular compromise before surgical removal affects the quality of the tissue significantly. Increased cold ischemic time results in increased fragmentation within the tissue samples, leading to reduction of DNA, RNA, and protein integrity. Jewell et al. (2002) determined the usability of nucleic acids extracted from banked human tissues for further molecular analyses. A total of 151 tissue specimens, stored for various times, were tested for DNA and RNA degradation. Overall, 80% of the stored human tissues had good-quality DNA, and 60% had good-quality RNA. The DNA and RNA degradation of lung tissue was stable for up to 5 hours after excision.

The volume of tissue is critical for the isolation of biomolecules. The minimum number of malig-

nant tumor cells required for molecular marker testing has not been well established; however, in general, larger samples with at least 200 to 400 malignant cells are preferred (Travis et al., 2011). Small tissue samples obtained by bronchoscopic biopsy and endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) are sufficient for detecting *EGFR* mutations in routine practice (Aravanis, Lee, & Klausner, 2017; Siravegna, Marsoni, Siena, & Bardelli, 2017; Wan et al., 2017). The *EGFR* mutations were consistently detectable in frozen FFPE tissue and cell smears when the tumor contained at least 20% cell population. Insufficient sensitivity may be noted if the tumor cell population is below this level.

Preparation of samples by macrodissection or laser capture microdissection prior to DNA extraction can significantly enrich tumor cell content and increase the utility of sequencing as a routine pretreatment test (Chowdhuri et al., 2012). Tissue samples with a volume greater than 8 mm^3 yielded at least 1 µg of DNA, and more than 80% of samples producing less than 1 µg were extracted from less than 4 mm^3 of tissue (Austin, Smith, Pritchard, & Tait, 2016). Nine squared millimeters of tissue could yield more than 1 µg of DNA.

A widespread intratumor heterogeneity has been observed (Jamal-Hanjani et al., 2017). Although driver mutations were almost always clonal, heterogeneous driver alterations do occur as a later event. Tumor evolution was reportedly found in more than 75% of the tumors.

Plasma Sequence/Liquid Biopsy

Tumor biopsies for detecting genetic mutations in advanced NSCLC are invasive, costly, and not always feasible for patients with advanced-stage disease. Liquid biopsy analyzes circulating tumor DNA (ctDNA) from blood samples and can be used to screen, diagnose, select treatments, monitor emergence of drug resistance clones, and predict prognosis (Aravanis et al., 2017; Siravegna et al., 2017; Wan et al., 2017).

Although patients with EGFR TKI–sensitizing mutations are initially responsive to EGFR TKIs, most tumors ultimately acquire resistance to the therapy. *EGFR* T790M mutation in exon 20 of the *EGFR* gene reduces the binding of a first-generation EGFR inhibitor, which is the most frequent mutation associated with resistance responsible for nearly 60% of cases (Arcila et al., 2011; Jenkins et al., 2017; Kuang et al., 2009; Nakamura et al., 2017; Sakai et al., 2013; Sequist et al., 2011; Watanabe et al., 2015).

Watanabe et al. (2015) evaluated the incidence and clinical significance of pretreatment of T790M in a larger cohort. The data revealed a sensitivity of approximately 0.001%. T790M mutation was detected more frequently in patients with a larger tumor size and in those with common *EGFR*-activating mutations.

Takahama et al. (2016) investigated 260 patients with *EGFR* mutation–positive NSCLC and acquired resistance to EGFR TKIs. Tumor tissue specimens were obtained at secondary biopsy from 18 patients treated with EGFR TKIs, and fluid samples were collected from 23 patients after the development of acquired resistance to EGFR TKIs. The assays detected a TKI-sensitizing mutation in 33 (80.5%) and T790M in 31 (75.6%) of these 41 specimens.

In September 2016, the FDA approved an osimertinib blood-based T790M companion diagnostic test for detection of the *EGFR* mutation T790M in patients with acquired EGFR TKI resistance. In studies, EGFR TKIs diminished the *EGFR*-sensitive mutant cfDNA with the treatment and EGFR TKI resistance accompanied by mutant cfDNA reappeared with the T790M mutation. T790M mutation in plasma was detected 15 to 344 days before disease progression (Oxnard et al., 2016; Sorensen et al., 2014).

The summarized concordance between mutations in the tumor tissue and cfDNA was approxi-

Highlights From the Panel Discussion

Dr. Durm: With the approval of osimertinib and its efficacy and tolerability, at the time of disease progression on erlotinib and gefitinib, it is now the standard of care to do the [mutation] testing. And if you don't find it with plasma-based testing, then you need to do a biopsy to confirm the absence of a mutation. If you've done your due diligence and performed plasma and tissue biopsies and they just don't have a mutation, that's OK. But the difference in their treatment and their overall prognosis is so much more different if you find that mutation. Not looking is simply not acceptable in this day and age.

mately 70% to 80%. The reported sensitivity of current platforms was 80% (Dagogo-Jack, Saltos, Shaw, & Gray, 2017). Plasma DNA genotyping had a sensitivity of 90% in detecting sensitizing *EGFR* mutations and 40% for the *EGFR* T790M resistance mutation (Thress et al., 2015).

Longitudinal *EGFR* mutations from 367 plasma samples from 81 patients with NSCLC treated with EGFR TKIs were studied (Lee et al., 2016). The concordance of plasma with tissue *EGFR* mutations was 87.9% for L858R and 86.2% for exon 19 deletion. A dramatic decrease of mutant copies (more than 50%) in plasma was seen during the first 2 months after treatment for the cases with sensitizing *EGFR* mutations. Emerging resistance with detection of T790M was found as a secondary mutation in 14 (28.6%) of 49 patients. Plasma T790M mutation could be used to monitor treatment response and to predict the resistance to EGFR TKI therapy.

THE WHEN, WHO, WHAT, AND WHY OF MOLECULAR TESTING IN NSCLC

With the development of improved technology to make sequencing more affordable, more efficient, and more convenient, the use of genomic sequencing in routine clinical practice will rapidly rise. Therefore, it will be increasingly important to define and identify which patients to test and the appropriate time these tests should be ordered.

Initial Biopsy and Workup of NSCLC

The initial workup for NSCLC often includes imaging and a tissue biopsy. Most patients present with symptoms including cough, shortness of breath, weight loss, or pain, but some patients can be asymptomatic at the time of diagnosis if their imaging was done for another purpose. Many patients will initially receive chest imaging, including chest x-ray or chest CT, with the most common findings including a parenchymal lung mass, mediastinal or hilar lymphadenopathy, or a pleural effusion. Additional imaging such as a PET scan can help identify additional sites of malignancy that may not be apparent on standard CTs. An MRI or CT of the brain is also commonly ordered to rule out the presence of metastatic disease.

The appropriate site to perform tissue biopsy will be determined based on the location of tumors, patient characteristics and clinical state, and the expertise of pulmonologists and interventional radiologists in the area. Typical biopsy strategies include CT-guided biopsies for more peripheral lung masses and bronchoscopy with or without EBUS for more central tumors. Other biopsy strategies can also be employed for metastatic lesions. Ultimately, the findings on imaging, in combination with the confirmation of NSCLC on tissue biopsy, provide the histologic diagnosis and stage for each patient with NSCLC.

When to Perform Molecular Testing

The appropriate time to conduct molecular testing continues to be an evolving discussion. Currently, testing is recommended at the time of diagnosis for all patients diagnosed with advanced-stage NSCLC, which includes all patients with stage IV or IIIB disease who are unable to tolerate curative treatment strategies. For patients with early-stage NSCLC (i.e., stages I–III), testing is encouraged but not mandatory, and the decision is left to the individual laboratory in collaboration with its local oncology team (Leighl et al., 2014).

At this time, outside of a clinical trial, molecularly targeted agents have no standard role in the treatment of early-stage NSCLC, and patients typically undergo therapy with curative intent (surgery or radiation with or without chemotherapy). Testing these patients at the time of diagnosis facilitates more rapid treatment in the setting of relapse and offers more information to clinicians about subsequent treatment options. It further assists in directing and enrolling patients in clinical trials that explore the role of targeted therapies in the adjuvant setting or as consolidation therapy following concurrent chemoradiation.

When deciding whether to perform molecular testing in these patients, a clinician should weigh the costs of testing against the benefit to the patients and practitioners. If patients with early-stage disease relapse and have not been tested previously, molecular testing should be sent at the time of recurrence. If patients with early-stage disease were tested at initial diagnosis and then develop a recurrence of their disease after a significant amount of time has elapsed, repeat testing should be considered, as the genomic profile of the tumor may have changed or the relapse could represent a new primary tumor.

Highlights From the Panel Discussion

Dr. Kiel: As far as tissue sample acquisition, when you do get the initial sample back from a patient and you're working it up for diagnostics and treatment potential as a predictive biomarker, how is that handled? Are most of the tests done locally at your institution? Do you send them out? What do you think most people are doing in clinical practice?

Dr. Durm: At our institution, the *EGFR*, *ALK*, and *ROS1* testing, as well as PD-L1 all are done in house at our pathology site. Some institutions set up contracts with outside vendors to conduct molecular testing.

Ms. Livers-Ertel: At our institution, it is sent out. So that brings about a really good point in my mind: for these patients—especially with advanced disease who are symptomatic—I don't really want to wait 7 to 10 days for that testing to come back. So there are situations when we're actually starting them on standard cytotoxic chemotherapy without knowing what those tests are going to reveal to us.

Dr. Durm: The ASCO guideline indicates the testing should be able to come back within 5 to 10 business days. And if it's longer than that, then sites should be taking some sort of measures to reduce that amount of time.

Origin of Current Guidelines

The current guidelines were developed as part of a joint effort between the CAP, the IASLC, and the Association for Molecular Pathology in 2013 and were later endorsed by the American Society of Clinical Oncology (ASCO) in 2014. These guidelines were an important step toward the standardization of molecular testing for *EGFR* and *ALK* in NSCLC (Leighl et al., 2014). As further molecular changes have been identified and additional therapies approved, these guidelines will need to be updated to include the most relevant targets and testing practices. A second source of expert guidelines for molecular testing in NSCLC can be found in the NCCN Guidelines. Although expert guidelines are important for standardization of testing and practice, they often cannot keep up with the rapid changes in NSCLC research. Therefore, some decisions about clinical practice and testing must be implemented prior to the adaptation of the guidelines and must take into account the most relevant literature and the individual characteristics and interests of each patient.

Who Should Be Tested

The current ASCO guidelines take into account testing for only *EGFR* mutations and *ALK* gene rearrangements. As targetable driver mutations are typically only found in adenocarcinomas, the recommendation for molecular testing is based on histology. According to current guidelines, all biopsy specimens that are purely adenocarcinoma or mixed histology with a component of adenocarcinoma should undergo molecular testing for *EGFR* or *ALK* regardless of clinical characteristics (Leighl et al., 2014). National Comprehensive Cancer Network guidelines further recommend testing for *ROS1* in patients with confirmed adenocarcinoma in at least part of their biopsy specimen (NCCN, 2017). There is also increasing interest in additional molecular targets (e.g., *BRAF*, *MET*, *RET*).

Consideration should be made for molecular testing in other histologic types, including squamous cell lung cancer and small cell lung cancer if the biopsy is limited and a component of adenocarcinoma cannot be ruled out. In these patients, it may be useful to consider clinical characteristics such as smoking history and age when deciding who to test, as they are more prevalent in younger patients who are light or never smokers. Furthermore, testing should be considered for less common tumors that may harbor *EGFR* or *ALK* changes, such as large cell carcinomas (especially if they show evidence of adenocarcinoma differentiation on IHC), sarcomatoid carcinomas, large cell neuroendocrine carcinomas, and NSCLC not otherwise specified. Testing is not recommended for fully excised surgical specimens that show no evidence of adenocarcinoma, as these tumors are highly unlikely to harbor targetable mutations (Leighl et al., 2014).

What to Test

Patients with advanced NSCLC can present with multiple metastatic lesions in addition to their primary tumor site. Questions often arise about the best site to biopsy to obtain the most accurate and useful molecular information. Current consensus is that primary and metastatic lesions are equally

suitable options for initial testing. In the case of a patient presenting with multiple, apparently separate primary lung adenocarcinomas, each tumor may be tested, but testing of multiple areas within a single tumor is not necessary (Leighl et al., 2014).

There is also the decision about the best types of specimens to test for *EGFR*, *ALK*, and *ROS1*. In general, tissue specimens are considered the best option for the most accurate information, although plasma-based samples are becoming increasingly more accurate and sensitive. Core biopsies are also typically preferred over FNA, as they yield more tissue for immediate and future testing. Pathologists should use FFPE samples or fresh, frozen, or alcohol-fixed samples to perform PCR-based testing for *EGFR* mutations. The guidelines also recommend that pathologists are involved in the selection of samples to be used for *ALK* and *ROS1* gene rearrangements. Fluorescence in situ hybridization with dual-labeled break-apart probes should be used to test for *ALK* and *ROS1*, although more recently, IHC testing for *ALK* has been validated and may be considered as an alternative to the more time-consuming and costly FISH assay. Cytologic samples are also acceptable for molecular testing, although cell blocks are preferred over smear preparations (Leighl et al., 2014).

With the improved efficiency and decreasing cost of NGS platforms, coupled with the expanding list of targetable genomic alterations, it is also acceptable to perform genomic sequencing in lieu of individual testing for each alteration. Most NGS assays will include standard *EGFR*, *ALK*, and *ROS1* testing in addition to a number of other targetable and nontargetable genetic alterations.

The downside to this approach is that it may be more costly than standard, individual testing for *EGFR*, *ALK*, and *ROS1*, and much of the information that it provides is currently not of use in standard clinical practice. Furthermore, these tests can take up to 2 to 4 weeks for results, and many practitioners and patients are unwilling to wait that long to begin treatment. As more targets are identified and more targeted drugs are developed, the cost-benefit ratio will likely shift to favor more broad-based NGS testing rather than individual gene testing, and some practices are already shifting to incorporate NGS into their initial treatment algorithms.

In the past few years, plasma-based testing has become more sensitive and accurate, and its popularity has grown rapidly. It is a convenient and less invasive way to perform molecular testing, and for this reason, many practitioners favor this over traditional tissue-based testing. However, it must be stressed that plasma-based testing should be considered complementary to tissue-based assays rather than an alternative standard option. If patients are tested with plasma-based assays and found to have targetable genomic alterations, it is acceptable to treat with appropriate TKIs in that setting. However, if targetable mutations are not detected, particularly in patients with a high likelihood of harboring these alterations (e.g., nonsmokers, young patients, those of Asian ethnicity), further testing with tissue-based assays would be highly recommended.

Testing for Other Genes

Testing for *EGFR*, *ALK*, and *ROS1* is currently considered to be part of routine clinical practice in advanced NSCLC, and such testing should be done in all adenocarcinoma patients regardless of clinical characteristics. However, there are a growing number of other molecular targets of interest. The FDA has recently approved therapies for some of these targets, and many others have shown evidence of efficacy in early-phase clinical trials. Current guidelines stress that *EGFR* and *ALK* testing should be prioritized over testing for other alterations, and *ROS1* should likely be prioritized next (Leighl et al., 2014). Part of the rationale for this approach is the amount of data showing the safety and efficacy of targeting these alterations with FDA-approved drugs in *EGFR*, *ALK*, and *ROS1*. The second reason is the frequency with which these alterations appear in NSCLC. Current estimates show sensitizing *EGFR* mutations in approximately 15% of patients with NSCLC, and this number increases to nearly 50% to 60% in Asian populations. *ALK* and *ROS1* gene rearrangements are seen in roughly 4% and 1% of patients, respectively (Dearden et al., 2013).

However, given the efficacy of targeting these genomic alterations compared with standard chemotherapy options and the often more tolerable toxicity profile, it is reasonable to test for additional targetable mutations despite their relatively rare

occurrence. Some of these targets include *BRAF*, *RET*, *MET*, and human epidermal growth factor receptor 2 (*HER2*), and there are many more under clinical investigation (NCCN, 2017). The FDA recently approved the combination of dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) for the treatment of *BRAF* V600E–mutated NSCLC based on an international, multicenter, open-label trial (FDA, 2017). In that trial, patients treated with the combination of both dabrafenib and trametinib had response rates exceeding 60%, with a relatively long duration of response.

Additional targets do not currently have FDA-approved therapies, although many of them have early-phase clinical trial evidence suggesting efficacy of either novel agents or agents approved for other indications. These data can help guide enrollment onto appropriate clinical trials or assist in garnering insurance approval for these medications off-label. Currently, many of these targets are not routinely tested for at the time of diagnosis; however, most NGS platforms include them as part of their gene panel. As treatment of these genomic alterations becomes more commonplace and more therapies are approved by the FDA, NGS will likely become the standard of care for initial molecular testing in advanced NSCLC.

Highlights From the Panel Discussion

Dr. Durm: Having more information may not necessarily help [one] patient, but it may help patients in the future. We can look at the prevalence of these mutations, and when we do come out later on with more drugs that may be active against them, we can do retrospective analyses and look back, which may give us information and help patients in the future.

Why Should We Test?

The identification of driver mutations and the development of TKIs to target them have drastically altered the landscape of treatment for NSCLC. Patients found to have targetable driver mutations have much better outcomes overall compared with those patients who are wild type for all these mutations. The toxicity profile of these drugs is typically better than that of chemotherapy as well, and thus quality of life can be improved in addition to clinical outcomes. As more driver mutations are identified and the resistance mechanisms to TKIs are more clearly defined, the available treatment options for these patients will continue to expand, making this approach even more appealing.

EGFR Gene Mutations

One of the first recognized, targetable driver mutations was *EGFR*, an important signaling pathway that regulates tumorigenesis and cell survival. Early studies found it to be overexpressed in the development and progression of NSCLC. Gefitinib, a TKI targeting this pathway, was evaluated in several clinical trials, with early studies suggesting more benefit associated with adenocarcinomas, Asian ethnicity, female sex, and never-smoker status (Fukuoka et al., 2011; Kris et al., 2003; Thatcher et al., 2005). However, at that time, the best biomarker for predicting outcomes was not known, although *EGFR* gene copy number, *EGFR* mutations, and EGFR protein expression were all being investigated.

The IPASS study was the first trial to demonstrate conclusively that patients with sensitizing *EGFR* gene mutations had improved response rates and clinical outcomes. In that trial, which was conducted in Asia, previously untreated never or light ex-smokers with advanced pulmonary adenocarcinoma were randomly assigned to receive either gefitinib or the combination of carboplatin and paclitaxel. This trial was designed as a noninferiority trial but actually demonstrated superiority for gefitinib for improving PFS over the chemotherapy arm (12-month PFS 24.9% vs. 6.7%). In a subgroup analysis, this benefit was exhibited in the patients with *EGFR* gene mutations alone, and the patients who were *EGFR* mutation–negative fared better with carboplatin and paclitaxel. A subsequent update evaluating OS showed no benefit for the targeted approach in patients with *EGFR* mutations, likely secondary to a high percentage of crossover in the chemotherapy group (Fukuoka et al., 2011; Mok et al., 2009).

Another study known as the EURTAC trial was then conducted in European patients with known *EGFR* gene mutations, randomly assigning patients either to first-line erlotinib or platinum doublet chemotherapy. This study again

demonstrated the superiority of an EGFR TKI, with a median PFS of 9.7 months in the erlotinib arm compared with 5.2 months in the chemotherapy arm (hazard ratio [HR], 0.37; Rosell et al., 2012). Based on the findings of this trial, the FDA approved erlotinib in 2013 for the first-line treatment of patients with *EGFR*-sensitizing mutations (exon 19 deletion or exon 21 substitution mutations, L858R). Gefitinib was also initially approved by the FDA for the first-line treatment of advanced adenocarcinoma of the lung, but its approval was later removed. Recently, the FDA has re-approved gefitinib for first-line use in NSCLC with exon 19 or L858R mutations.

Afatinib was the most recent TKI to be approved for the initial treatment of *EGFR* mutation–

Case Study 3: NSCLC With *EGFR* Exon 19 Mutation

A 48-year-old Asian woman who is a lifelong nonsmoker presents to her primary care provider with a dry cough for 8 months. She failed multiple rounds of antibiotics and underwent a chest x-ray, which showed abnormal findings, concerning for a lung mass. Subsequently, a chest positron-emission tomography/computed tomography (PET/CT) scan showed strong suspicion for widespread extensive malignancy. An ultrasound-guided biopsy of the liver lesion showed thyroid transcription factor-1 (TTF-1)–positive, CK7-positive, and CK20-negative stains, consistent with primary metastatic adenocarcinoma of the lung, and the tissue is sent for additional molecular testing including *EGFR*, *ALK*, *ROS1*, and PD-L1. Brain magnetic resonance imaging (MRI) is negative for metastases. An *EGFR* exon 19 deletion mutation was detected in the patient's tumor 1 week later.

Per the International Association for the Study of Lung Cancer (IASLC), clinicians must use *EGFR* and *ALK* molecular testing at the time of lung adenocarcinoma diagnosis for patients presenting with advanced-stage disease or for those experiencing disease progression who originally presented with lower-stage disease but were not previously tested (IASLC, 2014). Clinicians should use *EGFR* molecular testing to select patients with lung adenocarcinoma for EGFR-targeted therapy regardless of the disease or clinical characteristics or when an adenocarcinoma diagnosis cannot be excluded.

This patient was treated with erlotinib and had a good response for 18 months but developed slow tumor progression on every-3-month follow-up imaging. She did not have brain metastases at diagnosis but developed it at the time of disease progression while taking erlotinib. Subsequently, she was treated with whole-brain radiotherapy, which she tolerated well, and while on erlotinib, continued to have worsening cough and new-onset bone pain because of the new metastatic lesions. The role of next-line palliative systemic treatment was explained to her.

The most common type of drug mutation resistance in patients with disease that becomes refractory to EGFR TKIs is a secondary *EGFR* T790M mutation. A repeat tumor biopsy is recommended to identify whether there are any new genomic alterations in the tumor. In situations where a tumor biopsy sample cannot be obtained or would create a delay in changing treatment, liquid biopsies can be obtained. The sensitivity of these tests varies from 60% to 80%, but the specificity approaches 100% (Abbosh et al., 2017). In patients with a negative serum test, tissue biopsy should be performed if possible to detect T790M mutation. Blood-based testing methods for T790M mutation include polymerase chain reaction (PCR)-based testing, droplet digital PCR, and next-generation sequencing (NGS), as T790M mutation is observed in nearly 50% to 60% of patients who develop acquired resistance while on TKIs (Oxnard et al., 2014). Therefore, for patients whose tumor becomes T790M positive after progression on a EGFR TKI, osimertinib is recommended as the next-line option. Osimertinib was approved by the FDA in 2015 based on tumor response rate (60%) and duration of response data from clinical studies (Mok et al., 2017).

This patient was found to have a T790M mutation and was started on osimertinib. She continues to respond 1 year later. Close follow-up and monitoring for toxicities including gastrointestinal toxicities such as diarrhea, nausea, skin rash, cough, and fatigue and hematologic toxicities were explained to her.

positive NSCLC. This approval was based on two trials—LUX-Lung 3 and 6—which compared the irreversible second-generation EGFR TKI afatinib with combination platinum-based chemotherapy in Western and Asian populations, respectively. Afatinib improved PFS over combination chemotherapy in both trials, and a later pooled analysis of both trials suggested it may actually improve OS compared with chemotherapy in the subgroup of patients with exon 19 deletions. This improvement in OS was not seen in patients with L858R mutations. It should be noted that the toxicity profile of afatinib is generally less tolerable than either erlotinib or gefitinib, likely due to irreversible binding to wild-type *EGFR*, as well as mutant *EGFR* (Sequist et al., 2013; Wu et al., 2014; Yang et al., 2015).

There has been much literature on the mechanisms of resistance to first- and second-generation TKIs in *EGFR* mutation–positive NSCLC, and a number of resistance mechanisms have been identified. The most important of them is the T790M resistance mutation, which occurs in exon 20 of the *EGFR* gene. This is a substitution that changes the adenosine triphosphate binding pocket of the *EGFR* kinase domain and decreases its relative affinity for first- and second-generation TKIs (Suda, Onozato, Yatabe, & Mitsudomi, 2009). This mutation is thought to be present in approximately 50% of patients whose disease progresses on first-line treatment, and it is particularly important because an additional therapy has been developed to target this specific mutation.

In early 2017, the FDA approved osimertinib for patients with *EGFR*-mutant NSCLC whose disease has progressed on erlotinib, gefitinib, or afatinib. This approval was based on a phase III trial comparing second-line osimertinib with platinum and pemetrexed in patients who have previously been treated with a TKI and experienced disease progression. Median PFS was significantly longer with osimertinib (10.1 vs. 4.4 months, HR, 0.3) with a manageable toxicity profile (Mok et al., 2017).

The issue of the most appropriate way to test for T790M mutations in the setting of disease progression on first-line therapy has been of recent interest. Initially, this testing was done on tissue following repeat biopsy, but recently, improved plasma-based testing has been developed. A study by Oxnard et al. (2016) showed that plasma-based testing has an approximate 70% sensitivity for detecting T790M mutations when present in tissue. Furthermore, this study showed that patients had similar PFS regardless of whether their mutation is detected in the plasma or tissue. This suggests that if a T790M mutation is detected by plasma, the need for a repeat tissue biopsy may be obviated. However, if plasma-based testing is negative, a repeat tissue biopsy should be obtained in all patients in whom this is feasible.

Highlights From the Panel Discussion

Ms. Livers-Ertel: Plasma testing is great, and there's lots of it out there, but the gold standard of care is still to have tissue.

ALK Gene Rearrangements

ALK gene rearrangements are the second most commonly identified targetable driver mutations in NSCLC and occur in approximately 4% of patients with NSCLC (Blackhall et al., 2014). All patients with newly diagnosed adenocarcinoma of the lung should be tested for this rearrangement. Crizotinib is an oral small molecule inhibitor of *MET*, *ALK*, and *ROS1* and was the first TKI approved for use in patients with the *ALK* gene rearrangement.

This approval was based on a phase III study comparing crizotinib with platinum/pemetrexed chemotherapy as first-line treatment for patients with *ALK*-rearranged nonsquamous NSCLC. Median PFS was longer in the crizotinib arm (10.7 vs. 7 months, HR, 0.45), and objective response rate (ORR) was much improved as well (74% vs. 45%; Solomon et al., 2014). This led to FDA approval of crizotinib as first-line treatment for patients with advanced NSCLC with *ALK* gene rearrangements. Since that time, several additional ALK inhibitors have been developed, and both crizotinib and ceritinib are approved in the front-line setting. Ceritinib, alectinib, and brigatinib are approved for use following disease progression on crizotinib. In contrast to *EGFR*, there are a number of different resistance mutations that develop during treatment with *ALK* inhibitors, and sensitivity to later-generation TKIs differs by mutation. There is currently no standard guideline advising whether testing should be done for *ALK* resistance mutations at the time of disease progression on ALK inhibitors.

Highlights From the Panel Discussion

Ms. Livers-Ertel: This is a mechanism of treatment that is exceptional for our patients. When we are able to find, for example, an *EGFR* mutation, we're able to sit down with the patient and say, "We have a targeted therapy, and it isn't going to bring all those side effects of cytotoxic chemotherapy." There is a side-effect profile that comes along with it, but we're able to offer good quality of life and progression-free survival. So right at the beginning we're emphasizing being proactive and having a good patient relationship with the clinician to tell us these things. Because there may be a fear, especially when you're dealing with an advanced cancer, that if I report a symptom, they're going to change my treatment. So [we should provide] very clear, upfront education to these patients on what to expect.

Dr. Durm: They are still relatively new therapies. Some people who have had relatives with cancer or who had chemotherapy 10 or 15 years ago may have questions like, "How are you treating my lung cancer with a pill?" or "Am I not getting the best medicines?"

Dr. Kiel: Or when they're not getting side effects, they don't think it's working.

Dr. Durm: Right. "I feel so well, maybe we should change treatments." And that's not always the case. Those are obviously good problems to have. So we should provide education about what to expect and that this is considered to be a better treatment than first-line chemotherapy for patients who are fortunate to have [these mutations].

Dr. Kiel: One thing I usually like to clarify for patients too is that they will develop an acneiform rash. So if I have a 70- or 80-year-old in my room, to break the ice, I'll broach the subject and say, "Well, the main side effect of this drug is that it can make you look like you're 16 again." You get really bad acne on your face, your chest, and your back. But treating them with lotions and anti-acne creams just like you would a 14- to 18-year-old is probably the best supportive care you can do.

ROS1 Gene Rearrangements

ROS1 occurs in approximately 1% of patients with advanced nonsquamous NSCLC. Crizotinib is currently the only FDA-approved therapy for this alteration. This approval was based on a 50-patient phase I expansion cohort, which included only patients with a *ROS1* alteration. The ORR for that cohort was 72% with three complete responses, and the median PFS was 19.2 months. The safety profile was similar to that seen in previous ALK inhibitor studies and is generally considered to be manageable (Shaw et al., 2014).

IMPLICATIONS FOR THE ADVANCED PRACTITIONER

As the world of precision medicine and molecular profiling continues to rapidly expand, oncology advanced practitioners (APs) must have a firm understanding of genomics and be able to confidently educate their patients about the use of genomic testing to tailor therapy across all cancer types.

As the second most common cancer and leading cause of cancer death in the United States, lung cancer was one of the first types to have NCCN treatment recommendations that included genomic testing. Therefore, it is vital that oncology APs maintain current knowledge of evidence-based guidelines in advanced NSCLC and how to interpret molecular testing results in treatment planning. Advanced practitioners also need to understand the current limitations and ongoing clinical trials that seek to address these limitations and how to educate patients about the side effects of newly approved and emerging targeted therapies.

CONCLUSION

The identification of driver mutations in NSCLC and the development of multiple TKIs to target them have drastically changed the landscape of treatment in this disease. Current guidelines recommend testing for *EGFR*, *ALK*, and *ROS1* in all patients with adenocarcinoma at the time of diagnosis, and the list of targetable genomic alterations and effective therapies continues to expand. As testing and drug development continue to evolve, the utilization of broad-based testing with NGS will likely continue to expand, and as the study of resistance mechanisms continues to improve, repeat testing will become the norm to guide treatment with later-generation TKIs, as is already the case with *EGFR* T790M mutations. The treatment of advanced NSCLC will continue to be a rapidly evolving field, and this is exciting and encouraging for both patients and practitioners.

Disclosure

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