Overview: Non–small cell lung cancer (NSCLC) has become a prominent example of precision medicine among solid tumor malignancies. Clinical management of NSCLC now depends on surgical, chemotherapeutic, and radiation treatment regimens based on pathologic findings and clinical staging as well as targeted therapies based on molecular profiling. As molecular testing becomes increasingly important, preserving tissue for this purpose while rendering an accurate histologic diagnosis becomes a key consideration, particularly in advanced-stage NSCLC, in which small biopsy samples or aspirates are often the only specimen available. Next-generation sequencing panels are a powerful method of providing information relevant for both standard-of-care and investigational treatment options. However, taking advantage of the abundance of information gleaned from these panels requires careful annotation, prioritization, and reporting of molecular findings and their clinical significance. Although molecular profiling has traditionally relied on direct sampling of neoplastic tissue, blood-based diagnostics now offer the potential to provide some clinically useful information noninvasively.

Although tissue biopsies play a key role in NSCLC care and have a range of purposes, blood-based diagnostics in some instances offer the potential to noninvasively provide similar, clinically useful information. Lung cancer accounts for more cancer-related deaths in the United States than any other type of cancer.1 The management of lung cancer, particularly NSCLC, has evolved tremendously over the last 15 years. Among solid tumor cancers, NSCLC management has become a prominent example of precision medicine. Clinical management of NSCLC now depends on pathologic findings, clinical staging, and molecular profiling.2–4 Although early-stage disease is largely treated surgically (with or without adjuvant chemotherapy and/or radiation), targeted therapy based on molecular findings is the preferred treatment option for metastatic NSCLC when a targetable alteration is present. Molecular profiling has traditionally relied on direct sampling of neoplastic tissue. However, blood-based diagnostics may provide similar information by using noninvasive testing.

Pathology and staging

Pathologic evaluation of clinically identified lesions remains the gold standard for diagnosing malignancy. When tumors are excised, assessing pathologic stage is critical. In NSCLC, pathologic stage persists as the single best prognostic consideration, and the components of pathologic stage are the building blocks of determining clinical stage. Most pathologic assessment is performed on the basis of microscopic review of hematoxylin and eosin–stained slides, with specific criteria used to assess the tumor type, subtypes (as appropriate), pathologic stage, and any other key findings. Immunohistochemistry (IHC) is frequently used as an adjunct to hematoxylin and eosin review and can help to further characterize a lesion and refine the diagnosis. In some cases, IHC is necessary.

Key points

- Blood-based diagnostics in some instances offer the potential to noninvasively provide similar, clinically useful information.
- Among solid tumor cancers, NSCLC management has become a prominent example of precision medicine. Clinical management of NSCLC now depends on pathologic findings, clinical staging, and molecular profiling.
- Molecular profiling has traditionally relied on direct sampling of neoplastic tissue.
- Pathologic evaluation of clinically identified lesions remains the gold standard for diagnosing malignancy.
The approach to small samplings, such as fine-needle aspiration, bronchoscopic biopsy, and endobronchial ultrasonography–guided fine-needle aspiration, is markedly different from how a pathologist begins to assess a resected specimen.

- Pulmonary tumors are best classified by using the World Health Organization (WHO) system, which was revised in 2015.
- The approach indicated by the new WHO classification system places increased importance on the use of IHC, including for complete characterization of resected lesions.
- The overriding goal of pathologists should be to define, whenever possible, the histologic type of tumor present in a sample deemed to be malignant.
- The WHO classification divides epithelial tumors first and foremost into NSCLC and small cell lung cancer.
- NSCLC collectively describes numerous epithelial-derived tumors, of which the two most common histologic types are adenocarcinoma and squamous cell carcinoma.
- Proper identification of the appropriate histologic type is important because it affects prognosis and, in many cases, therapy selection as well as considerations for molecular testing.

Key points

- Increasingly, pathologists view the approach to classification of pulmonary lesions as distinct according to the type of sampling procured. The approach to small samplings, such as fine-needle aspiration, bronchoscopic biopsy, and endobronchial ultrasonography–guided fine-needle aspiration, is markedly different from how a pathologist begins to assess a resected specimen.

Tumor classification

Pulmonary tumors are best classified by using the World Health Organization (WHO) system, which was revised in 2015.4,7 The approach indicated by the new WHO classification system places increased importance on the use of IHC, including for complete characterization of resected lesions. This classification also codified some of the differences in approach and nomenclature for the consideration of small samplings versus resection samples.

The overriding goal of pathologists should be to define, whenever possible, the histologic type of tumor present in a sample deemed to be malignant. In resection samples, the availability of abundant materials can make this more straightforward; however, in small samplings, extensive characterization by IHC or other modalities can interfere with preserving tissue for molecular characterization for therapeutic decision-making. The WHO classification divides epithelial tumors first and foremost into NSCLC and small cell lung cancer.4,7 This is largely a historically derived nomenclature; in 1926 Barnard published findings suggesting that “oat cell carcinoma,” as it was then known, should be considered a bronchogenic carcinoma rather than a lymphomatous or sarcomatous lesion as had been previously thought.5,9 As the study of lung tumors advanced, it became clear that this type of tumor was distinct from other lung tumors, leading to this classification of “small cell carcinoma” and “non–small cell carcinoma,” which remains in use today. Further discussion of small cell lung cancer is beyond the scope of this article.

NSCLC collectively describes numerous epithelial-derived tumors, of which the two most common histologic types are adenocarcinoma and squamous cell carcinoma. Other histologic types are varied and typically rare, such as large cell carcinoma, pleomorphic carcinoma, and salivary gland–like tumors of the lung. Proper identification of the appropriate histologic type is important because it affects prognosis and, in many cases, therapy selection as well as considerations for molecular testing.10,11 For example, the role of tumor genotyping in pure squamous carcinoma is debated, whereas genotyping is recommended for all advanced nonsquamous NSCLC. In numerous circumstances, IHC is paramount in determining the correct histologic type. In particular, the solid variant of adenocarcinoma has substantial morphologic overlap with nonkeratinizing squamous cell carcinoma, and IHC can be crucial in making the distinction.12 This can typically be achieved by using a minimal set of IHC markers, consisting of a single adenocarcinoma and squamous marker, such as TTF-1 and p40.5,13 The diagnosis of squamous cell carcinomas that do not have keratinization should be confirmed with squamous IHC markers.7

An important change in the reporting of invasive adenocarcinoma is the
recommendation to include characterization of the histologic subtypes, which include lepidic, acinar, papillary, solid, and micropapillary and may correlate with histologic grade.\textsuperscript{14} The recommendation has been made to characterize lesions according to the predominant subtype and estimate the percentage of various subtypes in 5% increments. There is some evidence that the tumor subtype may be associated with prognosis in patients with early-stage resected disease, with the presence of higher-grade histologic types (micropapillary and solid) being associated with a higher incidence of occult lymph node metastases.\textsuperscript{15,16} In clinical practice, however, the predominant subtype of adenocarcinoma does not currently affect therapy decisions, and the clinical application of determining subtype has not been established in advanced-stage disease.

- **Staging**

In 2017, the American Joint Commission on Cancer published the eighth edition of the Cancer Staging Manual, which included several updates to the criteria used for staging of NSCLC.\textsuperscript{17,18} Key differences between the two versions include an additional tier of early-stage disease (T1c), reclassification of lesions greater than 5 cm but 7 cm or less in greatest dimension as T3 (instead of T2), and reclassification of tumors greater than 7 cm in greatest dimension as T4 (instead of T3). These and other revised staging criteria have become incorporated into routine practice, but some of the changes, particularly in synoptic reporting, may be unfamiliar to practicing oncologists. For example, spread through air spaces is now an optional component of the pathologic staging synoptic report, and its presence portends a higher risk for recurrence in tumors treated with limited resection.\textsuperscript{19-21} Another substantial change in the staging system includes separate measurements of invasive and lepidic components in adenocarcinoma. An additional area addressed directly in the new staging approach is classification of multifocal disease for the consideration of separate primary tumors versus intrapulmonary metastases, using a predominantly histologic-based approach.\textsuperscript{17,18,22} Growing evidence suggests that molecular testing can be a useful approach to determining the clonal relationship between multiple tumor nodules.\textsuperscript{23,24}

- **Specimen management**

Numerous logistical issues are also of note when the multiple priorities assigned to diagnosis small samplings of NSCLC are being considered. As noted, the need to balance IHC characterization (including consideration of metastases from non-lung sites) with preservation of material for molecular testing can be challenging, and numerous approaches can aid in the preservation of tissue.\textsuperscript{25} Given the clinical urgency in tumor classification and molecular testing in cases of advanced NSCLC, an understanding of the timelines for the technical and interpretive processes in pathology can aid in planning for individual patients. Histologic processing of biopsy samples is typically accomplished in 1 business day, allowing for a preliminary assessment of a sample on the following day. IHC staining can add 1 to 3 days or more to total assessment time, particularly if staining is done in stages to minimize tissue use for classification. Often, molecular testing is not initiated until the histologic assessment is complete, in case material is needed for additional IHC. Of particular note in the recently updated guidelines for the testing of NSCLC is the option to use cytopathology specimens other than cell blocks, such as smear preparations or touch preparations.\textsuperscript{2} Increasingly, molecular laboratories are validating this specimen type to increase patient access, shorten turnaround time, and reduce the need for repeat biopsies to successfully perform molecular studies in patients with NSCLC.\textsuperscript{26}

- **Molecular profiling**

- **Standard-of-care molecular biomarkers in NSCLC**

The current guidelines from the College of American Pathologists, International
The combination of BRAF inhibitor dabrafenib and MEK inhibitor trametinib was recently approved for NSCLC with an ALK or ROS1 rearrangement. Alectinib, ceritinib, and brigatinib are also approved for ALK-rearranged NSCLC.

The NCCN guidelines also recommend consideration of emerging targeted therapeutic options, including crizotinib for MET exon 14 skipping mutations or high-level MET amplification, cabozantinib or vandetanib for RET rearrangements, and ado-trastuzumab emtansine for ERBB2 mutations.

Association for the Study of Lung Cancer, and Association for Molecular Pathology recommend evaluation of EGFR, ALK, and ROS1 on all patients with lung cancer patients who have metastatic nonsquamous disease, irrespective of clinical characteristics. These guidelines do not recommend other genes, including BRAF, KRAS, RET, ERBB2 (HER2), and MET, as routine stand-alone assays outside the context of a clinical trial; however, in the United States a combination therapy is approved for patients with NSCLC who have BRAF p.V600E mutation, which may raise the impetus to consider a stand-alone assay for this target. Of note, multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond EGFR, ALK, and ROS1. For laboratories performing next-generation sequencing (NGS), it is recommended that BRAF, KRAS, RET, ERBB2, and MET be included. The National Comprehensive Cancer Network (NCCN) guidelines have one or more specific treatment recommendations for all of the genes except KRAS. These targeted therapies are indicated for patients with NSCLC who have metastatic disease with sensitizing molecular alterations. Tyrosine kinase inhibitors (TKIs) erlotinib, gefitinib, afatinib, and osimertinib are all U.S. Food and Drug Administration (FDA)–approved for the treatment of both EGFR exon 19 deletions and EGFR L858R mutations, whereas osimertinib is the only TKI approved for the treatment of commonly acquired resistance mutation p.T790M, and afatinib is the only TKI approved for uncommon EGFR driver mutations (e.g., G719X, L861Q). Crizotinib is FDA-approved for the treatment of NSCLC with an ALK or ROS1 rearrangement. Alectinib, ceritinib, and brigatinib are also approved for ALK-rearranged NSCLC. The combination of BRAF inhibitor dabrafenib and MEK inhibitor trametinib was recently approved for NSCLC with BRAF p.V600E. The anti–programmed death ligand 1 (PD-L1) antibody pembrolizumab is FDA-approved for NSCLC as a first-line therapy for high PD-L1 expression (tumor proportion score ≥ 50%) in the absence of an EGFR mutation or ALK rearrangement. This drug is also approved for patients who have progressed while receiving platinum-based chemotherapy or patients with EGFR or ALK alterations who have progressed while receiving an FDA-approved targeted therapy, with a tumor proportion score of 1% or greater.

The NCCN guidelines also recommend consideration of emerging targeted therapeutic options, including crizotinib for MET exon 14 skipping mutations or high-level MET amplification, cabozantinib or vandetanib for RET rearrangements, and ado-trastuzumab emtansine for ERBB2 mutations. The NCCN guidelines also point out that KRAS is associated with poorer prognosis and reduced responsiveness to EGFR tyrosine kinase inhibitor therapy, and that the presence of a KRAS mutation may identify patients who will not benefit from further molecular testing (owing to the low probability of overlapping targetable variants). Variants affecting many other potentially targetable genes are being evaluated in various clinical trials, including FGFR1 amplification, FGFR3 fusions, NTRK1 fusions, and PIK3CA and AKT1 mutations. NGS testing is increasingly enabling routine evaluation of those genes, as recommended by national guidelines, as well as many that are being evaluated in these trials.

Next-generation sequencing panels

NGS is a powerful tool that enables the simultaneous interrogation of many regions of human genome. In evaluating NSCLC samples, a wide array of information can be collected in a single test. This information may be relevant for standard-of-care treatment with FDA-approved drugs; off-label use of FDA-approved drugs based on clinical practice guidelines, clinical studies, and/or preclinical data; or consideration for enrollment in a clinical trial. Other information will have no immediate clinical utility, but panels often include targets without immediate clinical utility based on the possibility that such information may be useful in the future. As the volume of data from
NGS testing grows, so does the challenge of separating findings that are clinically meaningful and prioritizing their clinical utility. Molecular pathologists—in collaboration with their oncology colleagues—are tasked with evaluating this abundance of data, distilling down to what is clinically relevant, and communicating this information in the most cogent and manageable manner possible. A general algorithm for NGS data analysis can be illustrated as in Figure 1.

Filtering variants of no clinical utility
A great deal of information generated from NGS data is of no clinical utility and should not be reported because doing so would only make extracting clinically useful information more difficult for all practitioners. These unreportable findings include artifacts, synonymous variants, most intronic variants (other than splice site mutations and functional gene rearrangements), and benign germline polymorphisms.

The distinction between germline and somatic variants is not always clear (unless nonneoplastic tissue is also evaluated), and some germline variants can be clinically relevant, including those associated with cancer predisposition syndromes. Without a tumor-normal comparison, distinguishing somatic from germline variants relies largely on constitutional databases (such as 1000 Genomes Project, ExAC, dbSNP, ClinVar) and cancer-specific databases (Catalog of Somatic Mutations in Cancer [COSMIC], cBioPortal). However, information from these databases must be interpreted with caution because some somatic variants (e.g., JAK2 V617F) are included in germline databases and both deleterious and benign (e.g., KIT M541L) are included in cancer-specific databases. Primary literature may also be helpful in some instances. For example, even the acquired resistance mutation EGFR p.T790M can be seen rarely as a germline event, and this must be considered when identified at cancer diagnosis. Ultimately, tumor-only testing should not be used to infer germline status of an alteration, even when highly suspicious and germline testing and genetic counseling may be indicated in selected clinical situations.

Somatic variants are frequently inferred to represent driver or passenger mutations.

A driver mutation is one that confers a

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**Key points**

- A great deal of information generated from NGS data is of no clinical utility and should not be reported because doing so would only make extracting clinically useful information.
- The distinction between germline and somatic variants is not always clear (unless nonneoplastic tissue is also evaluated), and some germline variants can be clinically relevant.
- Without a tumor-normal comparison, distinguishing somatic from germline variants relies largely on constitutional databases and cancer-specific databases.
- Ultimately, tumor-only testing should not be used to infer germline status of an alteration, even when highly suspicious and germline testing and genetic counseling may be indicated in selected clinical situations.

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**FIGURE 1** General Approach for Curating Variants Detected by Using Next-Generation Sequencing.

COSMIC, Catalog of Somatic Mutations in Cancer; FDA, U.S. Food and Drug Administration; NCCN, National Comprehensive Care Network.
selective growth advantage for the mutated cell, whereas a passenger mutation has no growth advantage and is observed within a tumor because of its co-occurrence with a driver. Because passenger mutations are random events that are not selected for during oncogenesis, they generally are not recurrent, whereas driver mutations are. Therefore, the frequency of a particular variant within somatic mutations databases such as COSMIC and cBioPortal and the large cancer-specific studies (e.g., TCGA) are eminently useful in evaluating the potential oncogenesis of a mutation.

- Functional studies provide the most definitive evidence of oncogenesis; however, they are rarely available for unusual alterations, and identification of functional studies in the literature can be laborious. Although driver mutations are generally the most clinically relevant, passenger mutations may also be informative. For example, several studies have demonstrated a correlation between tumor mutation burden and response to immune checkpoint inhibitor therapies.29–31

### Classification of variants

Whole-genome studies have demonstrated a median of 888 and 15,659 mutations in NSCLC samples from nonsmokers and smokers, respectively.32 As described above, only a subset of these mutations represent driver mutations and only a tiny subset of these mutations offer potential clinical utility. Most molecular laboratories perform panel-based NGS that restricts evaluation to those genes or gene regions with recurrent driver mutations and potential clinical significance. However, even smaller, targeted panels can identify several mutations in a single sample. Classification of variants based on clinical significance based on availability of relevant therapies, national guidelines, and clinical/preclinical studies facilitates prioritization of molecular findings and appropriate clinical management.

Several classification schemas are used by various institutions performing clinical sequencing. MD Anderson Cancer Center’s Knowledge Base for Precision Oncology classifies therapies on the basis of the level of clinical or preclinical evidence supporting its efficacy for a particular tumor type and a particular type of variant.33 Other groups also incorporate clinical trial eligibility criteria.34 Guidelines for the interpretation and reporting of sequence variants in cancer were recently published in a joint consensus recommendation from the Association for Molecular Pathology, ASCO, and College of American Pathologists.35 These guidelines recommend grouping clinical and experimental evidence into four levels based on therapeutic, diagnostic, and/or prognostic significance.

### Other variant-specific information

Most clinical NGS reports describe each clinically significant variant. Descriptions often include the following:

- A precise, unambiguous description of each clinically significant variant. This is essential. A single variant can be designated in many different ways depending on variant nomenclature, the reference transcript being used, and other factors. The College of American Pathologists requires reporting of sequence variants by using Human Genome Variation Society nomenclature to include the gene name based on the HUGO Gene Nomenclature Committee, a standard versioned reference identifier to the transcript/protein, the reference genome assembly and version number, and chromosomal position.

- The expected functional effect of the variant (predicted or based on functional studies) illustrating that the variant is truly a driver mutation. For example, EGFR L858R mutations result in constitutive activation of EGFR and downstream growth signaling.

- A statement indicating whether the variant (or similar variants) has been described in the tumor type being evaluated, possibly with an estimate of the relative frequency.

- Prognostic significance (if any). For example, KRAS mutations have been associated with an inferior prognosis in NSCLC.36

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**Key points**

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- Most molecular laboratories perform panel-based NGS that restricts evaluation to those genes or gene regions with recurrent driver mutations and potential clinical significance.

- Several classification schemas are used by various institutions performing clinical sequencing.

- Guidelines for the interpretation and reporting of sequence variants in cancer were recently published in a joint consensus recommendation from the Association for Molecular Pathology, ASCO, and College of American Pathologists.
• Patterns of mutual exclusivity. For example, KRAS mutations are generally mutually exclusive with many other targetable molecular alterations. As a result, a KRAS mutation may indicate patients who will not benefit from further molecular testing.2

• Therapeutic implications, including FDA-approved indications, off-label therapeutic options, and therapies being investigated in clinical trials. The available evidence supporting or refuting the observed variant (or similar variants) being predictive of drug response should be summarized and appropriately cited.

• The effect of other variants also identified. For example, although an EGFR exon 19 deletion is associated with responsiveness to first-generation EGFR inhibitors, such as gefitinib, a co-occurring EGFR T790M mutation is associated with resistance.37

Information about the test
Basic information about the test being performed is also generally included in reports and is essential for understanding the limitations of the test:

• Limit of detection of the test. The limit of detection may differ between different types of variants, such as single nucleotide variant, insertional/deletion, copy number variant, and gene fusion.

• Genes and gene regions evaluated. Many reports list or reference a list of genes and possibly gene regions covered by the test.

• The types of variants detected including single nucleotide variants, insertional/deletion mutations, copy number variants, gene fusions, and gene expression. This is an important consideration a report is being interpreted. For example, a panel that includes MET sequencing for mutations may not provide information about MET amplification (copy number variants).

• Types of variants that may not be detected. For example, an amplification-based NGS that relies on primers targeting known gene fusions may fail to detect fusions involving novel or poorly described fusion partners or unusual fusion transcripts.

Biopsy versus liquid biopsy
The role of biopsies and liquid biopsies, Therapeutic implications, including FDA-approved indications, off-label therapeutic options, and therapies being investigated in clinical trials. The available evidence supporting or refuting the observed variant (or similar variants) being predictive of drug response should be summarized and appropriately cited.

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• Types of variants that may not be detected. For example, an amplification-based NGS that relies on primers targeting known gene fusions may fail to detect fusions involving novel or poorly described fusion partners or unusual fusion transcripts.
Key points

- Plasma genotyping could eventually play a similar orthogonal role in evaluating for the presence of disease spread.

- Across several data sets, it is apparent that detection in cfDNA of a previously identified tumor genotype (a marker of cancers that shed DNA into the plasma) is a marker of a relatively poor prognosis.

- Neoadjuvant therapy is one of several standard management strategies in planning multimodality therapy for locally advanced NSCLC.

- One reason neoadjuvant therapy is attractive is that it permits the testing of the resection specimen for degree of pathologic response.

- Of 32 patients with plasma available within 4 months of completing therapy, 17 (53%) had detectable tumor mutations in plasma cfDNA, and these patients had a dramatically higher rate of recurrence.

- Cancer recurrence after attempted curative therapy is a dramatic moment for a patient with lung cancer because recurrence often indicates that their cancer is no longer curable.

- Given the seriousness of lung cancer recurrence, biopsy to pathologically confirm recurrence is standard for patients more than 6 to 12 months out from curative therapy.

- Of 32 patients with plasma available within 4 months of completing therapy, 17 (53%) had detectable tumor mutations in plasma cfDNA, and these patients had a dramatically higher rate of recurrence. Given the seriousness of lung cancer recurrence, biopsy to pathologically confirm recurrence is standard for patients more than 6 to 12 months out from curative therapy. As discussed above for diagnostic biopsies, a noninvasive assay in some cases might serve as an alternative in patients with lung cancer too sick to undergo a recurrence biopsy. This would make the most sense in patients whose definitively treated lung cancer was known to harbor a pathognomonic lung cancer genotype (e.g., an EGFR mutation or ALK fusion). If this variant were detected in plasma at time of suspected recurrence, a trial of targeted therapy could serve as an alternative to biopsy, confirming recurrence. Similarly, if the pretreatment genotype is known, the presence of those markers could serve a similar purpose, although caution is warranted if the alteration is common to multiple malignancies (such as KRAS or TP53 mutations).

The staging biopsy

In planning curative therapy for lung cancer, staging biopsies, such as mediastinal lymph node biopsies or biopsies of suspected metastatic sites, are routine. Because staging imaging studies (PET, MRI) are imperfect, biopsies are a common supplement. For example, in a patient with an isolated rib lesion on PET, fine-needle aspiration of the bone lesion can confirm metastatic disease and inform prognosis. Plasma genotyping could eventually play a similar orthogonal role in evaluating for the presence of disease spread. Across several data sets, it is apparent that detection in cfDNA of a previously identified tumor genotype (a marker of cancers that shed DNA into the plasma) is a marker of a relatively poor prognosis. One could envision that detection of tumor DNA within the plasma cfDNA could similarly be a marker of poor prognosis in early-stage lung cancer, informing the chance of cure and contributing to cancer staging. This is a question that deserves further study in prospective data sets.

The recurrence biopsy

Cancer recurrence after attempted curative therapy is a dramatic moment for a patient with lung cancer because recurrence often indicates that their cancer is no longer curable. Given the seriousness of lung cancer recurrence, biopsy to pathologically confirm recurrence is standard for patients more than 6 to 12 months out from curative therapy. As discussed above for diagnostic biopsies, a noninvasive assay in some cases might serve as an alternative in patients with lung cancer too sick to undergo a recurrence biopsy. This would make the most sense in patients whose definitively treated lung cancer was known to harbor a pathognomonic lung cancer genotype (e.g., an EGFR mutation or ALK fusion). If this variant were detected in plasma at time of suspected recurrence, a trial of targeted therapy could serve as an alternative to biopsy, confirming recurrence. Similarly, if the pretreatment genotype is known, the presence of those markers could serve a similar purpose, although caution is warranted if the alteration is common to multiple malignancies (such as KRAS or TP53 mutations).
The biopsy for genotyping
It is well established that diagnostic lung cancer biopsies are frequently inadequate for the range of molecular studies now needed for treatment decisions,\textsuperscript{46,47} such as PD-L1 IHC and NGS. The diagnostic specimen may be small and much of the tissue could be exhausted during the performance of necessary studies to determine the diagnosis and histologic subtype. For this reason, a repeat biopsy is commonly required to permit complete molecular testing. Plasma cfDNA genotyping is an intuitive noninvasive option for such patients who are planning an additional biopsy. In 2016 the FDA approved the first plasma cfDNA genotyping assay for detection of EGFR mutations in patients with NSCLC who did not have tumor tissue available for genotyping.\textsuperscript{48} Furthermore, broader genotyping of a range of oncogenic drivers in cfDNA is now possible with commercially available plasma NGS technologies.\textsuperscript{49,50} Still, the sensitivity of plasma genotyping for driver mutations present within the tumor is only in the range of 60% to 80%.\textsuperscript{51} This means that negative plasma genotyping results must reflex to a biopsy for tumor genotyping. In some cases, it may be worth concurrently scheduling a biopsy procedure while waiting for plasma genotyping results because this shortens the time interval to molecular testing in the event of a negative plasma genotyping report.

The response biopsy
Biopsy at time of treatment response is not part of routine clinical practice but is increasingly used in some clinical trials to assess treatment effect. The aim is usually to assess a pharmacodynamic marker, such as adequate inhibition of a target, although tumor analysis is not always possible when a dramatic treatment effect results in little residual tumor for analysis.\textsuperscript{52} Plasma genotyping on therapy also can permit a noninvasive measurement of treatment effect.\textsuperscript{53} Many highly active targeted therapies can induce a rapid clearance of driver mutations from the plasma cfDNA, which is associated with a more favorable outcome.\textsuperscript{53} The clinical and scientific implications of such a plasma response still require further investigation.

The resistance biopsy
Biopsies for genotyping of lung cancer drug resistance have more recently emerged as a standard of care since the FDA approval of osimertinib for EGFR-mutant lung cancer and acquired drug resistance mediated by a specific resistance mutation, EGFR p.T790M.\textsuperscript{54} And yet, such resistance genotyping has long been used for clinical trial enrollment given the range of potentially targetable resistance mechanisms in EGFR-mutant lung cancer.\textsuperscript{55} Furthermore, such resistance biopsies are increasingly common as targetable resistance mechanisms can be seen with other targeted therapies, such as ALK and MET inhibitors.\textsuperscript{56,57} The convenience of plasma cfDNA genotyping makes it an ideal technology for testing for many of these resistance mechanisms, such that it is now an established standard for EGFR p.T790M testing in patients reluctant to pursue a resistance biopsy.\textsuperscript{58} However, as with initial genotyping, sensitivity is imperfect and a negative plasma result must reflex to a biopsy for tumor genotyping.

Conclusion
Precision medicine is exemplified by NSCLC in which management is tailored based on pathologic findings, clinical staging, and molecular profiling. Next-generation sequencing panels enable molecular profiling that includes information relevant for both standard of care and investigational treatment options. Taking full advantage of this abundance of information requires careful annotation, prioritization, and reporting of molecular data. Preserving tissue for molecular testing while rendering an accurate histologic diagnosis has also become a key consideration for pathologists and oncologists. Blood-based diagnostics now offer the potential to also provide clinically useful information noninvasively.

Key points
- It is well established that diagnostic lung cancer biopsies are frequently inadequate for the range of molecular studies now needed for treatment decisions, such as PD-L1 IHC and NGS.
- Plasma cfDNA genotyping is an intuitive noninvasive option for such patients who are planning an additional biopsy.
- In 2016 the FDA approved the first plasma cfDNA genotyping assay for detection of EGFR mutations in patients with NSCLC who did not have tumor tissue available for genotyping.
- Biopsy at time of treatment response is not part of routine clinical practice but is increasingly used in some clinical trials to assess treatment effect.
- Biopsies for genotyping of lung cancer drug resistance have more recently emerged as a standard of care since the FDA approval of osimertinib for EGFR-mutant lung cancer and acquired drug resistance mediated by a specific resistance mutation, EGFR p.T790M.
- The convenience of plasma cfDNA genotyping makes it an ideal technology for testing for many of these resistance mechanisms, such that it is now an established standard for EGFR p.T790M testing in patients reluctant to pursue a resistance biopsy.
References


