# Biomarker Testing for Patients With Advanced Non–Small Cell Lung Cancer: Real-World Issues and Tough Choices

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Overview

**Over the last decade, the treatment of patients with advanced non–small cell lung cancer (NSCLC) has become reliant on tissue and/or blood biomarkers to help guide treatment decisions. There are now multiple biomarker-defined patient subgroups, with evidence showing that treatment with targeted therapies has superior clinical outcomes when compared with traditional cytotoxic chemotherapy. However, rapid change in the field of precision oncology brings with it the challenge of translating recommendations into clinical practice. In this review, we discuss the major guidelines recommending biomarker testing in NSCLC, as well the logistical challenges to applying these guidelines to patients with NSCLC both in the United States and worldwide. The techniques commonly used for biomarker testing will be discussed, both for tissue- and blood-based biomarkers. Finally, we discuss the challenge of interpreting the results of biomarker testing and using these results to guide treatment decisions.**

## GUIDELINES FOR BIOMARKER TESTING IN NSCLC

As the treatment of lung cancer has become progressively more biomarker-driven, and with the rapid emergence of effective matched targeted therapies, organizations have made attempts to define best practices for which tests are necessary and in what target population. This practice has its own set of challenges in keeping up with a rapidly changing field. Although sensitizing mutations in the *EGFR* gene were first described in 2004,[1,2] followed by multiple trials showing that EGFR tyrosine kinase inhibitors (TKIs) were superior to chemotherapy in this subgroup of patients,[3,4] it was not until 2011 that the first provisional recommendation was issued by ASCO endorsing routine testing of all patients with lung adenocarcinoma for *EGFR* mutations.[5] In that same year, crizotinib was approved for treatment of anaplastic lymphoma kinase (ALK)–rearranged NSCLC,[6] illustrating the rapid pace of adoption of targeted therapies and the challenges guidelines would have keeping pace.

In 2013, the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) jointly issued guidelines for routine testing of patients with lung cancer for *EGFR* mutations and *ALK* gene fusions.[7] Beyond the biomarkers themselves, these guidelines also made detailed recommendations about the target population (all patients with advanced lung adenocarcinoma regardless of clinical indicators such as smoking status, race, or sex), the recommended testing approaches, and a recommended turnaround time of less than 14 days. This guideline established the standard of care for molecular testing in NSCLC that stands today, although now with expanded targets.

In 2018, the CAP/IASLC/AMP guidelines were updated to add the recommendation for routine testing for *ROS1* gene rearrangements[8] given the efficacy of TKIs such as crizotinib in this population.[9] However, the guideline committee completed its formal review just before the approval of dabrafenib and trametinib therapy for *BRAF+* NSCLC in June 2017,[10] and thus, this population was not included in the recommendations. The guidelines also recommended routine testing for T790M mutation in all patients with *EGFR* mutations who progressed on first- or second-generation EGFR TKIs and added endorsement of the use of cell-free DNA for testing when tissue was unavailable. Shortly afterward, ASCO issued an endorsement of the CAP/IASLC/AMP guidelines, but further extended the recommendations to include routine *BRAF* mutation testing.[11] Another commonly accessed source of recommendations is the National Comprehensive Cancer Network guidelines,[12] which currently mirror the ASCO recommendations.

There is also general concordance between guidelines in the United States and international organizations. The 2018 European Society for Medical Oncology (ESMO) Clinical Practice Guidelines similarly recommend routine testing for *EGFR*, *ALK*, and *ROS1*[13] and

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**PRACTICAL APPLICATIONS**

- Biomarker testing is necessary for determining the optimal treatment of patients newly diagnosed with NSCLC.
- Practical guidelines such as the CAP/IASLC/AMP, ASCO, and National Comprehensive Cancer Network guidelines are helpful for determining the most appropriate biomarkers and assays to use.
- Plasma-based assays have many advantages over tissue-based tests, being noninvasive, fast, and easily repeatable over time, but they may be less sensitive than tissue-based assays and therefore cannot serve as stand-alone testing for patients with NSCLC.
- There are many different types of tissue- and blood-based assays available for biomarker testing, all with their own advantages and disadvantages that clinicians should understand when deciding which assays to use.
- NGS reports contain large amounts of information that must be interpreted carefully before being used to make treatment recommendations.

commented that BRAF-targeting drugs were gaining approval in some European countries. In 2018, ESMO and the Chinese Society of Clinical Oncology issued joint Pan-Asian guidelines recommending routine testing of all advanced nonsquamous NSCLC for *EGFR* mutation, *ALK* rearrangement, *ROS1* rearrangement, and *BRAF* mutation, as well as PD-L1 immunohistochemistry.[14] The addition of PD-L1 immunohistochemistry (IHC) staining is based upon the improved outcomes with the immune checkpoint inhibitor pembrolizumab both in the second line for tumors with PD-L1 1% or more[15] and in the first line for patients with PD-L1 expression 50% or higher.[16] These guidelines were endorsed by the major oncology societies of China, Japan, Korea, Malaysia, Singapore, and Taiwan.

**TABLE 1.** Recommended Biomarker Tests for Patients With Newly Diagnosed NSCLC

| | Nonsquamous Histology | Squamous Cell Carcinoma |
|---|---|---|
| Minimum necessary | PD-L1 IHC, EGFR, ALK, ROS1, BRAF | PD-L1 IHC |
| Recommended* | RET, MET exon 14, HER2, KRAS, NTRK | |

Abbreviations: NSCLC, non–small cell lung cancer; IHC, immunohistochemistry; ALK, anaplastic lymphoma kinase; NGS, next-generation sequencing.
*These should be added if testing is done as part of a broad NGS-based panel.

There are also a number of emerging molecular targets and therapies in NSCLC, some likely nearing clinical practice (and already helpful for selection for clinical trials), such as *RET* rearrangements,[17] *MET* exon 14 skipping mutations,[18] and activating *HER2* mutations.[19] Molecular alterations occurring in other cancers can also present in a small proportion of NSCLC, such as *NTRK* gene rearrangements that are amenable to treatment with NTRK TKIs.[20] In addition, there may be merit in testing for *KRAS* mutations,[21] which are the most common oncogenic mutations in lung adenocarcinoma, are usually mutually exclusive of other targetable alterations, and so may be helpful in deciding which patients do or do not require broader testing.

In summary, all patients with advanced nonsquamous NSCLC, regardless of clinical characteristics such as age, race, or smoking status, plus some patients with squamous cell carcinoma such as nonsmokers or those under age 40, should undergo, at a minimum, testing for *EGFR* mutation, *ALK* and *ROS1* rearrangements, *BRAF* mutation, and PD-L1 IHC (Table 1). If next-generation sequencing (NGS) is being used for broader testing, additional alterations in genes such as *RET*, *MET*, *HER2*, and *KRAS* should also be assessed.

### Barriers and Access to Molecular Testing in NSCLC

Despite broad agreement on the importance of biomarker testing for patients with lung cancer, there is variable uptake in clinical practice. Even the most common targetable mutation (*EGFR*), for which testing has been part of standard practice since 2011, is not always assessed. EGFR testing rates have improved over time, from as low as 18% to 22% in 2010 to 2013,[22,23] to 59% to 61% in 2015 to 2016,[24,25] to more recent surveys suggesting testing rates in as high as 87% of patients with advanced adenocarcinoma in select markets.[26] ALK testing has been comparable, with testing rates rising from 32% in 2011 to 67% to 69% in 2018.[26,27] There is even less information on ROS1 or BRAF testing, with one study showing only 28% of patients being tested for ROS1.[26] Clearly, there is considerable room for improvement.

Outside the United States, the testing is even more variable, with data limited to only a few countries and no central data available, making extrapolations based on limited reports difficult. Rates of molecular testing, primarily EGFR, that have been reported in specific countries include Brazil (38%),[28] China (42% to 46%),[29,30] Switzerland (79%),[31] Italy (65%),[32] Spain (85%),[32] Germany (66%),[32] Australia (71%),[32] Japan (85%),[32] Korea (89%),[32] and Taiwan (91%).[32] A separate survey of oncologists from multiple developed countries in 2016 suggested that EGFR testing may be as high as 80%.[33] Although no data are available on

testing in most resource-constrained countries, it is reasonable to assume testing rates would be comparable or lower.

To what shall we attribute the lack of universal testing, even in the United States? There are a number of barriers to biomarker testing that must be reduced. Reflex testing rather than waiting for a physician order can reduce the time to initiating treatment.[34,35] Tissue samples from biopsies are often sufficient for diagnosis but inadequate for biomarker testing,[24,36] requiring rebiopsies that may be challenging from a risk, cost, and patient preference standpoint. Tests may fail due to technical reasons. Working with ancillary services such as pulmonology or interventional radiology to ensure enough tissue is obtained at diagnosis for testing can be very helpful. Similarly, optimizing tissue handling after biopsies are obtained to maximize available material for molecular studies would be equally important.

Payer coverage of testing may also be a barrier,[37] although in 2018, the U.S. Centers for Medicare and Medicaid Services approved a national coverage decision for NGS testing in patients with advanced NSCLC.[38] The U.S. Food and Drug Administration has also recently approved several NGS-based platforms for molecular testing in advanced malignancies, including Oncomine (testing EGFR, BRAF, and ROS1 only), MSK IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets), and Foundation One CDx. Concurrently, the Centers for Medicare and Medicaid Services proposed insurance coverage of Foundation One CDx and other similar NGS in vitro diagnostics for Medicare beneficiaries under the Parallel Review Program, designed to reduce the time of Food and Drug Administration approval of an in vitro diagnostic and its coverage determination by the Centers for Medicare and Medicaid Services.[39] Coverage through private payers, however, may still be variable.

Finally, most biomarker tests are still performed as a series of single gene tests rather than as a single broader panel,[24,40] which uses more tissue and potentially limits the number of tests that can be run. As more and more gene targets have emerged, it would be beneficial to switch to broader NGS-based assays that can evaluate all proven and emerging biomarkers in a single test. This should ultimately be significantly less costly, increase the number of patients who can have complete testing done, and reduce the need for rebiopsy.[41]

## TYPES OF BIOMARKER TESTING

Tumor tissue–based testing for therapeutically relevant alterations in lung cancer can be performed by several methods, depending on the testing platforms available to each institution. Molecular tests range from simple to highly complex. Simple tests are often designed to detect one type of mutation in one gene or could be low multiplex assays to detect the most common alterations in one gene or two. Complex tests, primarily NGS assays, can simultaneously detect multiple genetic alterations, including point mutations, insertions, and deletions, and, depending on the specific design, some may also detect copy number alterations, gene fusions, and other structural variants. Assays vary widely in the information they provide and sensitivity, specificity, comprehensiveness, tissue requirements, and turnaround times. As such, when ordering testing for a patient, it is important to be familiar with the type of assays and the tissue available for testing so that informed decisions can be made up front on tissue allocation and assay used. Importantly, given the multiple types of genetic alterations one must assess in patients with lung carcinoma, broader more complex assays are often most suitable for upfront assessment to avoid tissue exhaustion. Unfortunately, many of these assays are associated with very long turnaround times, which often surpass the time allotted by current guidelines for diagnosis. It is therefore important for each institution to design specific testing protocols for tissue triaging and allocation to accomplish basic testing with more rapid assays while still preserving valuable material for more extensive testing if needed.

Whether testing is performed in-house or at a commercial laboratory, two important definitions are universal and must be known prior to submission of the sample: the analytical sensitivity and the technical sensitivity of the assay.

1. The analytical sensitivity, often referred as the “limit of detection” or the technical sensitivity, is defined as the percentage of tumor cells that must be present in the specimen in order for a mutation to be identified. Sometimes, however, the analytical sensitivity can be defined as the lowest measurable amount of nucleic acid with a mutation that can be detected by the assay. This definition may cause considerable confusion given that most mutations are heterozygous, and, if present without gene amplification, only half of the tumor DNA will have the mutation. Thus, depending on the definition used by the laboratory, this will mean different tumor requirements. For example, if the technical sensitivity of a specific assay is quoted to be 10% of mutant DNA, this must be interpreted as requiring at least 20% tumor, as only half of the DNA will contain the mutation.
2. The diagnostic sensitivity relates to the comprehensiveness of the assay or the percentage of all mutations described for the gene and detectable by the given assay. For example, an assay for the detection of *EGFR* mutations that only tests for the most common mutations, *EGFR* exon 19 deletions, and the L858R mutation will have a diagnostic sensitivity of approximately 90% given that at least 10% of the *EGFR* mutations will occur outside of these two regions.

Testing for point mutations, insertions, or deletions is generally DNA based, extracted from formalin-fixed paraffin-embedded tissue. Numerous polymerase chain reaction (PCR)–based methods can be applied for rapid testing. Sanger sequencing, sizing assays, allele-specific PCR, real-time PCR, single-base extension assays, and mass spectrometry genotyping are among the many technologies successfully used for very targeted testing.[42-44] Newer NGS methods provide broader assessment capabilities, interrogating a comprehensive panel of clinically relevant genes, the whole genome, or whole exome, depending on design.

Targeted gene amplification and/or rearrangement assessment is often accomplished by fluorescent in situ hybridization. Other methods, such as reverse-transcription PCR, can identify specific gene fusions, but the assays must be sufficiently multiplexed to detect the several variants for the specific alteration. Alternatively, expression of the gene assessed by IHC can be used as a surrogate for fusion testing, depending on the specific antibody used and the robustness of performance. ALK IHC has now been incorporated in the recommendations as an equivalent alternative to fluorescent in situ hybridization for routine marker assessment.[8]

In recent years, NGS technology has emerged as a preferred method for comprehensive testing in lung carcinoma, as it enables the concurrent assessment of many alterations beyond EGFR, ALK, and ROS1 that are rapidly becoming clinically relevant. In the clinical setting, targeted NGS panels are preferred over whole-exome or whole-genome testing. Targeted testing provides higher coverage of genomic regions of interest to improve detection of relevant alterations and to allow critical molecular information to be available for therapeutic decisions at an adequate time frame.

Two basic types of NGS assays are commonly used in clinical molecular laboratories: amplicon-based and hybrid capture–based.[45] Amplicon-based assays use multiple PCR primers to directly amplify genomic regions of interest. Because of the multiplex primer design, these assays have limitations in the number of genes and regions that can be effectively covered. As a result, these assays are typically small panels that cover hot spots or highly selected regions of clinical interest. Most amplicon-based assays cannot reliably detect fusions or copy number alterations but may provide greater analytical sensitivity for key targets and better performance with very limited material. By contrast, capture panels use hybridization to capture larger genomic regions, allowing a broader assessment to include mutations, copy number alterations, and gene rearrangements, as long as they are incorporated in the design. Because these are generally more complex, turnaround time is often longer than for amplicon-based panels. For very limited samples, however, for which multiple tests cannot be performed, these assays are preferable for upfront comprehensive assessment.

Mutation assessment for secondary resistance mutations such as EGFR T790M and C797S may require the use of highly sensitive assays due to their subclonal nature. Allele-specific real-time PCR assays, PCR with locked nucleic acid or peptide nucleic acid clamping,[46] and digital PCR are some examples of such assays.[47] A list of common assays along with sensitivities and common variants detected are included in Table 2.

## WHAT TYPES OF PLASMA TESTING WORK? NSCLC AS A MODEL

The genomic complexity and growing number of targetable oncogenic subtypes of NSCLC support broad genomic testing at the time of diagnosis and at the point of acquired resistance. Thus, NSCLC serves as a model for the successful application of "precision medicine." Further, the advances brought about by checkpoint immunotherapy (CPI) in metastatic NSCLC and the emergence of predictive biomarkers for efficacy of CPI reinforce an evolving paradigm of upfront testing for both treatable genomic abnormalities and immune-related biomarkers. To this point, current national and international guidelines now recommend testing for oncogenic targets such as *EGFR* mutation, *ALK, ROS1, BRAF, RET, MET* and *HER2*, along with immune biomarkers such as PD-L1 and tumor mutational burden (TMB).[8,11-13] In view of the large number of actionable targets in NSCLC, plus other factors described in "Guidelines for Biomarker Testing in NSCLC" above, guidelines support the position that broad-based testing by NGS is recommended and preferred.

Thus, one of the key issues facing practicing oncologists in 2019 is not whether to test in patients with advanced NSCLC, but rather when to use tissue biopsy versus "liquid biopsy," primarily plasma-based circulating tumor DNA (ctDNA) assays, and whether ctDNA can now replace biopsy or rebiopsy in some clinical settings.

Testing by plasma ctDNA offers several potential advantages over tissue biopsy or rebiopsy:

- Liquid biopsy provides a global perspective by assessing tumor DNA shed into plasma from all tumor sites, thus potentially abrogating the issue of tissue heterogeneity.
- Liquid biopsy is relatively noninvasive, reduces costs and risks of complications associated with biopsy, and is highly acceptable to patients.
- Liquid biopsy is proficient in detection of both tumor-related genomic abnormalities at the time of initial diagnosis and at the time of acquired resistance.
- Tumor-associated genomic abnormalities detected by biopsy or plasma have proven to be equally actionable, as

**TABLE 2.** Review of Common Assays for Biomarker Testing

| Molecular Methods | Variant Types | | | | Sensitivity (%) | Turnaround Time |
|---|---|---|---|---|---|---|
| | Point Mutations | Small Deletions, Insertions | Copy Number Alterations | Rearrangements | | |
| Sizing assays | +/– | ✓ | | | | 2 to 3 days |
| PCR and Sanger sequencing | ✓ | ✓ | | | 20–50 | 3 to 4 days |
| PCR and pyrosequencing | ✓ | +/– | | | 20–50 | 3 to 4 days |
| PCR and mass spectrometry | ✓ | +/– | | | 1–10 | 3 to 4 days |
| PCR and single-base extension | ✓ | | | | 1–10 | 3 to 4 days |
| qPCR and digital PCR | ✓ | ✓ | | ✓ | 0.00001 | 2 to 3 days |
| Allele-specific PCR | ✓ | | | | | 1 to 2 days |
| FISH | | | +/– | ✓ | <1 | 2 to 3 days |
| NGS: targeted amplicon capture | ✓ | ✓ | | | 1–10 | 7–10 days |
| NGS: targeted hybridization capture | ✓ | ✓ | ✓ | +/–1 | 1–5 | 15–20 days |
| NGS: whole exome | ✓ | ✓ | ✓ | +/–1 | Variable | Weeks |
| NGS: whole genome | ✓ | ✓ | ✓ | ✓ | Variable | Weeks |

Abbreviations: PCR, polymerase chain reaction; qPCR, quantitative PCR; FISH, fluorescent in situ hybridization.

demonstrated by similar response rates to targeted therapies.

- Plasma testing can be repeated serially to monitor response and to detect emergence of acquired resistance prior to radiographic or clinical progression.
- New: TMB measured in tissue is emerging as a possible predictive biomarker for efficacy of CPI, as reflected by its incorporation into guidelines[12,13,48,49]; recent data quantifying TMB in plasma and associating high blood TMB (bTMB) with efficacy of CPI therapy have been reported.[50] If these findings are confirmed, "one-stop shopping" may be feasible in the near future, in which a single blood test couples identification of treatable driver oncogenes and TMB for CPI decision-making.

A detailed description of the available technologies and platforms for genomic testing is beyond the scope of this section of the article and is described in detail in the "Types of Biomarker Testing" section. Several recent reviews and consensus papers, including those recently published by the IASLC, provide essential background material on plasma-based testing and are referenced in this review.[51,52] In particular, the IASLC guidelines are a comprehensive review of the evolution of liquid biopsy technologies and, moreover, describe point-by-point pros and cons of tissue versus plasma testing in various clinical scenarios, including the so-called "plasma-first" algorithm (Figs. 1 and 2).

Here, instead, we focus on what is new in the clinical application of liquid biopsy for therapeutic decision-making in NSCLC, both for genomic testing and the emerging concept of bTMB.

### Detection of Mechanisms of Acquired Resistance to Oncogene-Related Therapies

Using *EGFR*-mutated NSCLC as an example, paradigms are already well established for therapeutic decision-making at the time of acquired resistance following initial response to first- and second-generation EGFR TKIs. Repeat biopsy or liquid biopsy in this setting detect the resistance mutation T790M in approximately 50% of cases. In T790M-positive cases, the third-generation EGFR TKI osimertinib is highly effective.[53,54] In T790M-negative cases, if a treatable bypass mechanism is identified, this information may factor into decision-making regarding chemotherapy or alternative targeted therapy.

However, now that osimertinib is approved for first-line therapy of *EGFR*-mutated NSCLC, it is increasingly important to identify acquired mechanisms of resistance in this setting. Preliminary reports have shown that following progressive disease to osimertinib first-line therapy, resistance mechanisms are quite different from those in the second-line setting when osimertinib is used in T790M-positive disease. In this regard, recent data have been presented on serial plasma ctDNA from the phase III FLAURA trial, which demonstrated superiority of osimertinib over the first-generation TKIs gefitinib or erlotinib.[55] As reported by Ramalingam et al,[56] at the time of acquired resistance to first-line osimertinib, no patients had evidence of T790M-mediated resistance by a commercially available plasma-based NGS. Instead, bypass mechanisms were found in approximately half of patients tested, including secondary *EGFR* mutations (9% overall;

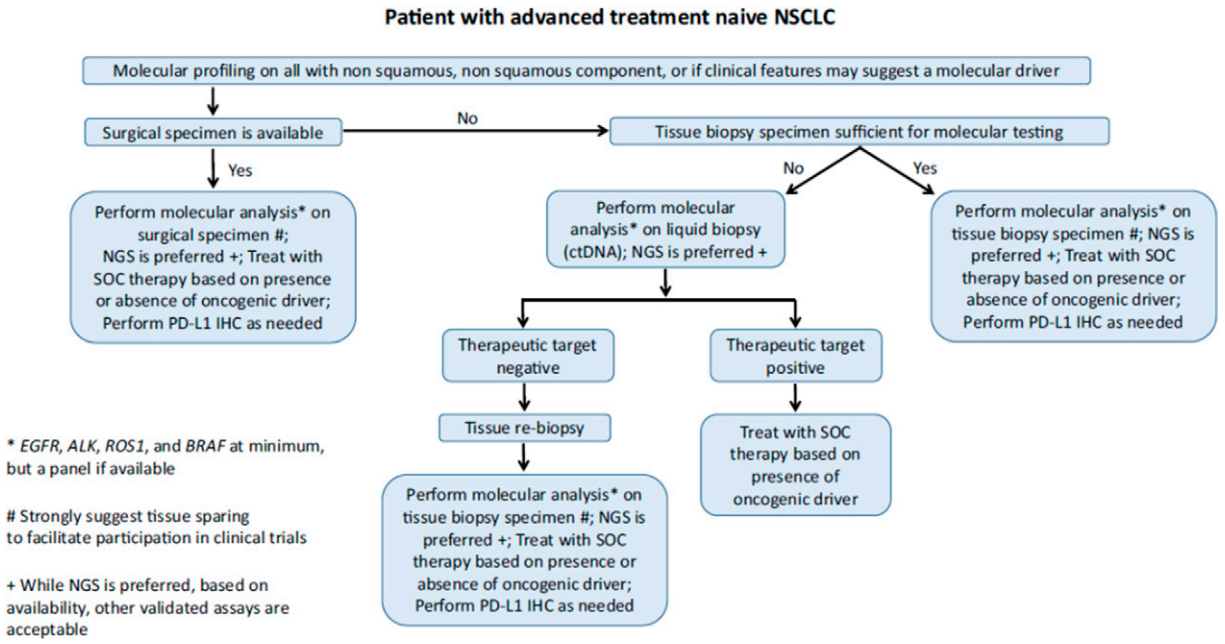


**FIGURE 1. Patient With Advanced Treatment-Naive NSCLC**

Abbreviations: NSCLC, non–small cell lung cancer; NGS, next-generation sequencing; SOC, standard of care; IHC, immunohistochemistry; ctDNA, circulating tumor DNA; ALK, anaplastic lymphoma kinase.

Reproduced with permission from Rolfo et al.[52]

7% C797S), *MET* amplification (15%), *PI3KCA* mutations (7%), cell cycle gene amplifications (10%), *BRAF* mutations (3%), and *HER2* amplification/mutations (3%).[56] Although the overall therapeutic implications of these findings remain to be determined, recent reports suggest that at least some of these abnormalities are treatable by targeted therapies.[57-59]

## NGS Determination of bTMB

PD-L1 testing by immunohistochemistry serves as a standard of care for CPI therapy for advanced NSCLC in several clinical settings. However, essentially all trials performed to date demonstrate a subset of patients who benefit even in complete absence of PD-L1 expression (PD-L1 $<$ 1%), suggesting the need for additional predictive biomarkers of CPI efficacy.[60,61] Of interest, several studies now show that tissue TMB by NGS, performed either by research-related whole-exome sequencing or commercially available comprehensive genomic profiling, is an independent predictor of CPI efficacy and surprisingly is largely nonoverlapping with PD-L1 expression levels.[48,49] Furthermore, analysis of tumor tissue by one commercially available TMB assay demonstrates comparability with TMB determined by whole-exome sequencing and also with predicted neoantigen load. Moreover, TMB by this assay has been reported to be equally predictive to neoantigen load for response to CPI therapy in advanced bladder cancer.[62]

To extrapolate these data into a plasma-based assay, meticulous validation procedures were required in a similar fashion to that for tissue-based TMB testing described above. Now, for the first time, analytic and clinical validation of a plasma-based NGS assay has been published using clinical samples collected during the randomized POPLAR and OAK trials of atezolizumab versus docetaxel in second-line and beyond therapy of advanced NSCLC. Samples from POPLAR were used as the test set and those from OAK for validation. At a cut point of 16 or more mutations/megabase of DNA for definition of high bTMB, this assay was predictive of response rate and progression-free survival in both trials, whereas in OAK, overall survival (OS) was equivocal at 16 mutations/megabase.[50] This assay is currently undergoing prospective validation in two randomized trials, BFIRST, a proof-of-principle study, and BFAST, a phase III trial comparing OS with atezolizumab versus first-line platinum chemotherapy in patients selected by bTMB 16 or higher. Preliminary data from BFIRST have been presented showing improved response rates with atezolizumab in cases with high bTMB (28.6%) versus low (4.4%; p = .0002) and a trend toward improved progression-free survival.[63,64]

Most recently, preliminary data on another plasma-based NGS assay were described in association with the MYSTIC phase III trial of durvalumab or durvalumab-tremelimumab versus platinum chemotherapy in first-line advanced NSCLC. In this otherwise negative trial, TMB was associated

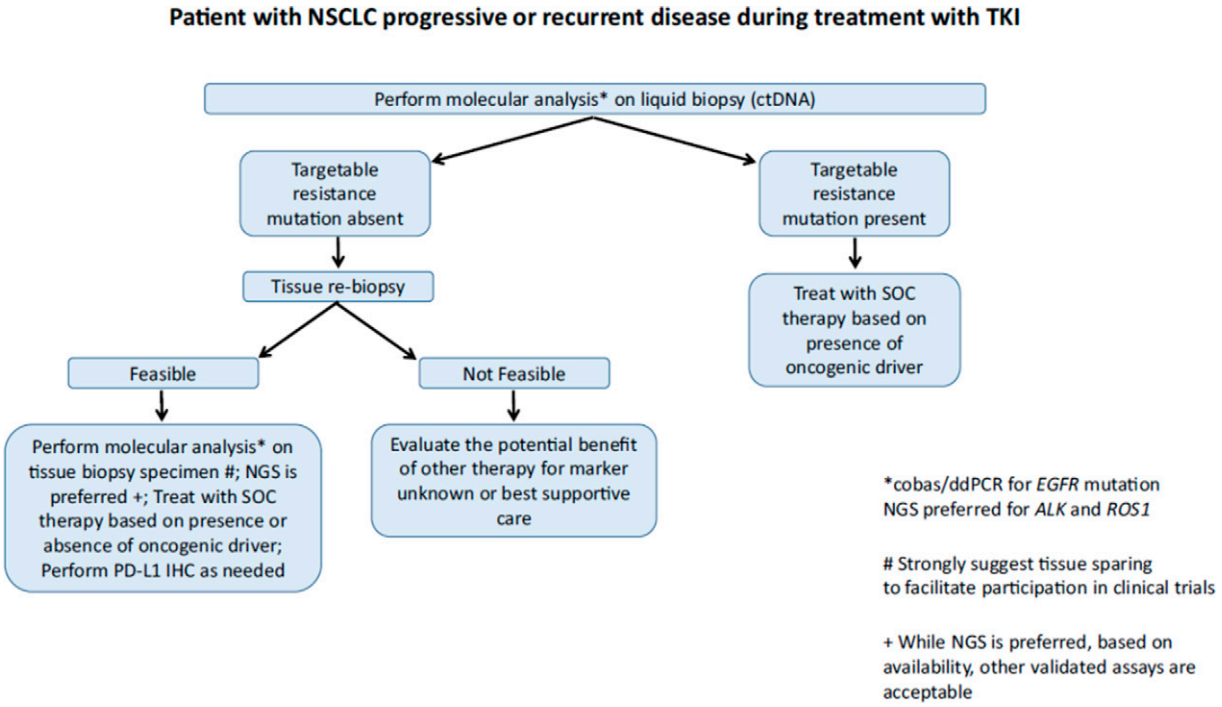


**FIGURE 2. Patient With NSCLC Progressive or Recurrent Disease During Treatment With TKI**

Abbreviations: NSCLC, non–small cell lung cancer; TKI, tyrosine kinase inhibitor; ctDNA, circulating tumor DNA; SOC, standard of care; NGS, next-generation sequencing; IHC, immunohistochemistry; ddPCR, digital droplet PCR; ALK, anaplastic lymphoma kinase. Reproduced with permission from Rolfo et al.[52]

with improved OS for the first time in CPI therapy.[65] At a bTMB at 16 or higher, median OS was 11.0 months (hazard ratio 0.80; 95% CI, 0.588–1.077) for durvalumab alone, 16.5 (hazard ratio 0.62; 0.451–0.8555) for durvalumab-tremelimumab, and 10.5 months for chemotherapy. Although these results for bTMB require confirmation, nevertheless they provide promise that a plasma-based TMB assay could be incorporated into routine clinical care. Along this line, the National Cancer Institute–sponsored lung cancer master protocol (LUNG-MAP), a unique private-public partnership for drug development, is incorporating bTMB into future substudies of CPI.

In summary, liquid biopsy has already emerged as a viable strategy for genomic testing in oncogene-driven advanced NSCLC, with defined roles both prior to first-line therapy and subsequently after development of acquired resistance. Serial plasma NGS facilitates monitoring and determination of mechanisms of acquired resistance. Tissue testing for TMB is already being incorporated into guidelines for NSCLC, and assessment by plasma-based NGS assays is under development.

## INTERPRETING AN NGS REPORT

Interpreting an NGS report requires thoughtful judgment and may pose several challenges. Different tumor types may have remarkably disparate pretest probabilities of harboring a clinically relevant biomarker. Oncologists reviewing these reports come from varied backgrounds and may have limited experience in determining the clinical significance of findings. Moreover, this interpretive process is a moving target, as new data emerge to introduce potential new targets over time, sometimes with new approved alternatives or at least promising data that suggest a novel consideration. These evolving options populate a continuum in the strength of the evidence supporting a biomarker-driven therapy for oncologists having different thresholds for whether and when to select a targeted therapy that may not have a well-established role in the current treatment algorithm for that cancer.

### Anatomy of an NGS Report

Though there is a wide range of vendors of multigene sequencing, each providing a report with a somewhat distinct format, they share some common features. Most begin by presenting a top-line summary of the key findings, generally distinguishing between biomarkers of established utility and those that are potentially clinically relevant but without clear consensus. Among those markers with a clearly demarcated role in clinical management, these reports typically highlight those for which a Food and Drug Administration–approved therapy for that particular tumor type is available or whether one or more commercially

available agents is available for that target but is approved for a different cancer type. Beyond this list, many NGS reports feature a summary of pertinent negative results that are clinically relevant but have not been identified.

In addition, many reports enumerate additional molecular alterations that may have a potential treatment option suggested based on a lower level of evidence. This support may be as strong as phase I or II data demonstrating clinical activity in a limited number of patients, but reports often suggest potential utility based on preclinical data or even a rationale based on a putative mechanism of action of a possibly biomarker-driven therapy.

A central feature of most NGS reports is a list of clinical trials for which a patient may be eligible based on the presence of an identified biomarker. These trials may focus on an established pathway and promising novel therapy, whereas others may have very broad inclusion criteria and very limited preclinical and/or clinical rationale. Some specify locations at which a trial is available and highlight ones within the region of the patient. NGS reports will typically conclude with an extensive list of references for the various treatment options discussed in the body of the report.

### Developing a Plan of Action From the NGS Report

There are two key questions that follow from an NGS report:

1. Is there an identified target (or potentially more than one) that should be considered as clinically relevant?
2. If so, how should treatment based on the molecular alteration(s) be sequenced compared with current standards independent of this molecular testing?

It is critical for oncologists and patients to understand that even as molecular oncology has delivered the promise of novel, personalized, biomarker-driven treatments for many patients with cancer, that promise is currently delivered for only a minority of patients. This is true even for a cancer like advanced nonsquamous NSCLC, for which there is currently an array of potentially actionable targets, and even more so for cancer types that rarely harbor a molecular alteration associated with effective biomarker-driven therapies.

**TABLE 3.** Brief Summary of Tiers Comprising ESMO Scale of Clinical Actionability

| Tier | Implication |
|---|---|
| I | Target suitable for routine use and recommend specific drug when specific molecular alteration is detected |
| II | Investigational targets that likely define a patient population that benefits from a targeted drug, but additional data are needed |
| III | Clinical benefit previously demonstrated in other tumor types or for related molecular targets |
| IV | Preclinical evidence of actionability |
| V | Evidence of relevant antitumor activity, not resulting in clinical meaningful benefit as single treatment but supporting development of cotargeting approaches |
| X | Lack of evidence for actionability |

For the majority of patients, standard treatments will offer more compelling evidence of clinical benefit than those based on molecular alterations, particularly if suggested targeted options are predicated only on preclinical or very limited clinical evidence thus far. Notably, though this research question has yet to be studied meticulously, there is a chance that selection of molecularly guided treatment may be clinically detrimental for patients if it supplants standard therapy options that are more effective. Many identified molecular alterations may be characterized as variants of unknown significance.

ESMO has developed a framework called the ESMO Scale of Clinical Actionability for molecular Targets (ESCAT) that articulates a range of tiers defining levels of evidence supporting a molecular alteration–based drug match.[66] A simplified version of this is presented in Table 3. The tiers range from the highest level, with strong prospective randomized trial evidence supporting the molecular alteration–based treatment and its appropriateness for routine clinical use, to investigational interventions with limited but supportive clinical evidence, to lower levels for which the value of the intervention remains hypothetical but with a compelling rationale, or the lowest levels, for which the available evidence shows no improved outcomes or has no evidence to support it.

The field of molecular oncology is remarkably dynamic. Accordingly, although NGS reports typically contain a relatively current summary of relevant reports and trials, there are a growing number of molecular alteration databases that collect information on a wide range of point mutations and structural variants along with information about treatment outcomes with various therapies. Although there are too many to cover in this review, some leading examples include Cancer Genome Interpreter,[67] Clinical Interpretation of Variants in Cancer,[68] MyCancerGenome,[69] and OncoKB.[70] These have varying degrees of curation, so results must be interpreted judiciously by the user, but they may provide additional and very current insight about a specific molecular alteration that is still being characterized but for which patterns may be emerging, along with putative treatment interventions.

This process of determining whether an actionable molecular alteration exists, and if so, how it compares to non–biomarker-driven standard therapies is illustrated in Fig. 3. Notably, a potential alteration-matched treatment may have very high-level evidence, corresponding to ESCAT evidence tier 1, which should lead to favoring it over nontargeted therapy

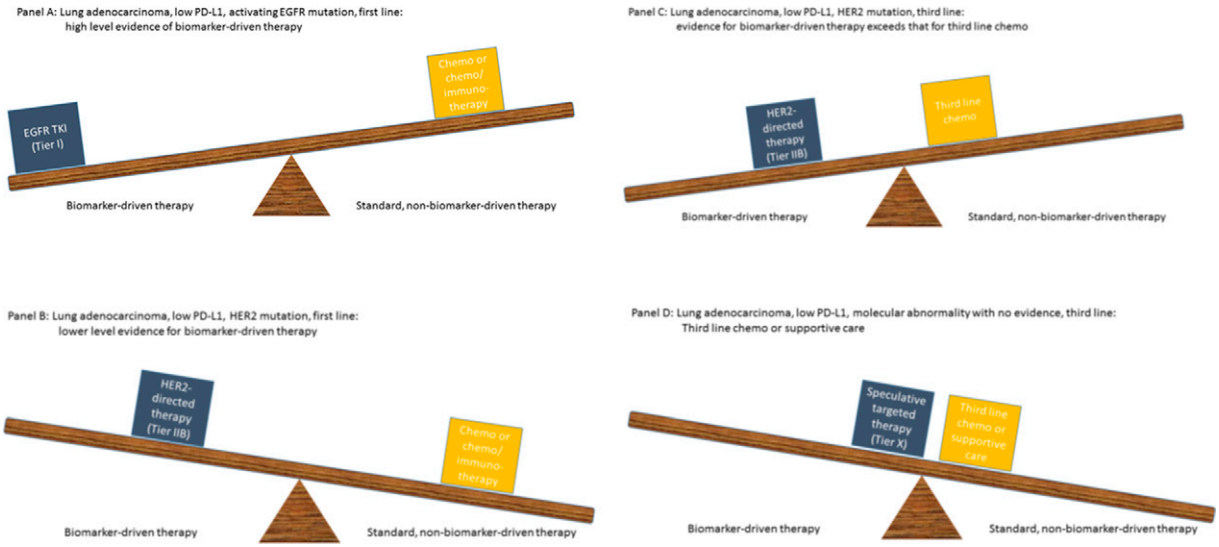


**FIGURE 3. Weighing Evidence Favoring Molecular Alteration-Based Therapy Versus Standard Nontargeted Therapy**
(A) Lung adenocarcinoma, low PD-L1, activating *EGFR* mutation, first line: High level evidence of biomarker-driven therapy; (B) Lung adenocarcinoma, low PD-L1, *HER2* mutation, first line: Lower level evidence for biomarker-driven therapy; (C) Lung adenocarcinoma, low PD-L1, *HER2* mutation, third line: Evidence for biomarker-driven therapy exceeds that for third-line chemotherapy; (D) Lung adenocarcinoma, low PD-L1, molecular abnormality with no evidence, third line: Third-line chemotherapy or supportive care.

(Fig. 3A), such as the finding of an activating *EGFR* mutation in advanced NSCLC. In other cases, the potential matched therapy is based on lower-level evidence, corresponding to ESCAT evidence tier IIB, such as with HER2-directed therapies in advanced NSCLC. In this setting, the evidence supporting standard first-line therapy is more compelling and should outweigh biomarker-driven treatment, at least outside of a trial setting (Fig. 3B); however, the limited evidence supporting targeted therapy makes it a strong later therapy choice (Fig. 3C). Finally, some molecular alterations may be identified but correspond to ESCAT tier X, with no evidence that it is clinically actionable (Fig. 3D). Treatments based on targeting this molecular alteration should not supersede evidence-based standard therapies, instead making this target primarily an appropriate subject of clinical trials, when available.

Finally, although synchronously identified driver mutations are typically mutually exclusive of one another, an NGS report may include several molecular findings. The decision-making process should reflect the same process of weighing the strength of each of the potentially relevant molecular abnormalities relative to standard treatment options as well as each other, as reflected in Fig. 3.


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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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