Genomic Testing in Lung Cancer: Past, Present, and Future

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Abstract

Precision medicine commonly refers to the selection of the most effective cancer treatments based on the presence of specific biomarkers (eg, genomic abnormalities) in a patient's tumor. Therefore, genomic testing is used to identify patients whose tumors harbor the vulnerability that is sensitive to corresponding targeted therapies. This approach allows for the selection of patients who have the greatest chance of deriving benefit from the treatments, reduces toxicity, and significantly improves outcome; precision medicine is recommended for advanced non-small cell lung cancer. This article reviews the evolution of genomic testing in lung cancer, from its development, including first success and failures, to its current use in the care of patients with lung cancer, and addresses future considerations, such as the expected increase of targetable abnormalities, the need to follow the genomic profile over time, and tumor heterogeneity.

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In the current era of cancer therapy, genomic testing of tumor samples and, more recently, blood, allows for the selection of patients most likely to derive benefit from certain treatments with reduced toxicity, and helps avoid unnecessary toxicities. Subsequent to the application of high-throughput genomic sequencing to lung cancer, many mutations had been identified in different histologic subtypes. Some of these mutations or other molecular alterations (eg, chromosomal rearrangements) represent therapeutic targets and can predict response to and outcome of specific lung cancer treatments. This article reviews the development of predictive biomarkers and the current status, clinical utility, and future perspective of genomic testing in non-small cell lung cancer (NSCLC); prognostic biomarkers are not discussed.

Predictive Biomarkers for Chemotherapy: A Story of Failure

Three biomarkers have been extensively studied to predict response to certain chemotherapy: ERCC1 for sensitivity to platinum agents; RRM1 for sensitivity to gemcitabine; and thymidylate synthase (TS) for sensitivity to pemetrexed. Unfortunately, no biomarker is currently recommended as a predictor of response to chemotherapy in patients with NSCLC.

Platinum-based agents are the backbone of chemotherapy in lung cancer through inducing DNA intrastrand and interstrand cross-links, which are mainly repaired by nucleotide excision repair (NER). Initially, biomarkers were searched for ability to predict the outcome of adjuvant chemotherapy. High DNA repair capacity eliminates cisplatin-induced DNA adducts and may lead to platinum resistance. ERCC1 is

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a rate-limiting enzyme in NER. An initial study of the samples from the International Adjuvant Lung Cancer Trial (IALT) reported that low ERCC1 expression was a poor prognostic factor in patients treated with surgery alone, but was predictive of greater survival benefit with cisplatin-based adjuvant chemotherapy.¹ However, the LACE-Bio project failed to validate this finding in other adjuvant chemotherapy trial samples.² When the same IALT samples used in the initial study were stained with the same (but different lot) 8F1 antibody, very different results were obtained, indicating variability of the antibodies between different batches. More importantly, it was discovered that only one isoform of ERCC1 was functional for NER and cisplatin resistance. Unfortunately, the different commercially available ERCC1 antibodies could not distinguish these various isoforms.² These results highlight the necessity of applying several methodological quality assurance steps before translating immunohistochemistry (IHC)-based biomarkers into clinical practice. It is mandatory to standardize techniques and validate them across cohorts and laboratories. This experience also underscores the crucial role of understanding the biology when designing biomarker assays. More recently, Soria et al³ reported that high ERCC1 protein expression as measured by targeted proteomic mass spectrometry was correlated with poorer prognosis in the Tailored Post-Surgical Therapy in Early Stage NSCLC (TASTE) trial, a biology-driven study in the adjuvant treatment setting in which patients with activated epidermal growth factor receptor (*EGFR*) mutations received 150 mg of erlotinib for 1 year; ERCC1-negative patients received 4 courses of cisplatin/pemetrexed, whereas ERCC1-positive patients underwent follow-up.⁴ These recent data are based on a small cohort of patients with a low rate of ERCC1 nonexpression, and will require validation before any clinically significant conclusion can be drawn.

RRM1 contributes to the synthesis of deoxynucleotide triphosphates (dNTPs), which is required for the DNA repair process. High RRM1 expression is thus associated with a better DNA repair capacity and predicted resistance to gemcitabine in the adjuvant and metastatic settings, as several retrospective studies have shown.⁵ No technical issues occurred with the detection of RRM1 expression, and these results were found to be very reproducible using IHC

or reverse transcription PCR. Nevertheless, the role of RRM1 was not confirmed in a prospective study, which showed no benefit associated with chemotherapy administered based on RRM1 expression compared with administration of standard random chemotherapy.⁶

Through maintaining the deoxythymidine monophosphate (dTMP) pool, TS, an enzyme targeted by pemetrexed, impairs DNA replication and repair. Retrospective trials and meta-analyses have consistently reported an association between a low TS expression and resistance to pemetrexed.⁵ However, prospective validation of these data is missing, and thus TS is not used in current practice.⁵

Therefore, no biomarker can currently be recommended for predicting NSCLC response to chemotherapy because of the failed validation of ERCC1 and RRM1 and the absence of validation for TS. These examples emphasize the absolute necessity of using the standardized treatment approach and of validating biomarkers before their use in clinical practice.

Predictive Biomarkers for Targeted Therapies Against Oncogenic Drivers

First Developments of Genomic Testing and Tyrosine Kinase Inhibitors

EGFR is a transmembrane receptor protein with tyrosine kinase activity. Its overexpression was found to be involved in the pathogenesis of various tumors, and therefore an attractive target for cancer therapy. Consequently, monoclonal antibodies and small molecule tyrosine kinases inhibitors (TKIs) were developed around 2000. The first clinical trials evaluated the activity of EGFR TKIs in previously treated NSCLC.^{7,8} These TKIs were further evaluated in combination with chemotherapy, but they did not improve outcome compared with chemotherapy alone.^{9,10}

Lung cancer care was revolutionized in 2005 by the discovery of activating mutations in the tyrosine kinases of *EGFR* as oncogenic drivers, and the sensitizing effect of EGFR TKI in lung adenocarcinoma bearing these mutations.¹¹ *EGFR*-sensitizing mutations occur between exon 18 and 21 and are mostly located in exon 19 (deletion) and 21 (point mutation L858R) of the tyrosine kinase domain. This discovery resulted in a dramatic change in the lung cancer treatment paradigm with the concept of targeted therapy. The overall response rate (RR) to EGFR

TKIs is 68% and the progression-free survival (PFS) is 12 months in patients with advanced NSCLCs that harbor an *EGFR*-activating mutation.¹² Subsequently, several other randomized trials have demonstrated the superiority of first-line treatment with *EGFR* TKIs compared with chemotherapy for patients with advanced *EGFR*-mutated NSCLC tumors.^{13–19}

The *ALK* gene was discovered as a translocation in anaplastic large cell lymphomas. In 2007, a gene rearrangement involving *ALK* was identified in lung cancer.²⁰ Several partners can be involved in the rearrangement, and all resulted in the translation of a fusion protein with constitutively active TKI activity. *ALK* rearrangement was found to be a predictor of high RRs and good outcomes to crizotinib, the first-generation *ALK* TKI.^{20,21} For patients with advanced NSCLC with *ALK* rearrangements, crizotinib results in a better RR (65% vs 20%; $P < .001$) and PFS (7.7 vs 3.0 months; hazard ratio [HR], 0.49; $P < .001$) than chemotherapy in the first-line setting.²¹

ROS1 is a receptor of the insulin receptor family with tyrosine kinase activity. First described in glioblastomas and cholangiocarcinomas, chromosomal rearrangements of *ROS1* genes were also identified in 1% to 2% of NSCLCs.^{22,23} Because *ALK* and *ROS1* tyrosine kinase domains have a high level of similarity, crizotinib was also found to be active in NSCLC harboring the *ROS1* gene rearrangement, with an RR of 72% and a PFS of 19.2 months in the US cohort,²² and these results were confirmed in the European cohort.²³

BRAF V600E mutation occurs in 1% to 2% of patients with lung adenocarcinomas, mainly in current and former smokers, in contrast with *EGFR* mutations and *ALK* rearrangements, which are mainly observed in nonsmokers.²⁴ For patients with *BRAF* V600E mutations, the NCCN Guidelines for NSCLC recommend the combination of dabrafenib with trametinib in the first-line setting.²⁵ Both dabrafenib and vemurafenib as monotherapy also show activity in NSCLC with *BRAF* V600E mutations, and are recommended in patients unable to tolerate the combination therapy recommended.^{25–29}

Current Clinical Use of Genomic Testing for Targeted Therapy

Recommendation for Molecular Testing and Treatment in First-Line Setting: Based on the findings discussed previously, testing for *EGFR*, *ALK*, *ROS1*,

and *BRAF* is recommended (category 1) at diagnosis in all patients with advanced nonsquamous NSCLC to help determine appropriate first-line therapy (Table 1). Patients harboring an *EGFR*-sensitizing mutation, *ALK* or *ROS1* rearrangements, or a *BRAF* mutation should receive an *EGFR* TKI (eg, gefitinib, erlotinib, afatinib), an *ALK* TKI (eg, crizotinib, ceritinib), a *ROS1* TKI (eg, crizotinib), or a *BRAF* inhibitor (eg, dabrafenib ± trametinib, or vemurafenib), respectively, in first-line treatment.^{25,30} To standardize biomarker testing in lung cancer, the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), and Association for Molecular Pathology (AMP) published guidelines³⁰ recommending that all patients with advanced NSCLC with an adenocarcinoma component be tested at diagnosis for *EGFR* mutations, *ALK* rearrangements, and *ROS1* fusions using the most accessible tissue (primary tumor or metastasis). These guidelines also state that testing can be considered in patients with pure squamous cell carcinoma (SCC) with clinical features that are associated with a high risk for *EGFR/ALK/ROS1* aberrations (eg, never/light smokers). These recommendations have been adopted by ASCO,³¹ NCCN,²⁵ and ESMO,³² although some do not yet recommend testing for *ROS1* rearrangement and *BRAF*. However, per current NCCN recommendations, these markers should be tested as targeted treatments are available. The ESMO guidelines will likely all include *ROS1* and *BRAF* in updated versions.

Mutations at Relapse: After an initial response or disease stabilization of approximately 11 months, most *EGFR* mutation-positive tumors eventually become resistant to *EGFR* TKIs. The T790M mutation was found to be a resistance mechanism that is acquired secondary to treatment with *EGFR* TKIs in almost 50% of cases.³³ Third-generation *EGFR* TKIs, such as osimertinib, have shown a RR of 77% in patients whose tumors bear a T790M mutation at resistance.³⁴ Other mechanisms of resistance to first-generation *EGFR* TKIs include small cell carcinoma transformation, *KRAS* mutations, and *c-MET* amplification, which have been clinically validated, and other mechanisms that require further validation, such as *HER2*-amplification, mesenchymal–epithelial transition, high expression IGF1R, and BCL2-like 11 (BIM) polymorphisms.

Liquid Biopsy: Although testing for a resistant mechanism, especially T790M, is required for osimertinib, rebiopsy may be clinically challenging. A noninvasive approach to detect molecular abnormalities has become an attractive alternative, such as genomic testing of peripheral blood, particularly based on cell-free DNA (ie, liquid biopsy), with the possibility of analyzing serial samples during treatment and at relapse. Liquid biopsy to detect *EGFR* T790M mutations at the time of acquired resistance to first-line *EGFR* TKIs is rapidly becoming the preferred procedure before performing a rebiopsy.³⁵ T790M detection in the circulating DNA has shown a sensitivity between 40% and 80%, depending on the technique used. However, because the sensitivity is relatively low, any negative results in the blood should lead to *EGFR* testing in the tumor sample. The specificity is 95% to 100%.³⁶ The FDA recently approved a test to detect *EGFR* T790M mutations in circulating tumor DNA (ctDNA) samples at the front line of treatment and at relapse (cobas *EGFR* Mutation Test v2, Roche Molecular Systems, Inc., Pleasanton, CA).³⁷

Evolving Targets for Testing

Other targetable oncogenic drivers have also been identified, including *RET* fusions, *HER2*, high-level *MET* amplification, and *MET* exon 14 splice junction mutations, for which new targeted therapies have been developed and are currently being evaluated worldwide. Cabozantinib and vandetanib were recently reported to show treatment benefit in patients whose tumors harbor *RET* fusions.^{38,39} Alectinib is also active in NSCLC with *RET* fusions,⁴⁰ but is currently not recommended in the NCCN Guidelines.²⁵ Accumulating evidence suggests that patients with NSCLC with *MET* exon 14 skipping mutation or high-level *MET* amplification may experience a significant clinical response to *MET* TKIs such as crizotinib.⁴¹ This splice-site mutation results in the deletion of exon 14 and loss of the Cbl-binding site on the *MET* receptor protein, resulting in the decreased degradation and high receptor expression level of this protein.⁴² *HER2* mutations may be tested; however, ado-trastuzumab emtansine has a category 2B recommendation in the NCCN Guidelines.^{25,43} Therefore, testing for *RET* fusions, *HER2* mutations, *MET* amplification, and *MET* exon 14 mutations should be conducted in all

patients who have negative results on *EGFR*, *ALK*, and *ROS1* mutation testing (Table 1), and possibly in parallel when tissue availability is not a concern (Figures 1 and 2). *PIK3CA*, neurotrophic tyrosine kinase receptor type 1 and 3 (*NTRK1* and *NTRK3*), and *KRAS* are not indicated in routine testing and not recommended in the NCCN Guidelines,²⁵ yet may be included as part of the multiplex panels used for *EGFR*, *ALK*, *ROS1*, *RET*, *BRAF*, *HER2*, and *MET* testing in patients who have access to clinical trials (Table 1).

Future Directions in Genomic Testing for Targeted Therapies

Biomarkers for *ALK* Inhibitors at Relapse?: Resistance mechanisms have also been identified in a patient with *ALK*-rearranged NSCLC treated with crizotinib, including several secondary mutations⁴⁴ (Table 2). Resistance to *ALK* inhibitors may also involve *ALK* amplifications; activations of bypass pathways, including the development of other mutations, such as *EGFR* and *KRAS* mutations; or *MET* amplifications. Second-generation *ALK* TKIs have been developed and showed high RRs—48% and 56% for alectinib and ceritinib, respectively—after the development of resistance to crizotinib.^{45,46} Brigatinib, another second-generation *ALK* TKI, showed a 71% RR and 12.3-month PFS in patients previously treated with crizotinib.⁴⁷ Resistance developed with second-generation *ALK* inhibitors

Table 1. Priority Molecular Testing at Diagnosis for Advanced Nonsquamous NSCLC

A Initial Testing (NCCN Category 1 ^a)	B Secondary Testing (NCCN Category 2A ^b Unless Otherwise Noted)	C For Clinical Trials Only
<i>EGFR</i> mutations	<i>RET</i> fusion	<i>PIK3CA</i> mutations
<i>ALK</i> rearrangement	<i>MET</i> exon 14 mutation	<i>KRAS</i> mutations
<i>ROS1</i> fusion	<i>HER2</i> mutations (category 2B ^c)	<i>NTRK1–3</i> mutations
<i>BRAF</i> mutations		

Testing A should be performed in all patients with advanced nonsquamous non-small cell lung cancer (NSCLC). Testing B can be performed secondary when testing A is negative, as targeted drugs for these drivers are recommended in the NCCN Guidelines for NSCLC.²⁵ Testing C can be proposed to patients whose tumor was found negative for biomarkers analyzed in testing A and B, and who have access to drugs targeting these oncogenic drivers in clinical trials.

^aBased upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

^bBased upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

^cBased upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Lung Cancer Genomic Testing

Figure 1. Primary genomic testing and targeted therapies for advanced NSCLC (category 1 recommendation in the NCCN Guidelines²⁵). Abbreviations: NOS, not otherwise specified; NSCLC, non–small cell lung cancer; SCC, squamous cell carcinoma. ^aIf combination is not tolerable.

also relies on specific resistance mutations of *ALK*.⁴⁸ Lorlatinib, a third-generation ALK inhibitor, is the only one with activity against the G1202R mutation and has a very high degree of central nervous system penetration, but induced a new mutation of resistance.⁴⁸ Although testing of these mutations is currently not recommended for determining treatment, guidelines may be adapted in the future because of the specificity of activity ALK inhibitors have against the different acquired mutations, and particularly given that G1202R-mutated *ALK* tumors

are resistant to all ALK inhibitors except lorlatinib. Resistance to ALK inhibitors may also involve activation of other pathways and development of other mutations, such as *EGFR* and *KRAS* mutations or *MET* amplifications.

Notably, second-generation ALK TKIs were developed for and mainly studied in patients with NSCLC that failed to respond to crizotinib treatment. However, at the 2016 ASCO Annual Meeting, results of the J-ALEX trial comparing alectinib and crizotinib were presented, showing an RR of

Figure 2. Secondary genomic testing and targeted therapies for advanced NSCLC (category 2A and 2B recommendations in the NCCN Guidelines²⁵). Abbreviations: NOS, not otherwise specified; NSCLC, non–small cell lung cancer; SCC, squamous cell carcinoma.

Table 2. Resistance Mutations to ALK TKIs^{44,48}

ALK Mutations	First-Generation TKI	Second-Generation TKIs			Third-Generation TKI
	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib
L1152R	x				
C1156Y	x				
F1174L	x				
L1196M	x				
L1198P	x				
D1203N	x				
G12102R	x	x	x		
G1269A	x				
F1174C/V		x			
G1202del		x			
I1171T/N/S	x		x		
V1180L			x		
E1210K + S1206C				x	
E1210K + D1203N				x	
C1156Y + L1198F					x
E1210K	x				

Abbreviation: TKI, tyrosine kinase inhibitor.

85.4% versus 70.2%, respectively, and significantly improved PFS with alectinib (HR, 0.34; CI, 95%, 0.17–0.71). Recently, findings from ASCEND-4 showed a doubled PFS of 16.6 versus 8.1 months for ceritinib versus platinum-based chemotherapy, respectively (HR, 0.55; $P<.00001$).⁴⁹ Thus, second-generation ALK inhibitors may have a future role in the first-line treatment setting, and this may change the profile of resistance in patients with ALK-rearranged lung tumors.

Other Oncogenic Drivers Still to be Targeted: The benefit of molecular testing and using targeted therapies based on molecular profiles have been confirmed in 2 large cohorts: the Lung Cancer Mutation Consortium study in the United States⁵⁰ and the Biomarkers France study.⁵¹ These studies clearly showed a better outcome for patients with actionable targets and targeted therapies. The biggest future challenges in targeted therapy will mainly be to develop efficient treatments for the numerous molecular abnormalities not targeted by available drugs. Currently, lung cancer still has few efficacy-proven targeted therapies. The role of chemists and pharmaceutical industries in developing drugs directed at these drivers will be essential. A promising option is the field of RNA chemistry and nanotechnologies.⁵² There is also a need for higher throughput screening of new drugs using patient-derived xenograft (PDX) models or, more recently, patient-derived cell line or organ-

oid models.^{53–55} In adenocarcinoma, many mutations remain untreatable, including the most frequently mutated TP53 and KRAS, but also less frequent mutations in known tumor suppressor genes and oncogenes.^{56,57}

In SCC of the lung, no targeted therapy has yet been shown to be efficacious. Based on the LUX-Lung 8 study,⁵⁸ the FDA recently approved afatinib for the treatment of patients with advanced squamous cell NSCLC following progression on platinum-based chemotherapy. This phase III study comparing second-line afatinib with erlotinib showed an overall survival of 7.9 versus 6.8 months (HR, 0.81; 95% CI, 0.69–0.95; $P=.0077$) and PFS of 2.4 versus 1.9 months, respectively (HR, 0.82; 95% CI, 0.68–1.00; $P=.0427$). Afatinib thus became a new second-line treatment option for advanced SCC, even though many options, including immunotherapy, are available for these patients and may be more efficient.⁵⁸ Several molecular abnormalities have been identified in SCC of the lung, but so far, no targeted therapy has been shown to be efficacious against these cancers. A multi-substudy randomized phase II/III trial based on genomic screening, the Lung-MAP trial, is currently assessing several biomarker-based therapies (eg, FGFR inhibitors, cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors, anti-hepatocyte growth factor monoclonal antibodies, PIK3CA

inhibitors) in SCCs of the lung (ClinicalTrials.gov identifier: NCT02154490).

Fewer data are available for small cell lung cancer (SCLC). Encouraging results of a phase I study were presented on rovalpituzumab tesirine (RovaT), an anti-DLL3 antibody combined with a cancer-killing agent, pyrrol benzodiazepine dimer, which damages DNA.⁵⁹ Approximately two-thirds of patients with SCLC have a high expression of DLL3 on their tumor cells, and RovaT is the first drug to target this protein. In this trial, among patients with recurrent SCLC, 39% with the highest levels of DLL3 showed a response to RovaT.⁵⁹ However, molecular testing is not recommended in the NCCN Guidelines for SCLC.⁶⁰

Tumor Heterogeneity: Tumor heterogeneity is not taken into account in patient care. However, tumors have a spatial and temporal heterogeneity at diagnosis, which is further increased by treatment.^{61,62} Due to tumor heterogeneity at diagnosis, tests for molecular markers in patients with NSCLC may be falsely negative, and multiple sampling within the primary tumor may be required to detect a molecular abnormality. This false-negative result is mainly a consequence of the high proportion of stromal cells, lymphocytic infiltration, and necrosis, resulting in a low tumor cellularity in the sample. In addition, although the driver may be a clonal event,⁶³ not all subclones within a single tumor will express the same driver. Moreover, a larger heterogeneity exists between tumor sites in patients with metastasis, and multiple site sampling would thus be required to assess the biology of the primary tumor and the metastasis.⁶⁴ Limitations in the effectiveness of targeted therapy are at least partly due to the heterogeneity of drivers within tumor cells in the same tumor or person.

ctDNA testing is proposed as a method to evaluate tumor biomarkers overall among the different cancer sites and to avoid multiple sampling.⁶⁵ Due to treatment effects on the biology of the tumor and the natural evolution of tumors over time, rebiopsy at relapse is required to determine the best treatment. When possible, a new biopsy should be performed at the relapsing site. However, this is sometimes technically challenging and ctDNA testing is often a good alternative. The main advantage of ctDNA testing is that it is noninvasive, but it may also reflect the cumulating sum of tumor DNA originating from

the different metastatic sites and may be performed repeatedly during the course of treatment and follow-up. Its main limitation is the lower sensitivity compared with tissue-based molecular analyses, due to the low proportion of ctDNA in total circulating DNA, and consequently the higher rate of false negativity.

Additionally, the presence of several potential oncogenic drivers is increasingly being detected as larger panels of next-generation sequencing (NGS) are used to analyze the tumors. When several molecular abnormalities are detected simultaneously, the role of oncogenic drivers and the best therapy for these patients will need to be determined. For example, 2 cases both presenting with a deletion of *EGFR* exon 19 and a *MET* amplification had a very different sensitivity to treatment, with one responding to an *EGFR* TKI and the other to crizotinib, indicating that the drivers were *EGFR* and *MET*, respectively.⁶⁶ To identify the driver, using PDXs or a patient-derived culture is potentially useful in the research setting because it allows for preclinical testing of tumor sensitivity to several drugs as monotherapy or in combination.⁶⁶ However, it will not be possible to use PDXs in clinical practice, and thus a practical way to determine driver mutations in the clinical setting is still needed.

Other Potential Applications of ctDNA: ctDNA testing may have broader applications in the future, with several preliminary studies indicating its potential role in monitoring disease status.^{67,68} ctDNA may predict residual disease and disease recurrence after treatments with curative intent, with its presence preceding the clinical signs of relapse.⁶⁷ The detection of somatic mutations in ctDNA after surgery has also been found to be correlated with disease stage and recurrence.⁶⁹ Detection of *EGFR* mutations in ctDNA during treatment for NSCLC harboring *EGFR*-sensitizing mutations has been shown to be associated with worse prognosis, and increased detection rates correlate with disease progression.⁷⁰ Similarly, longitudinal detection of *EGFR* TKI-sensitizing and resistance mutations in ctDNA has been found to correspond with disease status and to sometimes even precede the clinical signs of disease progression.⁶⁸ Thus, in the future, longitudinal detection and analysis of molecular abnormalities in the ctDNA may become a tool to monitor disease in both the curative and palliative settings.

Multiple Targets and/or Multitumor Approach

Studies: As NGS analyses of tumors are increasingly used, the current single gene/single treatment approach may be replaced by approaches that consider a broader spectrum of molecular and/or metabolic abnormalities (ie, DNA repair, stem cells, oxidative damage pathways). Several NGS platforms are now offering a panel of gene mutation and rearrangement testing, with a broad spectrum of potentially actionable cancer driver genes (eg, the FoundationOne validated panel by Foundation Medicine,⁷¹ and other panels commercialized by Illumina, Life Technologies, or Oncomine). Interestingly, such large panel NGS testing also provides the mutation load. Multiple-gene testing is more cost-effective than single-gene testing, not only in terms of monetary cost but also in terms of time and tissue. The cost issue will become increasingly significant, not only in terms of molecular testing but also because of the cost of targeted drugs and, particularly, new immunotherapy drugs. Overall, the annual cost for anticancer drugs is approximately \$100 billion USD, and is predicted to increase to \$150 billion by 2020. In the United States, the average price of a novel drug exceeds \$100,000 per year of treatment.⁷²

Several ongoing molecularly stratified umbrella clinical trials are investigating the possibility of performing panel NGS analyses and proposing bioguided treatment. Instead of performing multiple diagnostic tests to determine patient eligibility for many different studies, tumors are tested once for a broad range of potentially actionable biomarkers and patients are assigned to one clinical trial testing a specific drug.⁷³ Lung-MAP, as discussed previously, is using NGS panel gene analyses to match patients to substudies testing investigational treatments that may target the genomic alterations, or mutations, found to be driving cancer growth. Another example is the ongoing randomized phase II SAFIR02_Lung trial (ClinicalTrials.gov identifier: NCT02117167) comparing targeted treatment guided by high-throughput genomic testing versus standard maintenance chemotherapy in patients with metastatic NSCLC. In the case of targets detected in a small group of patients, it is particularly challenging to demonstrate significant benefit, and studies with a basket design are useful. In SAFIR02_Lung, a single biomarker and bioguided therapy is tested across multiples tumor types (ie,

cabozantinib efficacy is tested in tumors harboring RET with various histologies).

Immune Checkpoint Inhibitor Therapies: The Current Therapeutic Revolution

Current Biomarkers for Immunotherapies

Immunotherapy was recently established as an effective treatment for lung cancer.^{74,75} Checkpoint inhibitors such as the monoclonal antibodies against programmed death 1 (PD-1) and molecules against PD-1 ligand (PD-L1) are used to unblock the inhibitory mechanism of host immune response against tumor.⁷⁶ PD-1 and PD-L1 inhibitors, including nivolumab, pembrolizumab, and atezolizumab, have demonstrated their superiority as second-line chemotherapy after failure of platinum-based chemotherapy.^{74,75,77,78} Based on these findings, immune checkpoint inhibitors are recommended as preferred agents for second-line and subsequent therapy in patients with metastatic NSCLC (both squamous and adenocarcinoma histologies).^{25,79} In the first-line setting, pembrolizumab doubled PFS compared with chemotherapy in patients with advanced NSCLC with high PD-L1 expression (cutoff of 50%).⁸⁰ However, not all patients with NSCLC derive benefit from checkpoint inhibitor therapies, and the biomarkers to predict response to these therapies are being intensively investigated. The role of PD-L1 expression has been assessed by IHC to predict response to PD-1 and PD-L1 inhibitors,^{74,75,77,78} but variable and discordant results have been reported for PD-L1 expression regarding outcomes with various PD-1 and PD-L1 inhibitors. In adenocarcinomas, a greater benefit of nivolumab was seen when PD-L1 was detected, and the amplitude of benefit increased with higher levels of PD-L1 expression.⁷⁴ However, PD-L1 expression was not predictive of differential benefit for the same drug in SCC of the lung.⁷⁵ Pembrolizumab showed benefit compared with chemotherapy in first- and second-line therapy in patients with advanced NSCLC with PD-L1 expression on at least 50% and 1% of tumor cells, respectively.^{77,80} In the POPLAR trial, atezolizumab was beneficial compared with chemotherapy in the second line for patients with NSCLC with a minimum PD-L1 expression of 1% on tumor cells or tumor-infiltrating immune cells,⁸¹ whereas the other drug assays only considered tumor cell expression. However, the

phase III OAK trial comparing atezolizumab with docetaxel in the second-line setting for NSCLC showed benefit for all patients, even those whose tumor cells and immune cells did not express PD-L1.⁷⁸

The methodologies used in the different trials differed on several aspects, including type of antibodies, positive cutoff values, type of cells in which staining was assessed (ie, tumor vs inflammatory cells), and staining platforms. Based on these data, nivolumab and pembrolizumab are FDA-approved in the second-line setting in unselected patients with NSCLC for whom chemotherapy has failed, independent of PD-L1 expression status, whereas pembrolizumab in the first and second line is FDA-approved for patients expressing PD-L1 on at least 50% and 1% of tumor cells, respectively. Several other immune checkpoint inhibitors, including durvalumab, are currently being investigated.

It would be a challenge in routine clinical practice for a pathology laboratory to offer multiple PD-L1 testing for each patient using the numerous reported trial assays, not only because of the cost but also due to the availability of tissue. Therefore, there has been an international effort to evaluate the comparability of the different PD-L1 assays and to harmonize PD-L1 testing. The different antibodies used in the clinical trials included the following clones: 22C3 in tumor cells, 28-8 in tumor cells, SP142 in immune and tumor cells, and SP263 in tumor cells in trials assessing pembrolizumab, nivolumab, atezolizumab, and durvalumab, respectively. Multiple studies,^{74,75,77,78,80} including the Blueprint PD-L1 IHC Assay Comparison Project,⁸² showed that PD-L1 evaluation using the 22C3, 28-8, and SP263 assays was relatively comparable, whereas the SP142 antibody exhibited greater discordance with the 3 other assays. In addition, assessment of PD-L1 in tumor cells is more reproducible than in immune cells, which showed a high variability of staining. Two studies showed very similar results, with a lower staining of PD-L1 when using SP142 antibody compared with the other 3 antibodies.^{83,84} The French harmonization study of PD-L1 expression recently presented at the 2016 IASLC World Conference on Lung Cancer showed highly concordant results between different staining procedures, with SP263 antibody using the dedicated platform and kits, or even when using in-house optimized staining protocols.⁸⁵

Thus, based on the benefit with pembrolizumab compared with chemotherapy in patients with high PD-L1 expression,⁸⁰ PD-L1 expression testing is now recommended before first-line treatment in patients with metastatic NSCLC with negative or unknown test results for *EGRF* mutations, *ALK* rearrangements, and *ROS1* rearrangements.^{25,79} No specific IHC test is recommended, but the harmonization of PD-L1 assays is anticipated in the future.

Future Biomarkers for Immunotherapy

Regarding the selection of patients who would benefit from checkpoint inhibitors, many other biomarkers are currently being tested for their predictive value, including T-cell infiltrates, cytokines, mutation load, neoantigens, and DNA repair genes mutations. An overall high tumor mutation load has been associated with a durable clinical response,⁸⁶ although some responses were seen in patients with a low tumor mutation load. More specifically, the presence of neoantigens and a high rate of transversion were found to predispose patients to a higher sensitivity to checkpoint inhibitors.⁸⁶ The integration of CD8 T-cell infiltrate and mutation load is the most robustly reported biomarker so far. Thus, in the future, it is possible that reflex testing for PD-L1 expression and CD8 T-cell infiltration may be recommended at diagnosis for NSCLC, as well as the evaluation of mutation load obtained by NGS panel testing. Because only 30% of human cancers are infiltrated by CD8-positive lymphocytes, the combination of PD-L1 with vaccines, oncolytic viruses, and chimeric antigen receptor (CAR) T cells is currently being investigated.^{87,88} Also, in addition to the biomarkers of sensitivity to checkpoint inhibitors, mechanisms of resistance to PD-L1 inhibitors were recently identified as *JAK1* and *JAK2* mutations⁸⁹ or T-cell immunoglobulin mucin-3 upregulation,⁹⁰ thus indicating a potential need to overcome these abnormalities.

Conclusions

Currently, the NCCN Guidelines recommend that all patients with advanced nonsquamous NSCLC, or NSCLC not otherwise specified, and select patients with squamous cell histology be tested for *EGFR* mutations, *ALK* rearrangements, and *ROS1* fusions.²⁵ *RET* fusions, *BRAF* mutations, *MET* exon 14 mutations, and *HER2* expression (although with

a lower degree of evidence) also have available targeted treatments. Markers can be tested sequentially. However, the panel of oncogenic drivers and associated targeted therapies is increasing rapidly and will continue to do so, and the use of multiplex testing, already encouraged by the scientific community, will become standard.

In addition, PD-L1 expression testing is now recommended before first-line treatment in patients with metastatic NSCLC with negative or unknown test results for *EGFR* mutations and *ALK* and *ROS1* rearrangements.²⁶ No biomarker is currently recommended for the use of nivolumab or atezolizumab in patients with NSCLC, whereas a minimum tumor proportion score of 1% and 50% PD-L1 expression

is required for second- and first-line use of pembrolizumab, respectively. The existence of multiple PD-L1 assays matched to their therapeutics renders routine testing complicated, but the harmonization of PD-L1 assays is anticipated or already occurring. The clinical utility of other biomarkers for checkpoint inhibitor therapies remains to be validated.

Tumor heterogeneity will need to be integrated into patient care at diagnosis and over time to optimize treatment sequence and combinations. The emergence of cell-free ctDNA testing is a noninvasive alternative to tissue testing and may allow serial testing for monitoring of response and relapse, and for identifying the molecular mechanism of acquired treatment resistance.

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