



Update on emerging biomarkers in lung cancer

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Abstract: There has been considerable progress made in identifying oncogenic driver mutations in advanced lung cancer. The recognition that lung cancer is actually an umbrella classification that is comprised of multiple molecular subgroups has had a profound impact on how medical oncologists make treatment decisions. These mutations are clinically important as available targeted therapies can achieve significant responses and prolonged disease control. This review will summarize the current guidelines for biomarker testing and available therapeutic agents.

Keywords: Biomarkers; epidermal growth factor receptor (*EGFR*); anaplastic lymphoma kinase (*ALK*); *BRAF*; *RET*; *MET*; tumor mutational burden

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In 2004, several papers reported that the presence of epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC) predicted the response of those tumors to EGFR tyrosine kinase inhibitor (TKI) therapy (1-3). This news was followed by prospective clinical trials that confirmed better outcomes for NSCLC patients selected for gefitinib and erlotinib therapy on the basis of actionable EGFR mutations (4-8). This was followed by the discovery that the presence of anaplastic lymphoma kinase (*ALK*) gene rearrangements predicted the response of NSCLC to the ALK TKI crizotinib (9). The Food and Drug Administration (FDA) subsequently approved crizotinib for the treatment of advanced NSCLC harboring the *ALK* gene rearrangement with the Vysis ALK Break Apart FISH Probe Kit as a Companion Diagnostic for testing for the ALK translocation (10).

Over the past decade there has been a growing list of specific predictive biomarkers for actionable molecular abnormalities. An initial international guideline for testing EGFR and ALK in NSCLC was developed by the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular

Pathology and published in 2013 (11). A more recent update has added ROS1 as required with other predictive biomarkers recommended as parts of larger panels or when the required three biomarkers are negative: BRAF, MET, RET, ERBB2 (HER2), and KRAS (12). However, there has been a continuous pursuit of new predictive biomarkers and investigation of emerging biomarkers in lung cancer.

The ability to identify molecular subsets of advanced NSCLC has proceeded rapidly. Coupled with the expanding number of available and active targeted agents that have significant activity in oncogene-addicted cancers, biomarker testing has transformed the therapeutic landscape for patients with this disease. No longer is testing solely for EGFR and ALK acceptable in patients with advanced disease, given the number of identified mutations that can be targeted with a significant chance of clinically meaningful outcomes. In this article, we will only briefly review the well-known treatments for EGFR, ALK and ROS and instead will focus more on emerging targets such as BRAF, MET exon 14 and HER2 while also discussing how biomarkers are being developed to help guide immunology decisions.

The identification of driver mutations is not just a finding made possible by the expansion of elegant biomedical technology; it allows for a therapeutic intervention that actually benefits the lives of patients. Kris *et al.* looked at a multicenter effort to test patients with metastatic adenocarcinomas of the lung and test their tumors for a panel of actionable mutations (13). A total of 733 tumors were tested for 10 genes and 64% had an oncogenic driver. While it is true that 25% carried the KRAS mutation, currently felt to be undruggable, that still left over a third of patients with potentially actionable targets. Of the 28% of patients who went on to a trial or targeted therapy, the median survival was 3.5 years compared with 2.4 years for the patients with an identified driver who were not similarly treated with a targeted agent. The conclusion is that more expanded testing for actionable mutations can—if acted upon—significantly extend the lives of patients with these mutations.

While broad NGS testing in populations of advanced cancer patients remains controversial, within the specific confines of lung cancer the problem is the opposite: we have fairly robust data of efficacy and an increasing number of targets that need to be looked for (14,15). The frustration in the clinic is a longstanding one—tissue from patients with advanced lung cancer is often difficult to obtain and often scanty. And as the numbers of necessary biomarkers has expanded, more pressure has been placed on a limited biopsy sample to give important testing information. A number of strategies have been employed. There has been a prejudice in the clinic that core biopsies were better for genomic testing but recently there has been encouraging data that cytology samples were also fertile materials for testing for biomarkers (16). In addition, there has been increasing interest in using so-called ‘liquid biopsies’ to assay cell-free DNA. The interest stems from both the ability to hopefully acquire actionable information with less invasiveness than generally necessary for this group of patients, as well as the ease and ability to sequentially monitor allelic fractions to follow the course of the disease and elucidate resistance mechanisms (17).

With the proliferation of available commercial testing for cell free DNA as well as the ongoing questions of optimal use in the clinic, the IALSC recently released a statement paper to guide use and development of these technologies (18). The authors made recommendations in various clinical scenarios, such as treatment-naïve patients who lacked enough tissue for biomarker testing and patients who were progressing on their initial therapy. They emphasize that

a negative result from the peripheral blood needs to be confirmed with a tissue biopsy. If a T790 mutation is found in patients on a TKI for EGFR mutated lung cancer, the results are robust enough to trigger clinical action. Many other uses—assessing tumor mutational burden (TMB) and following the rise and fall of allelic fractions looking for correlation with therapeutic responses—remain promising avenues for investigation but are not yet felt to be standard of care (19).

EGFR, ALK and ROS mutated cancers share a number of similar features. They often are more common in non-smokers and women, although these clinical features are not robust enough to forego mutation testing in any patient with advanced adenocarcinoma (20). Patients often have significant and long-lasting responses to oral targeted agents with good quality of life, although these responses eventually are lost as the tumor develops acquired resistance (21). These oncogene-addicted tumors often have a low TMB, as would be expected from their mechanism of tumorigenesis (single driver mutation versus DNA damage from tobacco or UV radiation) and therefore have a low clinical response rate to immune checkpoint inhibitors (22-24). It is important for clinicians and pathologists to keep this fact in mind when deciding on initial treatment strategies; often programmed death-ligand 1 (PD-L1) results come back prior to the genomic results and therapy should not be initiated in the majority of instances until the genomic results have been reported.

There is fairly good consensus on the initial treatment approaches for EGFR, ALK and ROS mutated lung cancers. Osimertinib, a third generation TKI, is the recommended frontline agent for EGFR mutated lung cancer, providing a PFS of 18 months with excellent tolerability as well as significant activity in the CNS (25). Alectinib likewise is the frontline preference for ALK mutated lung cancer over the older drug crizotinib, although a number of very active competitors are being tested in the frontline setting (26). ROS1 remains very susceptible to crizotinib with often very prolonged responses (27,28).

Upon progression, it is becoming more common even outside of a clinical research trial to repeat a biopsy in order to look for new and actionable clinical targets. The continued growth of the use of liquid biopsy to test cell free DNA for mutations has allowed for both a better window on the processes that drive the development of acquired resistance as well as in some cases allowed patients to receive an additional targeted agent, often forestalling the need to go on to cytotoxic chemotherapy (29-31).

The recognition that additional mutations are actionable beyond the canonical three of EGFR, ALK and ROS has driven significant interest as well as drug development. BRAF was identified as a driver mutation in lung cancer in 2002 (32). These tumors, unlike EGFR, often arise in older patients with a tobacco history and can be seen in adenocarcinoma as well as squamous histology. In addition, their clinical outcome tends to be worse (33). Unlike the distribution in melanoma, the *V600E* mutation is found only 50% of the time. *V600E* mutations are significantly more prevalent in females than in males, often demonstrate an aggressive micropapillary histology characterized and are associated with shorter disease-free and overall survival rates (34).

Taking their cue from the activity of BRAF agents in melanoma, Planchard *et al.* looked at the activity of dabrafenib and trametinib in untreated patients with *V600E* mutated advanced lung cancer and saw significant activity (34). The overall response rate was 64% with 2 patients achieving a complete remission and 21 a partial remission. The median duration of response was 15.2 months and median progression-free survival was 14.6 months. The activity of this regimen led the FDA to approve the combination in June of 2017 for patients whose tumors harbor the *V600E* mutation.

Activating mutations as well as genomic amplification in the mesenchymal-to-epithelial transition MET gene have been recognized as a potentially important therapeutic target in NSCLC for many years but showing meaningful clinical activity has been a frustrating endeavor (35). However recent developments have put MET back on the list of actionable targets. Somatic mutations in the MET gene can cause *exon 14* skipping, and the resulting mutant receptor demonstrates increased c-MET signaling and downstream activation in multiple growth promoting pathways (36). These patients are often older than patients with EGFR mutations and are more frequently female. In addition, they can arise in histologies other than just adenocarcinoma, such as adenosquamous and sarcomatoid (37).

Crizotinib, approved for use in ALK-translocated lung cancer, was originally developed as a MET inhibitor and shows activity in *MET exon 14* skipping and high MET amplified lung cancer. Incidences of MET 14 skipping without MET amplification have been demonstrated which have responded to therapy with crizotinib and conversely MET amplification without *MET exon 14* also can exist and respond to TKI therapy (38).

MET is not just a *de novo* target at the time of disease

presentation; it is also one of the mutations that causes acquired resistance in patients with a different driver mutation treated with a TKI. Oxnard *et al.* looked at patients progressing on Osimertinib given second line following the development of a *T790* mutation and MET amplifications were one of the acquired resistance drivers that were discovered (39). With the increasing use of Osimertinib as the frontline choice in EGFR-mutated lung cancer (rather than second line following an earlier generation TKI) it remains to be seen if the spectrum of acquired resistance mutations will be similar. Nevertheless, targeting MET in the setting of acquired resistance is looking like an active strategy. Wu *et al.* recently published their experience in a phase Ib/II of capmatinib plus gefitinib in patients with MET dysregulation developing while on initial therapy with an EGFR TKI (40). Across the entire study the overall response rate (ORR) was 27% and in the phase II portion the ORR was 47% in patients with a MET gene copy number >6. The toxicity was manageable. Obviously, the identification of a population of EGFR patients who can benefit further from a targeted therapy at the time of acquired resistance will again push clinicians to obtain re-biopsy if possible, at the time of progression.

Liquid biopsy might be a very important tool to use in this situation, often obviating the need for patients to undergo repeat biopsy should an actionable mutation be discovered. Deng *et al.* recently published their experience of an EGFR patient progressing on TKI therapy who was found to have MET amplification on a liquid biopsy assay and who then responded to a combination of crizotinib and osimertinib (41). The expanded ability to look for therapeutic targets in patients losing their response to initial targeted therapy has the potential to delay the need for patients to progress to cytotoxic drugs and to hopefully maintain a superior quality of life.

The story of HER2 in lung cancer is quite different from that in breast, however there are a number of developments that are showing signs that these will also be meaningful targets. Early studies looked at a randomized trial of adding trastuzumab, a monoclonal antibody against HER2, to the same chemotherapy backbone in HER2 amplified lung cancer and failed to see any evidence of benefit (42). However, there were a small number of patients with high HER2 amplification who did seem to benefit from the addition of HER2 directed therapy. Later recognition that HER2 amplification and *HER2 exon 20* mutations reflected two different populations allowed a better understanding of the biology of the disease and how to target it. Li *et al.*

published their findings that HER2 amplification by FISH is found in approximately 3% of lung cancer adenocarcinomas and *HER2 exon 20* mutations are found in another 2% (43). None of the cases of *HER2 exon 20* mutations had overexpression.

Peters *et al.* recently published their experience looking at afatinib, an oral pan-HER2 blocking drugs, in heavily pretreated patients with *HER2 exon 20* mutations (44). Encouragingly, a number of patients had prolonged responses; 29% had a time to treatment failure of greater than 12 months. They also identified a particular mutation (*p.A775_G776insYVMA*) within exon 20 that also suggested a good response to therapy.

Ado-trastuzumab is a humanized monoclonal antibody that targets surface bound HER2 and has been approved for metastatic second line HER2 positive breast cancer. Li *et al.* recently published their experience using it as part of a basket trial of targeted agents at MSK (45). A total of 44 patients were identified with *HER2 exon* mutation and treated with Ado-trastuzumab. Forty-four percent of patients achieved a partial response with manageable toxicities. Responses were seen in patients with *HER2 exon 20* insertions and point mutations in the kinase, transmembrane, and extracellular domains. HER2 immunohistochemistry was 0–2+ and was not predictive of response. With a median progression free survival of 5 months, further exploration of HER2 targeted agents in patients with exon 20 are warranted.

Expanded ability of NGS to find tumor-agnostic and actionable mutations can also be applied to some patients with lung cancer. NTRK fusions are very rare in lung cancer but have been found in younger patients with a median age of 47 years and predominantly male (46). Drilon *et al.* reported their findings in a tumor-agnostic trial where patients with cancers containing TRK-fusions were treated with larotrectinib (47). Across the entire cohort, the overall response rate was 75% with 71% of the responses ongoing at one year. The responses were independent of regardless of the tumor type as well as the particular TRK fusion characteristics. Clearly testing for TRK as an individual, stand-alone test will never be a cost-effective assay but as part of a broader NGS panel it could make sense, given the outstanding clinical responses coupled with the fact that clinical predictors do not exist to help guide selection of patients for testing.

The use of testing for actionable biomarkers in patients with advanced lung cancer is not limited to a search for driver mutations. With the explosion of immunotherapy treatment options and significant clinical responses in many

patients, much attention has been given to identifying markers that predict for response. However, PD-L1 immunohistochemistry testing is a very different story than driver mutations: the expression is heterogeneous and often dynamic rather than static (48,49). Even with those limitations, patients who are discovered to have high PD-L1 levels at diagnosis have very high levels of clinical response to single agent pembrolizumab and that option was approved by the FDA in 2017 (50).

The majority of patients with advanced lung cancer however will not have either driver mutations that can be targeted with TKIs nor will they have very high tumor staining of PD-L1. For that reason, additional efforts have been made to identify a mutational signature predictive of clinical response. TMB has emerged as an attractive candidate. While standardization on methodology and agreement on definition remain issues, TMB can be measured when a sufficient number of genes have been studied and non-synonymous mutation numbers calculated (51). Snyder *et al.* looked at TMB in melanoma patients treated with Ipilimumab and found that patients with greater than 10 mutations per megabase had a significantly better response rate (52). Similarly, Rizvi *et al.* retrospectively looked at TMB in advanced lung cancer patients treated with immune checkpoint drugs and found that patients with high TMB had more robust response (53). The hypothesis is that patients with higher TMB develop more neoantigens that can serve as targets for a stimulated immune system. Findings from melanoma fit with this; immune checkpoint responses in cutaneous melanoma are very high and often long-lasting but responses to the same drugs in metastatic uveal melanoma, a tumor with one of the lowest TMB, remain very poor to the same drugs (54).

Recently Hellmann *et al.* published the results of Checkmate 227, looking at combination checkpoint inhibition with nivolumab and Ipilimumab versus nivolumab alone (or nivolumab with chemotherapy if the PD-L1 was <1) and cytotoxic chemotherapy (55). Patients with high TMB, regardless of the PD-L1 staining, had robust responses to ipilimumab and nivolumab and a much longer duration of response. Thus, TMB identifies a different population of patients than PD-L1 immunohistochemistry and has the potential to widen the number of patients able to benefit from immunotherapy and to put off being exposed to chemotherapy drugs. However, these findings pose new issues for pathologists and oncologists. TMB testing is more complicated than single gene or even panel assays and turn-around time and expense will be significant

issues. In addition, it is not unusual for lung cancer patients to lack enough tissue owing to small biopsies and thus the issue of re-biopsy for patients who do not have an adequate biopsy for TMB upfront will need to be addressed by clinicians and patients.

Because of the ongoing issues with tissue and specimen adequacy for the expanding questions being put on it, assaying cell-free DNA is an attractive option. Gandara *et al.* recently published their experience looking at TMB in patients treated on a clinical trial of Atezolizumab versus standard chemotherapy in lung cancer (56). They found that TMB as measured in cell free DNA was feasible to obtain and able to discriminate between populations likely to benefit. They also found that with rising levels of bTMB there was an improvement in progression free survival that favored the group treated with Atezolizumab. Lastly, while there was overlap, there were patients with high bTMB and low PD-L1 expression and vice versa, showing that like the Checkmate 227 data, high mutational burden and high PD-L1 expression can reflect separate patient cohorts.

Lastly, it must be mentioned that biomarkers need to be used to select patients for treatment—or against treatment. Like their colleagues who treat breast cancer, thoracic oncologists now have their own “triple negative” patients: patients with metastatic lung cancer who lack targetable driver mutations, have a PD-L1 <1 and low TMB. Additional molecular parameters are being looked at to help with further discrimination. Cristescu *et al.* recently looked at a genomics data set obtained over the course of a number of clinical trials involving the PD-1 inhibitor pembrolizumab. They found that, in addition to tumor PD-L1 staining, that TMB as well as T cell inflamed gene expression profile (GEP) described particular cohorts of patients with differential responses to immunotherapy (57). Notably, patients with low TMB as well as low GEP had extraordinarily low responses to pembrolizumab (0–9%), raising the difficult question whether—if identified pre-treatment—patients with these markers should be denied therapy with costly and ultimately always ineffective immune-oncology approaches.

Other biomarkers are being looked at as predictors of immune-oncology responders or failures. Skoulidis *et al.* recently published their look the efficacy of immune checkpoint inhibitors in patients with KRAS mutations and either *TP53* or *STK11/LKB1* mutations (58). They found that patients with KRAS mutations and TP53 had objective response rates to check-point inhibitors of 35.7% versus 7.4% if the co-mutation was *STK11/LKB1*. In addition,

patients with KRAS and *STK11/LKB* mutations had much shorter progression-free and overall survival than did patients KRAS positive and *STK11/LKBV* wildtype. Beyond further work to identify active drugs against *LKB1*, findings such as these can help be part of a genomic algorithm to help clinicians make decisions on treatment. Given that these patients have very low response rates to chemotherapy as well as immunotherapy, there is an important unmet need to identify up them upfront so that they can go on to clinical trials of novel approaches. And while it is hard to deny patients care, oncologists need to consider the expenses of immunotherapy and consider not treating patients whose tumors are unlikely to respond.

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Footnote

Conflicts of Interest: EH Bernicker has served on advisory boards for Guardant Health and Bristol Meyers Squibb. The other authors have no conflicts of interest to declare.

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