

# Non-Small Cell Lung Cancer in the Era of Personalized Medicine: Molecular Tests that Matter

CHRISTOPHER DEL PRETE, MD; CHRISTOPHER G. AZZOLI, MD, FASCO

## ABSTRACT

The diagnosis and treatment of lung cancer is entering a new era. With increasingly advanced diagnostic tools, we are more able than ever to pinpoint genetic changes in tumor cells that allow us to treat with highly effective, targeted therapy. In a growing number of patients, we are able to avoid cytotoxic therapies altogether. The recent advent of immunotherapy has led to a similar paradigm shift. This article will review the latest advances in tumor tissue and blood biomarkers directly as they relate to available treatments. Specifically, we will review activating and sensitizing gene mutations, gene fusions, PD-L1 tumor score, and close with an appraisal of the rapidly advancing field of peripheral blood biomarkers.

**KEYWORDS:** Non-Small Cell Lung cancer, peripheral blood biomarkers, molecular tests, immunotherapies

## INTRODUCTION

Lung cancer is one of the most common, and by far the deadliest form of cancer in both men and women in the United States. In 2019, it is estimated that 228,150 Americans will be diagnosed with lung cancer and 142,670 will die of the disease, accounting for approximately 25% of all cancer-related deaths, and making lung cancer, all by itself, the second leading cause of death in America after heart disease. The five-year overall survival rate for all-comers with non-small cell lung cancer is 15% or less.<sup>1</sup>

Amidst these grim statistics, a paradigm shift is underway in the diagnosis and treatment of advanced lung cancer. Increasingly, molecular tests performed on tumor tissue, and/or peripheral blood are driving therapeutic decision-making. In the context of metastatic disease in particular, therapies that target molecular pathways or harness the immune system to attack tumor cells are replacing traditional cytotoxic chemotherapies. These newer therapies are less toxic, more effective, and better differentiate malignant cells that carry particular mutations, protein expression, or immune susceptibilities compared to normal cells. This review will focus on the ever-expanding role of biomarkers in the selection of a growing array of gene-targeted drugs and immunotherapies for the treatment of advanced non-small cell lung cancer. It is essential that all physicians have

an awareness of the current biomarker landscape in lung cancer to better manage patients with this common disease.

## LUNG CANCER HISTOLOGY

After obtaining a tissue biopsy, the next step in the diagnosis of non-small cell lung cancer is to categorize the predominant histologic subtype. This is accomplished by histopathologic examination, though often aided by both immunohistochemical (IHC) and genetic analyses. The results of this pathologic analysis categorize non-small cell lung cancer into histologic subtypes: adenocarcinoma, squamous cell carcinoma, large cell neuroendocrine carcinoma, sarcomatoid or pleiomorphic carcinoma, undifferentiated, and mixed histologies. The vast majority of targetable mutations occur in the adenocarcinoma subtype. However, due to the potential to mis-categorize a small biopsy specimen, and the phenomenon of mixed histology, small biopsy specimens should be given the benefit of the doubt and tested for targetable mutations.<sup>2</sup>

## ACTIVATING AND SENSITIZING GENE MUTATIONS

Activating mutations occur in genes that code for intracellular proteins or receptors which drive cancer cell growth, survival, invasion and metastatic spread. They are analogous to a switch stuck in the “on” position, indifferent to the negative feedback that regulates normal cell behavior. In general, lung cancers driven by an activating oncogene are particularly virulent. So patients with such a cancer would have a poor prognosis if untreated. However, cancer cells carrying activating mutations also have a particular vulnerability. These cancers have been described as, “oncogene addicted,” meaning that they rely solely on a particular growth signal for survival, and that blocking this signal can trigger cell death.<sup>3</sup> Thus, the gene mutation both activates cancer growth, and also makes the cancer sensitive to a single gene-targeted drug. This paradigm provides the therapeutic rationale behind many of today’s targeted therapies. With proper diagnosis, lung cancers which may have a worse prognosis if untreated are flipped to dramatically better prognosis on appropriate treatment.

Targetable activating, sensitizing mutations can be identified in approximately one third of patients with lung

adenocarcinoma, and one fifth of unselected patients with NSCLC.<sup>4,5</sup> As a general rule, the presence of an activating, sensitizing mutation is more common in NSCLC in patients who never smoked cigarettes.<sup>6</sup> However, 90% of lung cancer patients are former or active smokers, and these mutations may still be found in heavy smokers. The clinical impact is such that you do not want to miss them, and patients should be tested for these gene mutations regardless of their smoking history.

### EGFR MUTATIONS

The epidermal growth factor receptor (EGFR) is a member of the tyrosine kinase family of cell membrane receptors which relays growth signals from the surface of the cell to the nucleus via downstream proteins including RAS, PI3K, mTOR and MAPK. This growth signal leads to a variety of anti-apoptotic and proliferative effects within the cell.

Approximately 10% of NSCLC patients will be found to have an activating mutation in *EGFR*, and most of these make the cell sensitive to drugs which block the signal. Over 90% of these mutations are substitution mutations in exon 21 and in frame deletions in exon 19.<sup>7</sup> The prognosis varies with the specific mutation, but in general the presence of an *EGFR* mutation is felt to be a favorable prognostic factor because the drugs blocking EGFR signal are so effective. A recent large cohort study of 1,656 patients with advanced NSCLC and *EGFR* mutation treated with targeted therapy revealed a median overall survival of 30 months and a 3- and 5-year survival of 40% and 20% respectively.<sup>8</sup> This represents a dramatic improvement in overall survival compared to patients with advanced NSCLC lacking an *EGFR* mutation.

Multiple tyrosine kinase inhibitors (TKIs) have been developed that target *EGFR* mutations including afatinib, erlotinib, gefitinib, and osimertinib, all of which are pills taken once daily. It is important to note that these drugs are considered first-line mono-therapeutic agents and as such are used in lieu of traditional chemotherapy or immunotherapy. While effective initially, these drugs do not cure the disease and inevitably cancer cells arise which are resistant to the drug. Resistance to EGFR TKIs can be caused by secondary mutations in *EGFR* which prevent the drug from binding to the protein, or by activation of bypass tracts which stimulate cell growth independent of EGFR.<sup>9</sup> Better initial treatments are being developed by studying the genetic mechanisms of acquired resistance to targeted therapy.

### ALK TRANSLOCATIONS

Translocations in the anaplastic lymphoma kinase (*ALK*) gene are both activating, and drug sensitizing. These translocations occur in approximately 3–7% of NSCLC cases and are associated with an excellent response rate to ALK TKIs, ranging from 57 to 74%.<sup>10</sup> Thus, similar to *EGFR* mutations, targeted therapy outperforms systemic chemotherapy by a wide margin in the vast majority of ALK-positive patients. Gene translocations can be harder to find

than gene mutations, and require techniques which look at chromosomes (fluorescence in situ hybridization (FISH), or protein immunohistochemistry (IHC)). More sophisticated genetic testing using next-generation sequencing can routinely detect both gene mutations and chromosomal alterations. Whether the *ALK* gene fusion is detected by FISH, IHC or next-generation sequencing, there are a number of drugs which are commercially available to target ALK-positive lung cancer, including alectinib, brigatinib, ceritinib, crizotinib and lorlatinib. Patients with advanced ALK-positive lung cancer treated with serial ALK inhibitors have a median survival time measured in years.<sup>11</sup>

### ACTIVATING-SENSITIZING GENE FUSIONS IN ROS1, NTRK, MET AND RET

Like *ALK*, other genes can be activated by chromosomal translocations. *ROS1*, or c-ROS oncogene 1, is a receptor tyrosine kinase which drives 1–2% of NSCLC patients. The tyrosine kinase domains of *ROS1* and *ALK* are highly conserved, meaning drugs such as crizotinib, ceritinib and lorlatinib are effective in both *ROS1* and *ALK*-positive lung cancers. In a similar story of overlapping efficacy, the drug entrectinib was recently FDA-approved for the treatment of lung cancers with either *ROS1*, or neurotrophic tyrosine receptor kinase (*NTRK*) genetic changes. The *TRK* proteins function during normal physiology as receptors for nerve growth factors, and when activated by gene fusions can drive lung cancers, as well as certain breast cancers and sarcomas. Both entrectinib and larotrectinib are FDA-approved for *NTRK*-positive lung cancer. *RET* and *MET* gene rearrangements are also both activating, and sensitizing; however, there are no FDA-approved drugs specific for these gene targets. Discovery of these genetic changes should prompt enrollment in a clinical trial, or off-label use of a drug approved for some other purpose (cabozantinib, lenvatinib and vandetinib for *RET*, and crizotinib for *MET*). Retrospective cohorts of *ROS1*-positive lung cancer include patients still alive at 5 years.<sup>12</sup> Because *NTRK*, *MET* and *RET* fusions are relatively rare, there is less known about long-term outcomes in these patients.

### BRAF V600E

The *BRAF* proto-oncogene codes for a kinase that when mutated activates the MEK signaling pathway, leading to anti-apoptotic signaling and proliferation. *BRAF* mutations are common in melanoma, but are found in only 1–3% of NSCLC. The combination of dabrafenib and trametinib is FDA-approved for the treatment of advanced lung cancer with *BRAF* V600E with response rates of 60%, and progression-free survival of 10 months.<sup>13,14</sup> It is important to note that, unfortunately, most *BRAF* mutations in lung cancer are not of the targetable V600E type. There is no targeted therapy for *BRAF* non-V600E, and patients with these mutations have a poor prognosis, with a reported 3-year survival rate of 0% compared to 24% of those with a V600E mutation.<sup>15</sup>

Notably, in contrast to the driver mutations discussed so far, *BRAF* mutations are more common in smokers.

### KRAS

The most common activating mutations in NSCLC are in the oncogene *KRAS*. Activating *KRAS* mutations are found in approximately 25–35% of patients with NSCLC and, like *BRAF*, are more common in current or former smokers. *KRAS* mutations are generally associated with poor outcomes, at least in part because there are currently no gene-targeted drugs available. Excitingly, this is likely to change with the development of inhibitors to the specific mutation, *KRAS* G12C, which occur in approximately 13% of lung adenocarcinomas and can be blocked by small molecules which bind covalently and exclusively to the mutant protein.<sup>16</sup>

### PD-L1

PD-L1 (programmed death-ligand 1) is a transmembrane protein expressed on tumor cells, stromal cells and macrophages that binds to PD-1 receptors on cytotoxic T-cells and effectively turns them off, halting anti-tumor effect. Thus, PD-L1 can be understood as a mask that cancers wear to hide from the immune system. (Please see the accompanying article by Hsu, et al in this edition of the RIMJ, “Immune Checkpoint Inhibitors in the Treatment of Gastrointestinal Malignancies: A Review of Current and Future Therapies.”)

The class of drugs that target this mechanism – also known as immune checkpoint inhibitors (ICPIs – includes pembrolizumab, nivolumab, atezolizumab and durvalumab. These monoclonal antibodies disrupt T-cell recognition of PD-L1, thereby enabling T-cell attack on tumor cells.

Due to the growing role of these therapies in modern oncology, and lung cancer in particular, it is now the standard of care in advanced non-small cell lung cancer to test all biospecimens for PD-L1 by immunohistochemical staining. After the stain has been applied, a pathologist calculates the proportion of tumor cells which are positive for PD-L1. The KEYNOTE-024 trial compared standard chemotherapy to pembrolizumab in patients with advanced NSCLC in the first-line setting in patients with a PD-L1 score of  $\geq 50\%$ , and who tested negative for *EGFR* or *ALK* genetic changes. The median overall survival (OS) in the immunotherapy group was not reached, but is estimated to exceed 24 months compared this to the typical 12-month median OS in patients treated with chemotherapy. The overall response rate to pembrolizumab was 45%, compared with 28% treated with chemotherapy.<sup>17</sup> Subsequent trials have combined chemotherapy with pembrolizumab and demonstrated survival benefit regardless of PD-L1 score.<sup>18,19</sup> However, the best biomarker package for selecting single-agent pembrolizumab remains testing negative for *EGFR* and *ALK* genetic changes and having a PD-L1 score  $\geq 50\%$ . These patients can avoid first-line cytotoxic therapy altogether, in favor of immunotherapy alone. More recent phase 3 data shows that the combination of low-dose ipilimumab + nivolumab (CTLA4 + PD1

blockade) is superior to chemotherapy even in PDL1 negative patients. This combination is not yet FDA-approved, perhaps because adding CTLA4 blockade adds side effects, and the overall survival comparison in the PDL1 negative cohort was not statistically significant because it was not part of the study’s statistical testing hierarchy (no alpha allocation). Also, ipilimumab + nivolumab was not reported to be superior to nivolumab alone for PD-L1  $> 1\%$ , and not reported to be superior to chemotherapy + nivolumab for PD-L1 negative patients.<sup>20</sup>

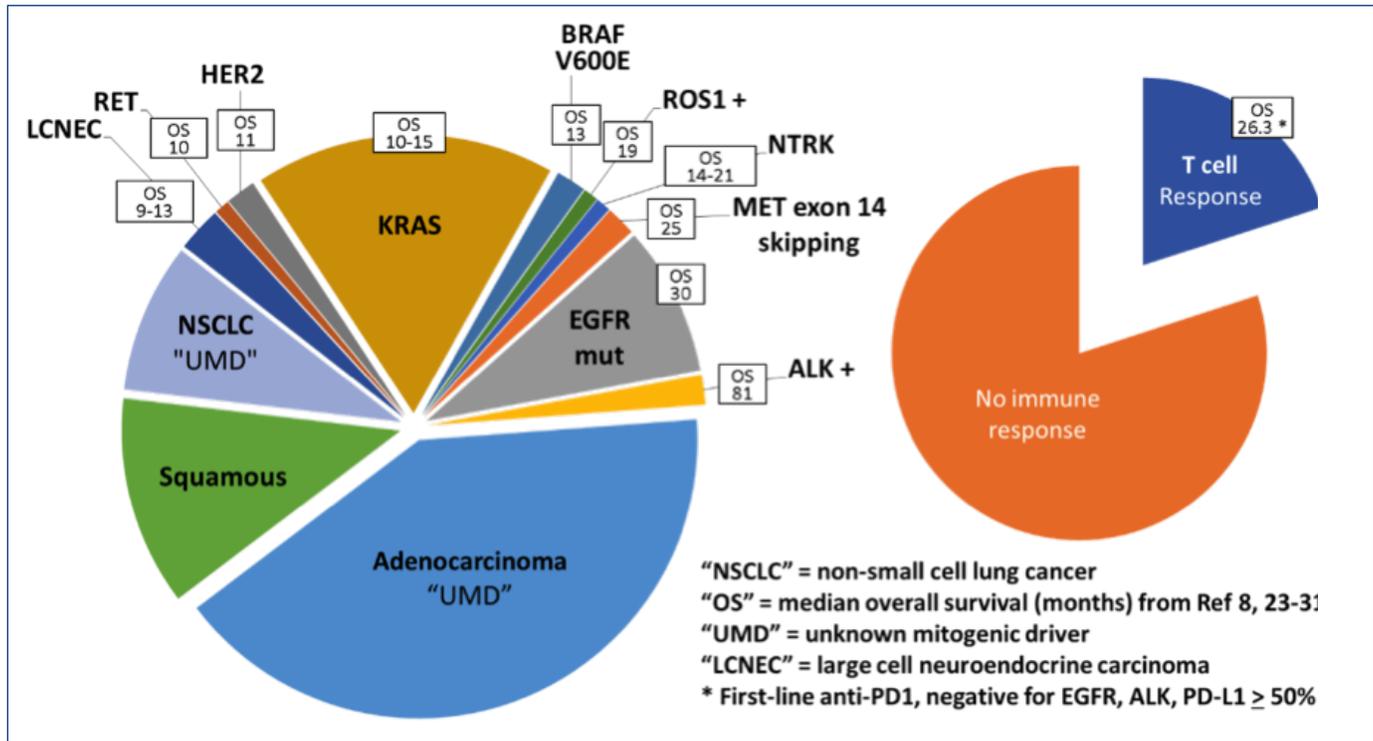
### FINDING MUTATIONS IN THE BLOOD

The molecular tests discussed so far rely on biopsy tissue to allow analysis of chromosomes, unique oncoproteins by IHC, and to obtain the tumor DNA for genetic analysis. The drugs which target these genes are then prescribed by medical oncologists to patients with advanced lung cancer as systemic therapy. It stands to reason that these same biomarkers may be found in the blood in patients with advanced disease. In fact, most patients with advanced non-small cell lung cancer will have circulating tumor DNA which can be detected in the blood. Blood-based testing is becoming increasingly popular and can speed up the process of molecular testing.<sup>21</sup> The problem is that up to 20% of patients with these diagnoses do not have circulating tumor DNA, and therefore extracting DNA from biopsy tissue for molecular testing remains the gold standard.

There is no blood test to select patients for immune therapy. Doctors and scientists at Brown University are studying extracellular vesicles which are found in both blood and saliva and can be used to classify metastatic cancers and tumor-host immune interactions. Extracellular vesicles – including exosomes (30–100 nm) and microvesicles (100–1000 nm) – are cell-derived membranous structures which originate from endosomes or are shed from the plasma membrane, and are involved in multiple cellular processes including intercellular communication and intercellular exchange of proteins, lipids and genetic material. Recent discoveries in immunology and cancer biology have established exosomes as an important mechanism by which cancer cells manipulate the tumor microenvironment and avoid immune-mediated elimination.<sup>22</sup>

A recent study looking at the role of extracellular vesicles in patients with melanoma found that melanoma cells release PD-L1-positive exosomes to amplify immunosuppressive signals to CD8+ T cells which would otherwise be limited to cell-to-cell contact. Since these exosomes can be isolated and measured in plasma and saliva, their detection and quantification can be used to distinguish clinical responders from non-responders to immune therapies.<sup>23</sup> Specifically, patients with metastatic melanoma responding to anti-PD1 immunotherapy (pembrolizumab) demonstrated increased levels of PD-L1 on circulating exosomes within 6 weeks of initiation of therapy. Ongoing research at Brown will evaluate exosomes as biomarkers of immune response in patients with advanced lung cancer.

Figure 1. Stage IV NSCLC: Clinically Relevant Subgroups



**CONCLUSIONS**

The treatment of advanced non-small cell lung cancer is evolving rapidly. Medical oncologists now prescribe a wide array of targeted therapies and immunotherapies based on biomarker science, making long-term survival possible in the context of a universally fatal disease. These advances depend on matching the right patient with the right drug, and have redefined non-small cell lung cancer as an ever-evolving collection of clinically relevant subgroups based on histology, molecular pathology results, and observed benefit from immune therapy (Figure 1). This paradigm has resulted in a feedback loop of discovery and therapeutic progress. In this way, molecular pathology will continue to redefine non-small cell lung cancer into subgroups with unique treatment opportunities and, with continued progress, the potential for using drug therapy for cure.

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**Authors**

Christopher Del Prete, MD; Department of Medicine, Division of Hematology/Oncology, Warren Alpert Medical School of Brown University, Providence, RI.

Christopher G. Azzoli, MD, FASCO; Associate Professor of Medicine, Warren Alpert Medical School of Brown University; Director of Thoracic Oncology, Lifespan Cancer Institute, Providence, RI.

**Correspondence**

Christopher Del Prete, MD  
 593 Eddy Street, George 310  
 Providence, RI 02903  
[cdelprete@lifespan.org](mailto:cdelprete@lifespan.org)