

Lung Cancer Biomarker Speak: Teach Me the Language

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Abstract

A vital role of the advanced practitioner is to analyze biomarker testing reports and explain to patients how the results may impact their treatment. At JADPRO Live Virtual 2021, presenters reviewed the importance of molecular testing in non-small cell lung cancer and how to interpret molecular pathology reports.

There are currently nine actionable biomarkers in non-small cell lung cancer (NSCLC), and the list of targeted therapies continues to grow. During JADPRO Live Virtual 2021, Beth Sandy, MSN, CRNP, and Jennifer Morrisette, PhD, FACMG, reviewed the importance of molecular testing in NSCLC and described different molecular testing strategies and techniques. The presenters also discussed biomarker testing reports and indications in NSCLC.

“Targeted therapy is often superior to chemotherapy and immunotherapy in most patients with biomarker-positive disease, but you can’t give a targeted therapy unless you test for and find the mutation or biomarker,” said Ms. Sandy. “It’s critical to find these biomarkers and treat them upfront. You have to test.”

HISTOLOGY

As Ms. Sandy explained, NSCLC is by far the most common type of lung

cancer and comprises 84% of cases (approximately 10% to 15% of NSCLC cases are never-smokers). Another 13% of lung cancers are small cell lung cancer, a very aggressive type that is rarely diagnosed in never-smokers (Scagliotti et al., 2009).

According to Ms. Sandy, mutation drivers play a much more significant role in the pathophysiology and treatment of NSCLC compared with small cell lung cancer. Adenocarcinoma is the most common histologic subtype of NSCLC and most common in never-smokers. It’s also the subtype most likely to have an actionable mutation.

The second most common type is squamous cell carcinoma, which is more common in smokers. Large cell carcinoma is the least common type.

WHY DO MOLECULAR TESTING?

The importance of molecular or biomarker testing in lung cancer has been demonstrated in several stud-

ies. One meta-analysis of patients with driver mutations who received chemotherapy as opposed to targeted therapy in the front-line setting showed a significant decrease in survival (Singal et al., 2019). Patients with biomarker-positive lung cancer who received the proper targeted therapy had an overall survival of 18.6 months vs. only 11.4 months for those receiving standard chemotherapy.

“A 7-month improvement in overall survival highlights just how important it is to find these mutations and treat them,” said Ms. Sandy.

Data from the US Oncology Network, however, suggest that molecular testing remains underutilized (Robert et al., 2021). The study of 3,474 patients between 2018 and 2020 showed that *ALK* and *EGFR*, the most common targetable mutations at the time, were tested only 76% of time, and *ROS1* and *BRAF* were tested only 73% and 59% of the time, respectively. Although PD-L1 was tested for 83% of the time, because of the simplicity of immunohistochemistry staining, less than half of patients were tested for all five of the top biomarkers at the time.

According to Ms. Sandy, more recent data from the US Oncology Network shows that only 45% of eligible patients are receiving a broad sequencing panel per National Comprehensive Cancer Network (NCCN) guidelines.

“More than 50% of adenocarcinoma cases will have an actionable mutation,” said Ms. Sandy. “There is no reason not to test for all of these

mutations when we have drugs approved to treat them. If we don’t test, we can’t target these mutations and improve outcomes” (Table 1).

THE IMPORTANCE OF BROAD MOLECULAR TESTING IN NSCLC

Non-small cell lung cancer is a heterogeneous disease, which is defined by oncogenic drivers. Although approximately 31% of patients have an unknown driver mutation, mutations are identified most patients, and many of these are targetable.

According to Dr. Morrisette, oncogenic mutations are often mutually exclusive, but patients with multiple primary tumors may carry different driver mutations. In addition, progression of cancer often leads to a second driver mutation.

As Dr. Morrisette explained, different assays can be performed, and these assays may differ between the types of variants that can be detected based on the assay targets. Broad molecular testing is recommended in NSCLC because there are multiple potentially actionable markers. Performing a separate test for each of the biomarkers would be difficult, time consuming, expensive, and can lead to tissue wastage, said Dr. Morrisette.

Broad molecular profiling also allows the detection of many actionable mutations in a single or few assays. This test is often called next-generation sequencing or massively parallel sequencing.

Guidelines from the NCCN, the Association of Molecular Pathology, American Society of Clinical

Table 1. Approved Agents/Regimens in Lung Cancer

Mutation	Agent
<i>EGFR</i> exon 19 or 21	Osimertinib: preferred first-line treatment Gefitinib, erlotinib, afatinib, dacomitinib Erlotinib + bevacizumab or ramucirumab If <i>EGFR</i> exon 20 insertion: Amivantamab, mobocertinib If <i>EGFR</i> uncommon mutations (G719X, S768I, L861Q): Afatinib approved
<i>ALK</i>	Alectinib, brigatinib, lorlatinib: preferred first-line Lorlatinib second/third line Crizotinib, ceritinib (not used much anymore)
<i>ROS1</i>	Crizotinib, entrectinib, ceritinib, lorlatinib
<i>KRAS</i> ^{G12C}	Sotorasib
<i>BRAF</i>	Dabrafenib/trametinib
<i>MET</i> exon 14 skipping	Capmatinib, crizotinib, tepotinib
<i>RET</i> rearrangement	Selpercatinib, pralsetinib, cabozantinib, vandetanib
<i>NTRK</i> (extremely rare)	Entrectinib, larotrectinib

cal Oncology (ASCO), and the College of American Pathologists (CAP) recommend that patients with advanced or metastatic NSCLC be tested with broad molecular testing. In addition, some of these guidelines recommend testing patients with other lung carcinoma histologies if there are clinical features that may indicate a higher probability of an oncogenic driver. These guidelines also suggest that testing should be performed before the initiation of therapy, said Dr. Morrisette, which may necessitate rapid single-gene testing of common targetable oncogenic drivers in certain clinical circumstances.

TYPES OF BROAD GENOMIC PROFILING

There are different types of broad genomic profiling, including DNA-based sequencing panels and RNA-based sequencing panels. As Dr. Morrisette explained, DNA-based sequencing panels nearly always detect single nucleotide variants (e.g., *KRAS* G12C, *EGFR* L858R) and relatively small insertions and deletions (e.g., *EGFR* exon 19 deletion). DNA-based sequencing panels may also detect copy number changes, fusions, and metadata, such as tumor mutational burden or microsatellite instability, and this information has important treatment implications.

In contrast, RNA-based sequencing panels nearly always detect fusion genes that are secondary to gene rearrangements, and they are often targeted panels designed to be partner agnostic. RNA-based sequencing panels may also detect deletions within a gene and different types of gene expression data.

“Fusion genes can be difficult to detect using standard DNA testing and may require an RNA-based test to detect these important driver mutations, including the *EML4-ALK* rearrangement,” said Dr. Morrisette.

When deciding what type of test to perform, Dr. Morrisette underscored the importance of detecting actionable fusion genes. Although relatively rare alone, recurrent targetable gene fusions comprise nearly 10% of patients with NSCLC. These include fusions involving *ALK*, *ROS1*, *RET*, *NTRK1*, *NTRK2*, and *NTRK3*, and *MET* splicing isoforms.

Although RNA-based sequencing detects the direct result of gene fusion and exon deletion

events, Dr. Morrisette noted that fusion gene testing using RNA can be labile if the tissue isn't treated appropriately. DNA-based sequencing, on the other hand, detects exactly where the DNA molecules are joined. However, if the breakage occurs in a large intron, the fusion can be missed. According to Dr. Morrisette, DNA-based sequencing is more likely to lead to false-negative gene fusion results (Davies & Aisner, 2019).

“If there is no driver gene detected, and you've only performed DNA-based next-generation sequencing, you should consider reflexing to an RNA-based test to be able to detect whether or not there are driver genes present,” she said.

LIQUID BIOPSY VS. TISSUE-BASED TESTING

Liquid biopsies are a minimally invasive way to detect mutations due to cancer cell death, which releases DNA into the bloodstream. As Dr. Morrisette explained, this method detects fragments of DNA that are circulating in the patient's peripheral blood and is useful when samples from the primary tumor are insufficient or inaccessible or for monitoring response to treatment.

“Liquid biopsy has increasingly become a standard of care in NSCLC,” said Dr. Morrisette. “It's non-invasive, can be used to monitor disease, can be used if tissue is inadequate, and positive results are actionable. Liquid biopsy may also pick up mutations from multiple metastatic sites.”

On the other hand, variants may be missed with this approach. Dr. Morrisette noted that it's not uncommon for a variant to be detected with tissue-based testing and be missed with liquid biopsy testing.

“Liquid biopsy has a high positive predictive value, but if a driver gene is not detected, ASCO and CAP guidelines recommend reflexing to tissue biopsy, as there can be biological reasons for a false negative test result,” she added.

According to Dr. Morrisette, advantages of tissue-based testing include the ability to assess tumor and cancer cell content with an associated pathological diagnosis associated with the tissue tested, and assessment of nucleic acid quantity and quality. Because there is only one testing site, however, that site may not be the most representative site of the cancer, and it's difficult to monitor

Table 2. Where Can You Find Out More Information About a Gene/Variant in the Report?

Database	Website	Best use(s)
Oncokb (MSK)	Oncokb.org	Gene and variant descriptions with references; targeted therapies, includes FDA approved content
PubMed	pubmed.ncbi.nlm.nih.gov	Literature search
ClinVar	ncbi.nlm.nih.gov/clinvar/	Clinical relationship with variants
COSMIC	cancer.sanger.ac.uk/cosmic	Catalog of somatic mutations in cancer based on literature
IARC TP53 database	p53.iarc.fr/TP53GeneVariations.aspx	Example of a gene specific database that characterizes <i>TP53</i> mutations
My cancer genome	Mycancergenome.org	Curated variant descriptions, therapies, mechanisms

disease through multiple invasive tissue collections, she said (Table 2). ●

Disclosure

Dr. Morrisette reported financial relationships with Bayer, Novartis, and ThermoFisher. Ms. Sandy reported financial relationships with Amgen, AstraZeneca, Jazz Pharmaceuticals, Merck, and Takeda.

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