Review Article

Narrative Review
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# Lung Cancer Genomics

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The landscape of lung cancer treatment is rapidly evolving with the use of genomic testing which helps identify specific mutations or resistance mutations for these heterogenous tumors. Advanced lung cancer has a very poor prognosis but identifying other treatment options based on genomic profiling of the tumor can lead to improved outcomes. Evidence of benefit for genomic testing in lung cancer has now resulted in this test becoming part of national guidelines. There are challenges with genomic testing which need to be understood as well as understanding how to apply test results. These results can help identify treatment options or may serve as predictors to respond to specific therapies. **Conclusion.** In the current era of precision medicine, it is imperative clinicians be familiar with genomic testing and be able to offer it to their cancer patients, specifically those with advanced lung cancer.

## Introduction

Cancer is a leading cause of death worldwide with lung cancer representing the highest with an estimated 2.09 million cases in 2018. The most common cause of cancer death is from lung cancer representing 1.76 million deaths in 2018 as well (1). Tobacco use is responsible for approximately 22% of all cancer deaths and causes nearly 90% of lung cancers (2, 3). Lung cancer is categorized as small cell lung cancer and the more common non-small cell lung cancer (NSCLC) which represents 80-85% of lung cancer cases. Non-small cell lung cancer is further sub-divided based on histologic types to adenocarcinoma, squamous cell carcinoma, large-cell neuroendocrine carcinoma, and pulmonary carcinoid tumors (4). In the U.S., the 5-year survival rates for all people with all types of lung cancer is 18% (5). The survival rate is directly related to the stage at diagnosis with people diagnosed at earlier stages having a higher rate of survival; 92% 5-year survival rate for stage IA versus 10% 5-year survival rate for stage IVA (6). The traditional modalities of treatment have included surgery, radiation and chemotherapy. However, the landscape of cancer treatment, specifically in lung cancer, has been rapidly evolving over the past 5 years to now incorporate immuno-oncology treatments as well as targeted therapies based on molecular alterations. Immuno-oncology treatments and targeted therapies for patients with known driver mutations have led to improved responses when compared to chemotherapy alone. These successes have led to utilizing these therapies in the first-line setting for certain patient populations. These newer advances have led to better outcomes as well

as improved quality of life in patients with late-stage lung cancers.

Large-scale comprehensive sequencing efforts such as The Cancer Genome Atlas (CGA) have led to the discovery of various mutations and pathways which may play a role in the pathogenesis of lung cancer and may offer a target for potential treatment (7). These sequencing efforts, or genomic testing, have helped clinicians understand the heterogenous nature of lung cancer as well as expanded the field of precision oncology.

This review article will highlight the role of genomic testing in making treatment decisions for patients with lung cancer.

## Genomic Sequencing

Since the early days of the Human Genome Project, there has been a continuous decrease in costs for next-generation sequencing (NGS) with more attention towards clinical implementation of whole genomes. Increased adoption has resulted in increased actionable therapeutic insights (8). As a result, more clinicians have utilized NGS for their patients with advanced disease especially when other treatment options are no longer available. One main question is when the appropriate time is to order NGS for a patient.

Although this answer is less clear for other cancers, the National Comprehensive Cancer Network (NCCN) guidelines recommends broad molecular profiling upfront in advanced or metastatic patients with the goal of identifying rare driver mutations for which effective drugs or clinical trials may be available (9). Several targetable genes are known to be altered in NSCLC including *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *HER2*, *RET* and *NTRK1*. Upfront NGS can be more cost-effective and faster than multiple single gene or limited gene testing. A study presented at the 2018 American Society of Clinical Oncology Annual Meeting predicted that in the United States, using Centers for Medicare and Medicaid Services reimbursement, NGS resulted in a savings of almost $1.4 million compared with exclusionary testing, $1.5 million compared with sequential testing, and more than $2.1 million compared with panel testing. NGS was also less expensive with commercial payers as well (10).

## Solid Tumor vs. Liquid Biopsy

Another consideration when ordering NGS is the method to which to obtain the testing. Genomic analysis of tumor tissue is the standard technique for identifying DNA alterations in malignancies (11). However, obtaining tumor tissue is always not feasible and in some instances, major complication rates with thoracic biopsies have been reported at 5.2% (12). NGS of circulating tumor cell-free DNA (cfDNA) represents a relatively non-invasive method of identifying potential targetable mutations from peripheral blood. In a retrospective study of twenty-eight patients with advanced solid tumors with paired NGS tissue and cfDNA, concordance was 91.9-93.9%, however the concordance rate decreased to 11.8-17.1% when considering only genes with reported genomic alterations in either assay (11). A prospective study evaluating plasma cfDNA in detecting oncogenic drivers for lung cancer demonstrated a tissue NGS concordance of 96.1% that directly led to matched targeted therapy in 21.9% (46/210) with clinical response. The authors also noted a shorter turnaround time for plasma NGS compared to tissue NGS with median time to result of 7 days compared to 20, respectively (13). One limitation of plasma NGS genotyping included the low concentration of cfDNA shed into the peripheral circulation (14). This would then suggest that a negative finding on cfDNA may not exclude the presence of a targetable driver. Despite the differences in concordance, the U.S. Food and Drug Administration (FDA) did approve the first

liquid biopsy test which can detect the presence of a T790M mutation in patients with metastatic epidermal growth factor receptor (EGFR) mutation-positive non-small cell lung cancer, who have progressed on or after an EGFR tyrosine kinase inhibitor (15).

## Results from Genomic Testing

NGS has helped identify many genomic alterations in lung cancers. According to researchers from the CGA and others, the most commonly mutated oncogenes in lung adenocarcinoma are *KRAS* (in 33% of tumors), *EGFR* (in 14%), *BRAF* (in 10%), *PIK3CA* (in 7%), and *MET* (in 7%). Data from the CGA have also shown a higher prevalence of *EGFR* mutations than of other mutations in specimens from groups with a low rate of transversion (16). Table 1 demonstrates recurrent molecular alterations in lung adenocarcinoma, squamous-cell carcinoma and small-cell carcinoma.

Genomic analyses can also discover clonal evolution as well as resistance genes. Subclones may be intermixed within one tumor sample or regionally separated within a primary tumor and metastatic sites (17). One possible scenario is when an *ALK* fusion-positive tumor treated with an *ALK* inhibitor continues to progress due to evolution of an *EGFR* mutation-driven subclonal cancer cell population (18). Figure 1 demonstrates three scenarios for evolution of the *ALK* fusion after *ALK* inhibition.

Long-term treatment results of the impact of NGS on treatment decisions and patient outcomes are still underway. Clinical trials such as NCI-MATCH and ASCO's TAPUR studies will provide some outcome data once available. However, smaller studies have been published including a retrospective study of 234 stage IIIb/IV NSCLC patients who had NGS testing in Israel. 62% performed tissue NGS and 38% performed

**Table 1. Recurrent Molecular Alterations in Lung Adenocarcinoma, Squamous-Cell Carcinoma, and Small-Cell Carcinoma.***

| Type of Alteration | Adenocarcinoma | Squamous-Cell Carcinoma | Small-Cell Carcinoma |
|---|---|---|---|
| Cell-cycle mutations | *TP53* (46%), *CDKN2A*(4%) | *TP53* (91%), *CDKN2A* (17%), *RB1* (7%) | *TP53* (92%), *RB1* (75%) |
| | *RTK/PI3K-MTOR* signaling | *RTK/PI3K-MTOR* signaling | *RTK/PI3K-MTOR* signaling: *PTEN* (5%) |
| | *KRAS* (33%), *EGFR* (14%), *BRAF* (10%), *STK11* (17%), *MET* (8%), *NF1* (11%), *PIK3CA* (7%), *RIT1* (2%) | *PIK3CA* (16%), *PTEN* (8%), *HRAS* (3%) | |
| Other mutations | Oxidative stress response: *KEAP1* (17%), *MYC* pathway; *MGA* (8%) | Oxidative stress response: *CUL3* (6%), *KEAP1* (12%), *NFE2L2* (15%) | Epigenetic deregulation: *EP300* (11%), *CREBBP* (10%) |
| | Aberrant splicing: *U2AF1* (3%), *RBM10* (8%) | Squamous differentiation: *NOTCH1* (8%), *ASCL4* (3%), *NOTCH2* (5%) | Neuroendocrine differentiation: *NOTCH1* (15%), *NOTCH2* (5%), and *NOTCH3* (9%) |
| Rearrangements | *ALK* (3–8%), *ROS1* (2%), *RET* (1%), *NTRK1* (3%), *NRG1* (2%), *BRAF* (3% in those who never smoked), *ERBB4* (1%) | *FGFRs* (rare) | *RB1* (13%), *TP73* (7%), *CREBBP* (4%), *PTEN* (4%), *RBL1* (3%) |
| Amplifications | *TTF1* (14%), *TERT* (18%), *EGFR* (7%), *MET* (4%), *KRAS* (6%), *ERBB2* (3%), *MDM2* (8%) | Chr3q: *SOX2* (43%), *TP63* (29%), *PIK3CA* (38%), *HES1* (26%)† | *MYC* family members (16%): *MYC, MYCN, MYCL1, SOX2* (27%), *FGFR1* (8%), *IRS2* (2%) |
| Deletions | *CDKN2A* (20%) | *CDKN2A* (27%), *PTEN* (3%) | *TP53, RB1, CDKN2A,* Chr3p (e.g., *FHIT, ROBO1*)† |
| Commonly altered pathways | MAPK and PI3K signaling, oxidative stress response, cell-cycle progression, RNA splicing and processing, nucleosome remodeling | Squamous-cell differentiation, oxidative stress response, MAPK and PI3K signaling | Cell-cycle regulation, PI3K signaling, regulation of nucleosome transcriptional and remodeling, NOTCH signaling and neuroendocrine differentiation |

* Percentages represent the prevalence of mutation and were obtained from the cBioPortal for Cancer Genomics (www.cbioportal.org).[10,11]
† Chromosomes 3q and 3p are cytogenetic bands.

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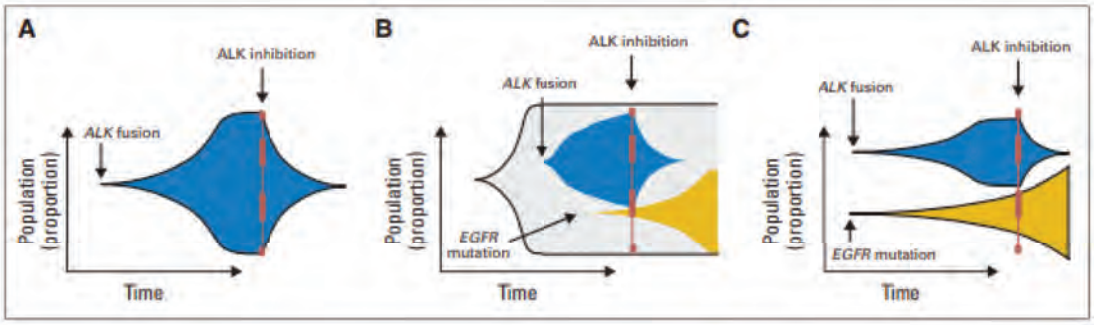


Figure 1. These scenarios for evolution of an anaplastic lymphoma kinase gene (*ALK*) fusion after *ALK* inhibition. (A) *ALK* fusion is a truncal event shared by all cancer cells, and *ALK* inhibition is effective. (B) *ALK* fusion and epidermal growth factor receptor gene (*EGFR*) mutation are later branched events that are only present in a fraction of the cancer cells. *ALK* inhibition clears cancer cells that carry the *ALK* fusion but leaves *ALK* fusion-negative cancer cells, including cells that carry *EGFR* activating mutations, to proliferate. (C) *ALK* fusion and *EGFR* mutation are both trunk events in separate primary tumors and progress in close proximity. *ALK* inhibition attenuates the growth of the primary tumor that carries *ALK* fusion leaves the *EGFR* mutated primary to progress. Reprinted with permission from Journal of Clinical Oncology (18).

liquid NGS. 91 patients had received targeted therapy based on NGS analysis, 75 received therapy based on NCCN guidelines, 9 off-protocol, and 7 received immunotherapy due to high tumor mutational burden (TMB) found on NGS. Median overall survival for this group was 25.7 months (19). Numerous case reports and case studies have also been reported in the literature highlighting positive responses to genomic-based therapy. Our group published a report on a patient with metastatic NSCLC who harbored a *PTEN* and *STK11* mutation from NGS testing who had a response to temsirolimus for almost 20 months (20). Although each case is unique and not all patients will benefit from NGS based therapy, these results highlight the heterogenous nature of metastatic lung cancer and will help identify specific patient populations that will benefit from such treatment.

In addition to providing genomic mutation results, NGS now also provides biomarkers which can help identify those patients who may respond to immunotherapy. Aside from the correlation of PD-L1 expression and response to checkpoint inhibitors, other markers are also present which may serve as predictors to respond to immunotherapy. Lung cancer genomes have a high tumor mutational burden (TMB) compared to other cancer types which is attributed to cigarette smoke exposure (21). Recent data reviewing 151 patients with any type of cancer who underwent NGS, had a TMB assessment and treated with immunotherapy were reviewed for response rate (RR), progression-free survival (PFS) and overall survival (OS). Higher TMB was independently associated with better outcome parameters. The RR for high TMB (>/= 20 mutations/mb) vs. low to intermediate TMB was 58% vs. 20%, median PFS was 12.8 vs. 3.3 months and median OS was not reached in the high TMB group vs. 16.3 for the low to intermediate group (22). A phase III trial specific to non-small-cell lung cancer showed that 1-year PFS rate was 42.6% with nivolumab plus ipilimumab versus 13.2% with chemotherapy in patients with a higher TMB (≥10 mutations per megabase) (23). Other predictors to respond to immunotherapy seen on NGS testing include identification of repair pathway defects such as MMR deficiency and mutation in DNA polymerases POLE

and POLD1 which are surrogate markers for TMB (24).

Obtaining these data points are instrumental in helping to identify which patients will respond to targeted or immunotherapy. One study demonstrated that out of 4064 patients with non-small cell lung cancer, 871 (21.4%) had an alteration in *EGFR*, *ALK* or *ROS1*. Among those with a driver alteration, improved OS was observed in those treated vs not treated with targeted therapies (median, 18.6 months vs 11.4 months, respectively). TMB of 20 or more was also associated with improved OS when treated with checkpoint inhibitors (16.8 months vs. 8.5 months, respectively). This study further illustrates the positive value of genomic testing in improving treatment responses in select patients as well as the importance of genomic databases for data collection and interpretation (25).

## Discussion

As we continue to gain a better insight into the heterogenous nature of lung cancers, we must accept that treatment is no longer "one-size fits all." Standard treatments with surgery, radiation and chemotherapy certainly still have their place, however, it is essential to deepen our understanding of each unique cancer patient's disease so we can offer them the best treatment option available. The field of precision medicine is rapidly growing and NGS is a significant part of that growth. As costs for NGS testing has decreased this has allowed greater access for clinicians and patients. The turnaround time can vary usually between 7-21 days depending on the test ordered and whether it is a solid tumor biopsy or liquid. Results can also be difficult to interpret if clinicians do not have much experience. Developing molecular tumor boards can help create a platform where cases are discussed, and treatments are reviewed based on current evidence (26). This may also help enroll patients into more clinical trials as well. In the future, with the increase usage of NGS, more relevant mutations can be discovered which can lead to further drug development. In addition, databases can capture multiple data points and outcome data to help create potential algorithms to identify patients most likely to respond to a specific therapy.

## Conclusion

Long-term data from current clinical trials such as NCI-MATCH and TAPUR will be available to help identify successful targetable mutations or biomarkers for various cancers. The past 5 years have seen rapid growth in the field of oncology, specifically in lung cancer treatments. With the success of immunotherapy and targeted therapies, we will without doubt see patients with advanced lung cancers living longer.

**Conflict of Interest:** The author declares that he has no conflict of interest.

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