NSCLC: An Update of Driver Mutations, Their Role in Pathogenesis and Clinical Significance

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ABSTRACT
Lung cancer is the most common malignancy in the US and causes the most cancer-related deaths. Non-small-cell lung carcinoma (NSCLC) accounts for the majority of cases. NSCLC historically was considered one entity, reflected by platinum-based therapy as the standard of care; however, with the discovery of EGFR mutations and ALK rearrangements, the landscape of treatment has become more personalized reflecting genomic heterogeneity. The molecular basis for tumor genesis was recognized and became a new method of classification. The availability of tumor sequencing and testing for these mutations is also becoming more accessible outside of major academic institutions. Targeted therapies offer alternatives to dangerous cytotoxic chemotherapy with equal or better efficacy. With these changes, driver mutations will play an increasing role in the diagnosis and treatment of NSCLC. In this review we will examine the characteristics of several NSCLC driver mutations and gene rearrangements and emerging data on therapies directed against them.

KEYWORDS: NSCLC, Driver Mutation, EGFR, ALK, ROS1, RET, BRAF, FGFR, MET, Targeted therapy

BACKGROUND
Lung cancer is the most common malignancy in the US and is also responsible for the most cancer related deaths. The American Cancer Society estimates there will be 221,200 new cases and 158,000 deaths from lung cancer in 2015. Approximately 85% of all lung cancer is NSCLC and the median age of diagnosis for NSCLC is approximately 70 years. Worse yet, more than half (approximately 57%) of all lung cancers are at an advanced stage at diagnosis. While the treatment of early stage lung cancer remains surgical resection and close follow up, advanced lung cancer is still a very mortal disease requiring aggressive and toxic treatments. This presents as a problem in many patients, as they do not have a performance status compatible with the aggressive standard-of-care chemotherapy and radiation regimens. However, many of the existing targeted therapies are better tolerated than standard chemotherapy and this approach may provide more treatment options for these frail patients than currently exist.

Following the human genome project, many genes were implicated in the development of cancer. Continued research into the genetics of lung cancer has led to the discovery of mutations and gene rearrangements influencing oncogenesis also known as, “Driver Mutations.” [Table 1] This phenomenon is best described in Non-Small Cell Lung Cancer (NSCLC), specifically adenocarcinoma. As a result, many prognostic tools and medications have been developed. In this review we discuss the most prominent driver mutations and gene rearrangements of NSCLC and the current agents both available and under development which target them.

EGFR
EGFR is the most well established driver mutation in NSCLC. Epidermal Growth Factor Receptor is a cell signaling, trans-membrane protein intimately involved in proliferation. Mutations occur in the kinase region and lead to unregulated phosphorylation and activation of cell survival/proliferation pathways. There are multiple therapies available that work against this mutation: Erlotinib and Afatinib are FDA-approved for EGFR mutated tumors.

The incidence is highest in female, non-smokers, with adenocarcinoma histology. Though this gene has multiple known mutations, 90% of those present in adenocarcinoma are of exon 19 and L858R point mutation in exon 21. This mutation is favored in Asian populations with its frequency reaching 62%, as opposed to 10% in US populations.

Table 1. Common Genomic Alterations in NSCLC

<table>
<thead>
<tr>
<th>Type of alteration</th>
<th>Frequency</th>
<th>Therapy FDA Approved?</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR Mutation</td>
<td>10-35%</td>
<td>Yes</td>
<td>Prognostic and Predictive</td>
</tr>
<tr>
<td>ALK Rearrangement</td>
<td>3-7%</td>
<td>Yes</td>
<td>Predictive, Not prognostic</td>
</tr>
<tr>
<td>ROS1 Rearrangement</td>
<td>1%</td>
<td>Yes, but for a different mutation</td>
<td>Predictive, Not prognostic</td>
</tr>
<tr>
<td>RET Rearrangement</td>
<td>1%</td>
<td>Yes, but only for other cancers</td>
<td>Not Predictive or Prognostic</td>
</tr>
<tr>
<td>BRAF Mutation</td>
<td>1-3%</td>
<td>Yes, but only for other cancers</td>
<td>Not Predictive or Prognostic</td>
</tr>
<tr>
<td>FGFR-1 Amplification</td>
<td>20%</td>
<td>No</td>
<td>Prognostic (Negatively) only</td>
</tr>
<tr>
<td>MET Amplification</td>
<td>2-4%</td>
<td>Yes, but for a different mutation</td>
<td>Prognostic (Negatively) only</td>
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</table>
NCCN guidelines now recommend routine testing for this mutation for all new cases of metastatic adenocarcinoma, with consideration in squamous cell patients who are never smokers or have mixed histology. Treatment with Erlotinib or Afatinib should be offered as upfront therapy to all patients who harbor this mutation.

It should be noted that insertions to exon 20 have been deemed tyrosine kinase inhibitor (TKI) resistant through clinical trials and EGFR inhibitors will be far less effective in patients with these mutations. For patients with this insertion, conventional chemotherapy is favored.

A mutation of the 790th amino acid from T to M is found in up to 50% of patients who initially responded to treatment with erlotinib but subsequently progressed despite continued treatment. T790M can be the sole driver mutation in EGFR mutated cancers; however, this is rare and only accounts for approximately 5% of all EGFR mutated NSCLC. New TKIs that are mutant specific and can specifically target this mutation are currently under development; rociletinib is furthest along in development but currently there are no approved agents for use against the T790M mutation.

**ALK**

Anaplastic Lymphoma Kinase, is a CD receptor (CD246) that plays a large role in the development of neurons. The ALK gene products are known to promote cell growth/proliferation and inhibit apoptosis at baseline. Rearrangement and fusion of this gene with EML4 is amplified and leads to a fusion protein product that is produced in an unregulated quantity and subsequently causes excessive cell proliferation. It is found in 5–7% of NSCLC. Again found predominantly in never-smokers and almost exclusively in adenocarcinoma, this is the second, and only other genetic aberration other to have an FDA-approved therapy in advanced NSCLC. Crizotinib, an ALK inhibitor, was approved for treatment of metastatic NSCLC in 2011 and response rates were comparable to EGFR positive tumors being treated with Erlotinib.

NCCN guidelines now recommend routine testing for this gene fusion (2p23) by FISH for all new diagnoses of metastatic lung adenocarcinoma. Treatment with crizotinib should be offered as upfront therapy to all patients. In ASCO 2014, Siraj M Ali et al, presented their experience with ALK rearranged lung carcinomas (LC) as detected by a clinical next generation sequencing (NGS)-based assay. Genomic profiling of 1,070 lung carcinomas was performed. Of 1,070 total lung carcinomas profiled, 47 harbored ALK rearrangements (4.4%). Of the 28 ALK rearranged specimens also tested by ALK FISH, 9 (32%) were negative, and 19 were positive. Twenty-two patients were treated with crizotinib and had response data available; 19 responded by investigator assessment. Of the 9 cases which were negative by FISH, 5 patients responded to crizotinib, 2 patients did not, and...
the response data for the remaining 2 patients is unavailable. Targeted NGS may be more sensitive for the detection of ALK rearrangements than FISH. In light of the responsiveness of ALK NGS+/FISH- tumors to crizotinib, the use of FISH as the gold standard for ALK detection in LC warrants prospective study.\(^{11}\)

Several resistance mutations have been described.\(^{12}\) In April 2014 a second agent, ceritinib, was approved for treatment of ALK positive NSCLC. Ceritinib is a second generation TKI that is approximately 20 times more potent than crizotinib.\(^{13}\) Preclinical studies suggested that ceritinib had significant activity against cells that were either sensitive or resistant to crizotinib, including resistant tumors with the most common L1196M and G1269A resistance mutations.\(^{14}\)

**ROS1**

ROS1 is a tyrosine kinase receptor belonging to the insulin family. Having striking similarity to ALK, this gene promotes proliferation and inhibits apoptosis, develops mutations via a rearrangement that leads to unregulated production of fusion proteins which retain the kinase domain,\(^{15}\) and preferentially affects young, never-smokers with adenocarcinoma. ROS1 translocations are identified by a FISH break-apart assay, again much like ALK. Though similar to ALK in many respects, the notable difference is the frequency at which ROS1 is found in tumors which is 1–2% of all NSCLC.

Interestingly, this gene rearrangement has increased sensitivity to the well-established ALK inhibitor, crizotinib.\(^{16}\) Retrospective analysis of crizotinib suggest that it may actually be a stronger inhibitor of ROS1 than it is of ALK. Initial data is so promising that it is highly likely that this will be the third mutation to be formally included in national guidelines for the treatment of advanced adenocarcinoma of the lung following EGFR and ALK.

**RET**

RET is a proto oncogene that becomes pathologic upon rearrangement with other genes leading to fusion proteins.\(^{17}\) Normally RET, a tyrosine kinase, responds to growth factors and promotes cell proliferation. RET mutations are present in 1–2 % of lung adenocarcinoma and not found in SCC. This pathway is currently better understood in thyroid cancer and the only FDA-approved agent against this mutation is the multiple kinase inhibitor vandetanib which targets RET among other kinases.\(^{18}\) Initial trials of this agent in RET positive NSCLC were disappointing. At this time there are no recommended treatments for RET positive NSCLC patients. Clinical trials with other RET inhibitors are ongoing.

**BRAF**

BRAF is a member of the Raf kinases which regulate the MAP kinase pathway. Better known for its role in melanoma, BRAF mutations are seen in 1–4% of lung adenocarcinoma.\(^{19}\) There are several mutations identified,\(^{20}\) of which the most clinically significant is a point mutation, V600E. This mutation causes phosphorylation in the absence of normal signal and unregulated cell growth. V600E accounts for 90% of BRAF positive melanoma, but only accounts for 50% of BRAF positive lung adenocarcinoma. This is problematic as the only two FDA approved BRAF targeting drugs, vemurafenib and dabrafenib, were developed to target this specific mutation. A phase II trial treating V600E positive lung adenocarcinoma with dabrafenib demonstrated clinical efficacy. Responses were relatively durable and the side-effect profile was consistent with what is observed patients with melanoma. This data suggests that current BRAF inhibitors could be as efficacious in V600E positive lung adenocarcinoma as they are in melanoma.

**FGFR1**

Fibroblast growth factor receptor-1 is another tyrosine kinase which plays a role in cell proliferation. Contrary to other mutations, FGFR1 amplification is associated with smoking and with worse overall survival. This amplification is found in approximately 15–20% of lung squamous cell carcinomas.\(^{21}\) Actionable driver mutations are rare in lung SCC compared to adenocarcinoma and there is heightened interest in FGFR1 amplification. Gene copy number is evaluated by fluorescent in situ hybridization, though the number of copies needed for the gene to be significant is not known.\(^{22}\)

TKIs for this mutation are currently under development and in phase 1 trials. A study of the TKI “BGJ398” in patients with FGFR1-amplified lung SCC showed partial response in 15% of patients. There are no approved FGFR inhibitors at this time.

**MET**

MET amplification is found in 2-4% of untreated NSCLC but found in up to 20% of EGFR positive cancers that have developed resistance to TKIs.\(^{23}\) Subsequent studies confirmed that amplification of MET is integral to development of this resistance.\(^{24}\) MET is located on chromosome 7 and amplification can be detected by fluorescent in situ hybridization. In a recent phase I trial, crizotinib showed activity in patients with MET amplified NSCLC and had a response rate of 33%.\(^{25}\) This may evolve into a new second line treatment for TKI resistant EGFR positive patients.
FINAL THOUGHTS

There exist other mutations not mentioned in this article. DDR2, PIK3C, AKT and PTEN mutations are all suspected to be driver mutations and are currently under investigation as well. As yet there are no clear therapies or prognostic implications for tumors positive for these mutations and further development is needed. Additionally, these mutations are found more commonly in squamous cell lung cancer and may offer hope of targeted therapy for a tumor type that currently lacks effective agents.

Although individual rates of mutations are small, when added together they account for a large percentage of new NSCLC diagnoses, particularly in non-smokers with adenocarcinoma. Many new agents offer equivalent or better OS and PFS with less toxicity than chemotherapy. NGS analysis is a useful tool for discovery of these mutations and potential treatments on or off clinical trials. We recommend its use in patients who have progressed through standard therapy but retain a good performance status. Personalized medicine is the future of oncology and genomic analysis will play a large role in cancer prognosis and therapy.

References


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