Genomic profiling toward precision medicine in non-small cell lung cancer: getting beyond EGFR

Abstract: Lung cancer remains the leading cause of cancer-related mortality worldwide. The application of next-generation genomic technologies has offered a more comprehensive look at the mutational landscape across the different subtypes of non-small cell lung cancer (NSCLC). A number of recurrent mutations such as TP53, KRAS, and epidermal growth factor receptor (EGFR) have been identified in NSCLC. While targeted therapeutic successes have been demonstrated in the therapeutic targeting of EGFR and ALK, the majority of NSCLC tumors do not harbor these genomic events. This review looks at the current treatment paradigms for lung adenocarcinomas and squamous cell carcinomas, examining genomic aberrations that dictate therapy selection, as well as novel therapeutic strategies for tumors harboring mutations in KRAS, TP53, and LKB1, which, to date, have been considered “undruggable”. A more thorough understanding of the molecular alterations that govern NSCLC tumorigenesis, aided by next-generation sequencing, will lead to targeted therapeutic options expected to dramatically reduce the high mortality rate observed in lung cancer.

Keywords: non-small cell lung cancer, precision medicine, epidermal growth factor receptor, Kirsten rat sarcoma viral oncogene homolog, serine/threonine kinase 11, tumor protein p53

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide. In the United States alone, lung cancer is expected to affect more than 224,000 people in 2014, representing 13.5% of all new cancer cases with a 5-year survivorship of 16.8% (http://seer.cancer.gov/csr/1975_2011/) and will be responsible for an estimated 160,000 deaths. The high mortality associated with lung cancer is due to the frequent presence of regional and distant metastasis at diagnosis (78% of diagnoses), that carries 5-year survival rates of 25% (regional) and 4% (distant), as well as increased incidence in relapse following treatment and resistance to standard therapeutics. These challenges necessitate a thorough understanding of the molecular biology of lung cancer toward the discovery and development of novel therapeutic approaches. The advent of genomic technologies and, more recently, next-generation sequencing (NGS), allow for a more comprehensive look at lung cancer, with the promise of therapeutically actionable discoveries.

Lung cancer is separated into two major histological categories: small cell lung cancer and non-small cell lung cancer (NSCLC). Of the two, NSCLC accounts for the vast majority of lung cancer cases. This review will focus on the molecular drivers and genomics-enabled treatment strategies in NSCLC. NSCLC is further divided into histological subtypes: lung adenocarcinomas (LAC) that arise in cells that line
the alveoli; squamous cell carcinomas (SCC); and large-cell carcinomas. Adenocarcinomas and SCC account for the majority of NSCLC cases. In addition to distinct histological features, adenocarcinomas and SCC differ in terms of their molecular drivers, pathogenesis, and disease progression, and they require differential treatment strategies.

Genomics-enabled precision medicine

The molecular landscape of many tumor types is currently being explored by NGS. A more thorough understanding of the molecular alterations in tumors offers opportunities not only to discover driver events, but also to predict therapeutic strategies that might benefit patients based on the individual tumor biology (precision medicine). Genomics-based therapeutic selection has become the standard-of-care for LAC patients, with mutant epidermal growth factor receptor (EGFR) and anaplastic lymphoma receptor tyrosine kinase (ALK) gene rearrangements dictating therapies (gefitinib/erlotinib and crizotinib, respectively) with improved response rates over conventional chemotherapy.\(^5,6\) While these have been hailed as therapeutic successes, the majority of lung tumors (>75%) do not harbor these molecular alterations. The discovery of recurrent genomic alterations has also paved the way for multiplexed biomarker tests. Platforms such as Sequenom® or SNaPShot® are now available to identify therapeutically actionable molecular alterations in lung cancer.\(^9\) In addition, the application of genomic technologies may also enable more the accurate identification of NSCLC histological subtypes from limited tissue samples, an area of current clinical need. With the cost of NGS rapidly diminishing, and time-to-results getting shorter, the genome-wide characterization of an individual tumor toward therapy selection is now an imminent possibility. Importantly, the discovery of therapeutic options in prevalent, previously “undruggable” genes such as Kirsten rat sarcoma viral oncogene homolog (KRAS), serine/threonine kinase 11 (STK11/LKB1), and tumor protein p53 (TP53), as well as the prediction of combinatorial therapies suggested by tumor alterations, will forward precision medicine toward the reduction of lung cancer mortality.

Molecular landscape of NSCLC

Lung adenocarcinoma

LAC are the most common histological subtype of lung cancer, and are characterized by abnormal growth of peripheral glandular epithelial tissue. LACs are highly heterogeneous, demonstrating high rates of somatic mutations and genomic rearrangements.\(^10\) Comprehensive molecular profiling of 230 LAC tumors and matched normal tissue by The Cancer Genome Atlas (TCGA) Research Network\(^11\) identified mutations to several oncogenes (KRAS [33%], EGFR [14%], BRAF [10%], MET [7%], and RIT1 [2%]) and tumor suppressors (TP53 [46%], STK11 [17%], KEAP1 [17%], NF1 [11%], RB1 [4%], and CDKN2A [4%]). Chromatin-modifying genes, SETD2 (9%), ARID1A (7%), and SMARCA4 (6%), and mutations in RNA splicing genes, RBM10 (8%) and U2AF1 (3%), were also identified (all gene names with symbols discussed can be found in Table S1). Figure 1A shows the frequency of molecular alterations that will be discussed in this review as established or emerging therapeutic targets. Common alterations in key pathways were also identified: mitogen-activated protein kinases (MAPK) activation (76%); phosphatidylinositol 3-kinases (PI3K)–AKT–mammalian target of rapamycin (MTOR) activation (25%); TP53 alteration (63%); cell-cycle regulation dysfunction (64%); oxidative stress pathway modification (22%); and mutations in chromatin or RNA splice factors (49%).\(^9\) As a growing number of these genes and pathways are therapeutically targetable, identification of genomic alterations in an individual tumor should predict which therapy is more likely to elicit a response.

Squamous cell lung carcinoma

SCC is a distinct subtype of NSCLC occurring in approximately 30% of cases and is the second most common type of NSCLC behind adenocarcinoma. Common driver mutations dictating therapeutic selection — such as EGFR and ALK — while prominent in adenocarcinoma, are rarely found in SCC and targeting agents for these mutations are mostly ineffective in SCC.\(^5,6\) Until recently, the molecular drivers of SCC remained unknown and few targeting agents have been in development. In 2012, TCGA published a study profiling the genetics of 176 SCC samples and found that SCC had a high mutation rate of 8.1 mutations per megabase with the most frequent mutation found in TP53 (83%).\(^12\) Additionally, they found nine other significant mutated genes: CDKN2A (15%); PTEN (8%); PIK3CA (16%); KEAP1 (12%); MLL2 (20%); HLA-A (3%); NFE2L2 (15%); NOTCH1 (8%); and RB1 (7%). Figure 1B shows the frequency of molecular alterations that will be discussed in this review as established or emerging therapeutic targets. Notably, these mutations represent a set of frequently mutated pathways including cell-cycle control, oxidative stress, cell survival, apoptotic control, and squamous cell differentiation. This study suggested that ~64% of the cases contained a targetable genomic alteration (defined
by a US Food and Drug Administration-approved agent and the mutation present in the RNA). These data provide important information regarding the molecular drivers of lung SCC and allow for the development of targeted therapeutic opportunities.

**Drug targets and precision treatment strategies**

**Standard of care**

While targeted therapies are approved for treatment of the small population of adenocarcinoma patients carrying specific mutations, the bulk of treatment options for patients with SCC, large-cell carcinoma, and LAC are focused upon standard treatment with cytotoxic drugs and surgical resection. Pathological diagnosis and staging are critical in determining the course of treatment. Current treatment recommendations indicate surgical resection without chemotherapy for patients with early-stage, nonmetastatic disease (stage IA–IB), surgical resection with postoperative chemotherapy for patients with local metastatic disease (stage IIA–IIIB), and chemotherapy for patients with nonresectable, metastatic NSCLC (stage IV). Platinum-based chemotherapy (cisplatin, carboplatin), combined with antimetotics (vinorelbine, vinblastine, docetaxel, and paclitaxel) or antimebolites (gemcitabine, pemetrexed), is the treatment of choice for patients with stage IIA–IV, shown to provide significant overall survival benefits in several clinical trials. Concurrent thoracic radiation therapy for patients has been found to have benefit for patients with metastatic disease, and is particularly beneficial for patients harboring brain metastasis. More recent data found that a combination of cisplatin with pemetrexed provided improved survival for patients with nonsquamous NSCLC histology (LAC, large-cell carcinoma), compared to cisplatin with gemcitabine, leading to the current recommendation of this combination for nonsquamous tumors. As such, defining the NSCLC histological subtype through pathological review and the routine application of immunohistochemical staining for specific markers of SCC (p63, cytokeratins 5 and 6) and LAC (thyroid transcription factor-1, napsin A, cytokeratin 7) has become the standard of care.

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**Figure 1** Frequency of selected molecular alterations in lung adenocarcinomas and squamous cell carcinomas.

**Notes:** The frequency of selected molecular alterations as reported in The Cancer Genome Atlas for (A) lung adenocarcinomas (230 samples) and (B) squamous cell carcinomas (178 samples). Red represents gene amplification, blue represents homozygous deletion, green represents mutation, and a black triangle represents a gene fusion. This figure is adapted from an OncoPrint figure generated at [http://www.cbioportal.org](http://www.cbioportal.org).

**Abbreviations:** TP53, tumor protein p53; EGFR, epidermal growth factor receptor; STK11, serine/threonine kinase 11; ALK, anaplastic lymphoma receptor tyrosine kinase; RET, ret proto-oncogene; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PTEN, phosphatase and tensin homolog; CDKN2A, cyclin-dependent kinase inhibitor 2A; FGFR1, fibroblast growth factor receptor 1.
Selective clinical trials for eGFR-resistant NSCLC

Table 1

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Note: *Identifier from ClinicalTrials.gov.

Abbreviations: eGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor; Hsp90, heat shock protein 90; PI3K, phosphatidylinositol 3-kinases; MTOR, mammalian target of rapamycin; HDAC, histone deacetylase inhibitor.

**Anaplastic lymphoma receptor tyrosine kinase (ALK)**

ALK is a receptor tyrosine kinase in the insulin receptor superfamily. Although its role in normal tissues is not well characterized, activating mutations, transforming rearrangements leading to gene fusions (most common), and resistance to EGFR TKIs, resulting in increased kinase affinity for ATP, thus decreasing the sensitivity to ATP-competitive inhibitors. Several second-generation EGFR TKIs have been developed to combat T790M resistance. These second-generation EGFR TKIs differ from erlotinib and gefitinib by being both mutant-selective and irreversibly binding into the ATP pocket of EGFR. Several of these second-generation EGFR TKIs have entered the clinic (Table 1), demonstrating significant responses rates and progression-free survival as a first-line treatment, as well as showing promise in patients with acquired resistance. Alternative pathways have been clinically validated to cause resistance, such as activation of BRAF, allowing for continual activation of downstream effectors despite EGFR inhibition. MET expression after TKI resistance has been proposed to downregulate the expression of BIM (BCL2L11), which is involved in apoptosis. The epithelial–mesenchymal transition, characterized by a loss of E-cadherin expression and the increased expression of fibronectin and vimentin, is associated with EGFR TKI resistance. The transition to small-cell lung cancer from TKI-resistant EGFR adenocarcinoma has been demonstrated. Although less commonplace than the EGFR T790M mutation, therapeutic approaches and clinical trials are ongoing (Table 1) to overcome these resistance mechanisms.
aberrant expression of the ALK gene have been described in several different tumor types: anaplastic large-cell lymphoma; neuroblastoma; glioblastoma; colorectal cancer; ovarian cancer; inflammatory myofibroblastic tumor; and NSCLC.\textsuperscript{6,39–41} In NSCLC, ALK rearrangements produce an inversion in which one of several possible 5’ fusion partners and its promoter region are moved upstream of the ALK kinase domain, resulting in a fusion gene.\textsuperscript{42,43} In 2%–7% of NSCLC patients, the inversion event fuses ALK with ephidermoderm microtubule-associated protein-like 4 (EML4), though fusions with KIF5B and TFG have also been described.\textsuperscript{6} The resulting fusion gene encodes a protein with a ligand-independent, constitutively active kinase domain capable of driving tumor progression, proliferation, survival, and migration through the downstream activation of the MAPK, JAK–STAT, and PI3K/AKT pathways.\textsuperscript{6,42–44} The oncogenic capabilities of EML4–ALK have been described in vitro and in vivo, and they have been successfully suppressed by certain small-molecule inhibitors to ALK.\textsuperscript{43,45,46}

These characteristics have led to the clinical application of small molecules to inhibit EML4–ALK fusions present in some LAC patients. Crizotinib (PF-02341066), a clinically approved, well-tolerated small-molecule inhibitor to the tyrosine kinase activity of ALK and hepatocyte growth factor receptor (HGF/MET) is the first ALK inhibitor developed, and it remains a standard TKI for ALK-positive patients.\textsuperscript{5,47} A clinical study by Kwak et al.\textsuperscript{48} involving 82 patients with ALK-positive NSCLC evaluated the effectiveness of ALK inhibition with crizotinib, where 57% of patients showed a complete or partial response (14/26 complete; 45/46 partial); a partial response by Response Evaluation Criteria in Solid Tumors (RECIST) criteria was defined by at least a 30% change in tumor burden, while 33% (27 patients) had stable disease. A Phase I trial involving 149 ALK translocation-positive NSCLC patients showed a reduction in tumor size by >90%, with 61% displaying an objective response.\textsuperscript{49} ALK-positive NSCLC patients have shown both increased progression-free survival and response rates with crizotinib in Phase I and II clinical trials.\textsuperscript{50} As crizotinib inhibits multiple tyrosine kinases, it is also being used clinically to treat adenocarcinomas that harbor a ROS1 rearrangement, a genomic aberration observed in 2% of NSCLC patients.\textsuperscript{51}

To date, numerous second-generation small-molecule inhibitors of ALK are currently being developed or are undergoing clinical trials to improve efficacy and combat crizotinib resistance (Table 2). Ceritinib (LDK378), clinically approved in 2014, is a second-generation ALK inhibitor shown to overcome crizotinib resistance in preclinical and Phase I clinical trials of NSCLC patients harboring ALK rearrangements.\textsuperscript{42,49} In a recent Phase I/II clinical trial,\textsuperscript{52} crizotinib-naïve NSCLC patients with ALK rearrangements were treated with alectinib (CH5424802), a well-tolerated, selective ALK inhibitor with 93.5% having an objective response. A dual ALK and EGFR inhibitor, AP26113 showed anti-ALK kinase activity in a Phase I/II study.\textsuperscript{53} Inhibitors of heat shock protein 90 (Hsp90), a chaperone protein involved in ALK synthesis, have shown promise in reducing ALK protein levels in preliminary studies of ALK rearranged NSCLC.\textsuperscript{42} Specifically, ganetespib (STA-9090), a Hsp90 inhibitor, has shown efficacy in a Phase IIb/III study in combination with docetaxel.\textsuperscript{42}

### The ret proto-oncogene (RET)

RET, the ret proto-oncogene of the cadherin superfamily, encodes a receptor tyrosine kinase involved in neural crest development, growth, and differentiation.\textsuperscript{54} RET mutations are implicated in several different diseases including multiple endocrine neoplasia (types IIA and IIB), Hirschsprung disease, medullary thyroid carcinoma, and NSCLC.\textsuperscript{51}

In a study involving 1,876 patients with lung carcinomas, fluorescent in situ hybridization and reverse transcriptase polymerase chain reaction were used to detect RET gene rearrangements. A total of 1.2% (number = 22) of cases were found to be positive for RET rearrangement, and all cases were LAC.\textsuperscript{60} RET rearrangement is correlated with younger patients (<60 years of age), adenocarcinomas with no other known oncogenic drivers, small primary tumors, and a history of nonsmoking.\textsuperscript{51–53} In NSCLC, chromosomal rearrangements result in the fusion of RET’s C-terminal region to the N-terminal of several proteins (KIF5B, CCD6, NCOA4, TRIM33), resulting in constitutive activation of the RET kinase domain\textsuperscript{51} and oncogenic activity.\textsuperscript{53} In 19

### Table 2 Selected clinical trials using ALK inhibitors

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*Identifier from ClinicalTrials.gov.

**Abbreviations:** ALK, anaplastic lymphoma receptor tyrosine kinase; EGFR, epidermal growth factor receptor; Hsp90, heat shock protein 90.
of the 22 cases, RET was fused with KIF5B, and in 3/22 cases, the fusion partner was CCD6.53 KIF5B is the most prevalent fusion partner, though RET fusions with NCOA4 and TRIM33 have been identified.52,54

To date, limited treatment options are available for patients harboring RET rearrangements. Carbozantinib (XL-184), a multi-TKI and RET inhibitor, is undergoing Phase II clinical trials to determine its efficacy in NSCLC patients with RET fusion-positive advanced NSCLC (NCT01639508). Clinical data available on the first three patients treated with carbozantinib indicated a partial response in two of the three patients, and one had prolonged stable disease for 31 weeks. All three were progression free during treatment.31

Vandetanib is a RET/VEGF/EGFR inhibitor approved for the treatment of medullary thyroid cancer. It was shown to decrease metastasis size and led to remission in a 58-year-old patient with metastasized LAC.53 A Phase II clinical trial is currently recruiting patients to study the safety and efficacy of vandetanib in advanced NSCLC patients with RET gene rearrangements (NCT01823068). Other small-molecule inhibitors currently being investigated for efficacy in RET-positive LAC patients include ponatinib (Phase II), levatinib/E7080 (Phase II), MGCD516 (Phase I/IIb), and sunitinib (Phase II).

Defined molecular targets in SCC

While molecular targets for SCC have been limited in the past, current research has identified several notable targets including the fibroblast growth factor receptor (FGFR) family kinases and the PI3K/AKT pathway.

Fibroblast growth factor 1 (FGFR1)

FGFR are a family of tyrosine kinases that, under normal cellular function, play an important role in development, angiogenesis, and proliferation.55 Of the four FGFRs (FGFR1–4), FGFR1 has been found to be frequently deregulated in SCC by amplification or receptor activation.55,56 One study found amplifications in chromosome 8p12 in 22% of SCC patients.56 Additionally, FGFR1 amplification and high serum basic fibroblast growth factor (bFGF) levels have been associated with poor prognosis57,58 and increased proliferative rate.59 The oncogenic role of FGFR1 was demonstrated in preclinical studies of FGFR1-amplified cell lines. Inhibition of FGFR1 signaling through decreased FGFR1 expression via FGFR1-specific small hairpin (sh) RNA,56 or the inhibition of bFGF through neutralizing mAbs,59 results in growth inhibition. Similarly, multitargeted small-molecule inhibitors like nintedanib,60 ponatinib,61 and the FGFR-specific inhibitor AZD454762 have demonstrated antitumor and antiangiogenic effects in FGFR-amplified preclinical studies, which has led to clinical studies of FGFR1 inhibitors (Table 3).

Nintedanib (BIBF 1120) is a multitargeting TKI that blocks vascular endothelial growth factor receptor, platelet-derived growth factor receptor, and FGFR signaling.60 It is currently in a Phase I/II clinical trial (NCT01346540) in combination with platinum-based chemotherapy for recurrent SCC NSCLC patients. A completed Phase I trial employing nintedanib with carboplatin/paclitaxel in advanced NSCLC found partial responses in 27% of patients; however, two of three patients with squamous histology responded.61 Ponatinib is another multi-targeting TKI, initially developed to target aberrant BCR-ABL, but has since been found to also inhibit FGFR and preferentially inhibit growth of FGFR1-amplified primary lung cancer cells.64 Ponatinib is currently in Phase II/III clinical trials (NCT01761747) for SCC NSCLC or for SCC of the head and neck with confirmed FGFR1 amplifications; however, this trial has currently suspended enrollment due to an increased risk of blood clots.

AZD4547 is a TKI specific to FGFR1–4, but it also results in mild inhibition of VEGF4.62 In preclinical models, AZD4547 showed cytotoxic and cyostatic effects in cell lines with FGFR1 amplifications.62 A current Phase II clinical trial (NCT01795768) is examining AZD4547 in breast, squamous lung, and stomach cancers with FGFR1 or 2 amplifications. This trial is specifically looking at tumor growth and tumor ERK1/2 phosphorylation as a proof-of-concept study. Notably, a closed Phase I clinical trial (NCT00979134) found partial response in lung SCC patients with high FGFR amplification and mild, reversible adverse effects to AZD4547 treatment.

**Table 3** Selected clinical trials for FGFR1 in SCC

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**Note:** *Identifier from ClinicalTrials.gov.

**Abbreviations:** FGFR1, fibroblast growth factor receptor I; SCC, squamous cell carcinomas; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor.
Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA)

The PI3K are a family of lipid kinases that are generally activated by receptor tyrosine kinases and contribute to cell proliferation, growth, and differentiation. The kinases are heterodimers consisting of a 58 KDa regulatory subunit (PIK3R1, 2, or 3) and a 110 KDa catalytic subunit (PIK3CA, B, or D) which, when mutated, are implicated in a number of cancers including lung SCC. Upon activation, PI3K functions through activating the AKT/MTOR pathway to drive cell growth and survival. The tumor suppressor, PTEN, is one of the main antagonistic regulators of the PI3K, and mutational inactivation of PTEN leads to hyperactivation of PI3K signaling and increased cell growth. Deletion of PTEN and LKB1 in GEMMs induces NSCLC tumors of SCC histology. Current TCGA data revealed mutations or amplifications in the PI3K/AKT pathway in 43% of lung SCC samples including 16% with altered PIK3CA, 16% AKT3 mutations, and 15% PTEN alterations. Additionally PIK3CA is amplified in 33%–43% of SCC cases. However, because of the high mutation rate of SCC, it is possible that many of these mutations are passenger mutations rather than drivers. One study found that samples with alterations in PIK3CA had no other common driver mutations (eg, KRAS and EGFR), thus indicating a potential therapeutic response from targeting the PI3K pathway in SCC.

PI3K inhibitors have been developed for clinical trials in other cancers, but yet they are still limited for lung SCC. Current PI3K inhibitors including pan-PI3K inhibitors, isofrom-specific PI3K inhibitors, AKT inhibitors, MTOR inhibitors, and dual PI3K/MTOR inhibitors are currently in clinical trials (Table 4), and they are being evaluated alone and in combination with standard platinum-based chemotherapy treatments.

Two pan-PI3K inhibitors, buparlisib (BKM120) and PX-866, are being evaluated in SCC lung cancer.

Table 4 Selected clinical trials for PIK3CA in SCC

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Note: *Identifier from ClinicalTrials.gov.

Abbreviations: PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; SCC, squamous cell carcinoma; PI3K, phosphatidylinositol 3-kinases.

The pan-PI3K inhibitor buparlisib binds to the ATP-binding site in the lipid kinase subunit of all PI3K isoforms; in preclinical trials, it has shown antiangiogenic and antiproliferative effects preferentially in PIK3CA mutated cell lines. Phase I trials in Japanese patients with advanced solid tumors demonstrated stable disease and partial responses to buparlisib treatment alone. Phase II trials have been initiated in patients with pretreated metastatic NSCLC (including SCC) with an activated PI3K pathway; however, no results have been reported (NCT01297491, NCT01820325). PX-866 is an irreversible pan-class I PI3K inhibitor that has shown lasting PI3K inhibition and antitumor effects in vivo in PIK3CA-mutated SCC of the head and neck. In Phase I and II clinical trials for PX-866, alone or in combination, for patients with advanced solid tumors, it was found that 79%–85% of patients had stable disease for the period of the trial. However, these trials saw no correlation between PIK3CA status and response rate. An ongoing Phase I and II clinical trial (NCT01204099) in NSCLC and SCC of the head and neck is evaluating the response of PX-866 and docetaxel combination therapy. Preliminary data from this study found that the treatment was well tolerated, and patients with PIK3CA mutations maintained progression-free disease longer than those with KRAS or both KRAS and PIK3CA mutations.

The alpha class I PI3K isoform-specific PI3K inhibitor, BYL719, may be a promising treatment for patients with PIK3CA mutations and copy number gain. In initial clinical trials (NCT01219699), BYL719 was well tolerated and showed preliminary efficacy in patients with PIK3CA-mutated solid tumors. Although no clinical trials are underway for lung SCC patients, there are studies in progress for previously treated head and neck SCC (NCT01602315) and esophageal SCC (NCT01494058). However, preclinical data also found that although PIK3CA-mutated cell lines are sensitive to the treatment, PTEN inactivation is associated with insensitivity to BYL719, indicating that patients with lung SCC may benefit from a pan-PI3K inhibitor due to the frequency of PTEN mutations.

Emerging targets in LAC

Kirsten rat sarcoma viral oncogene homolog (KRAS)

Across 230 primary LAC, 33% were observed to harbor a KRAS mutation by NGS in TCGA. As such, KRAS mutation represents one of the most frequent alterations in this tumor type. KRAS mutations are prevalent in other malignancies such as pancreatic cancer; where KRAS mutations are present in up to 95% of cases. As a guanosine triphosphatase...
(GTPase), KRAS functions as a molecular switch that, once activated, functions to propagate signal transduction pathways. KRAS signaling associates with numerous tumor-related signaling pathways including MAPK signaling, PI3K/akt signaling, and RAC and RAL signaling. The prevalence of KRAS mutations in many tumor types, including LAC, has made it an attractive therapeutic target. However, despite early hopes based upon in vitro and in vivo experiments using mutants of the KRAS homolog, HRAS, direct targeting of KRAS has been unsuccessful to date. More recently, a better understanding of mutant KRAS signaling and KRAS function has led to novel therapeutic strategies for this molecular subgroup.

To date, KRAS mutation status has not been proven to be prognostic to treatment with adjuvant chemotherapy. There are, however, multiple clinical trials that are recruiting and running, and which are targeting lung tumors with activating KRAS mutations through the immunological targeting of mutant KRAS, RAS-related downstream signaling, and G2 checkpoint inhibitors (Table 5). In 2004, Lu et al. described a yeast-based immunotherapy in which yeast expressing mutant RAS proteins could illicit tumor killing in lung cancers harboring the KRAS mutation. The use of this immunogenic therapy (GI-4000) is currently in clinical trial for KRAS-mutated lung cancer (NCT00655161). A Phase II study of the GI-4000 KRAS vaccine in patients with LAC harboring common KRAS mutations demonstrated that GI-4000 could be tolerated and elicit an immunogenic response in patients. Another therapeutic avenue to combat KRAS-driven lung cancer is the inhibition of downstream signaling pathways – more specifically, MAPK and PI3K. In vivo studies with KRAS-driven lung tumorigenesis have demonstrated therapeutic responses with combinations of MEK inhibitors and PI3K inhibitors. In 2008, Engelman et al. demonstrated synergistic tumor shrinkage in mutant KRAS cancers employing NVP-BEZ235, a PI3K and Mtor inhibitor, and ARRY-142886, a MEK inhibitor. There are currently several clinical trials exploring both the safety and efficacy of combinations of MEK inhibitors and PI3K inhibitors.

In vivo mouse studies have highlighted other therapeutic combinations with MEK inhibition that are capable of suppressing KRAS-driven tumorigenesis, including MEK with BCLXL inhibitors, MEK and insulin-like growth factor 1 receptor inhibitors, and MEK with JAK/TK1 inhibitors. However, it is important to consider the contributions of other tumor-associated mutations, as mutations to the STK11/LKB1 tumor suppressor reduced the efficacy of MEK inhibition, while TP53 did not within murine genetically engineered mouse models (GEMM) expressing mutant KRAS. Regardless, as the signaling pathways governed by KRAS are better understood, novel therapeutic strategies will continue to improve responses against KRAS-driven lung tumorigenesis.

A third avenue to inhibit KRAS-driven tumorigenesis employs G2 checkpoint inhibitors. The G2/M checkpoint serves to ensure DNA integrity prior to the cell entering mitosis. The inhibition of proteins that govern the G2 checkpoint such as ATM, CHK1/2, PLK1, and WEE1 has attracted significant interest toward cytotoxic treatment. PLK1 is overexpressed in KRAS-mutant tumors, and these tumors were sensitive to PLK inhibitors. Recently, Weisberg et al. demonstrated that WEE1 inhibition combined with Mtor inhibitors selectively inhibited tumors that were positive for mutant RAS. The WEE1 inhibitor AZD1775 is currently in clinical trials in combination with DNA-damaging agents in pancreatic cancer, which is a KRAS-driven tumor type (NCT02037230). Lastly, the use of Hsp90 inhibitors in KRAS-driven tumorigenesis is under investigation. In vivo studies have suggested that mutant KRAS tumors are sensitive to Hsp90 inhibitors. Of interest, the efficacy of an Hsp90 inhibitor in NRAS-driven melanoma was dependent on inhibition of WEE1. Thus, a better understanding of the role of mutant RAS in the regulation of G2 and DNA damage checkpoints may facilitate new therapeutic strategies in RAS-driven tumors.

Table 5 Selected clinical trials for mtKRAS tumors

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<td>DNA damage</td>
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<td>NCT0207940</td>
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Note: *Identifier from ClinicalTrials.gov.

Abbreviations: mtKRAS, activating KRAS mutations; Hsp90, heat shock protein 90; Mtor, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinases; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor.
Tumor protein p53 (TP53)

The tumor suppressor, TP53, is one of the most frequent genes mutated in cancer. TP53 is altered (mutated or deleted) in 46% of LAC and 90% of SCC according to TCGA. While the frequency of TP53 alteration is well recognized, therapeutic options based on this alteration have been scarce in lung cancer. One reason for this is an incomplete understanding of TP53 biology in the context of TP53 mutation. TP53 is mutated across the entire coding sequence of the gene leading to everything from TP53 deletions to oncogenic, gain-of-function mutations. In situations where the TP53 protein is deleted, reintroduction of the wild type gene has been considered as a therapeutic strategy. Gene transfer of wild type TP53 by retroviral vector was used in lung cancer clinical trials as early as 1996. This Phase I study showed no toxic effects of the vector, and tumor regression was noted in three of nine patients. Since then, a number of clinical studies have attempted to reestablish wild type TP53 function by gene transfer.

Small molecules that inhibit the growth of cancer cells harboring TP53 mutations are also being explored. PRIMA-1 selectively inhibits the growth of mutant TP53 cells by restoring TP53 to a wild type conformation. This restoration improves the therapeutic efficacy of DNA-damaging agents such as cisplatin, radiation, and other chemotherapeutic drugs. The safety and effectiveness of APR-246, an analog of PRIMA-1, is currently under investigation in clinical trials (NCT02098343) (Table 6).

Another way in which mutant TP53 is being targeted therapeutically is the development of tumor vaccines. The ability to therapeutically harness the host immune response is under intense investigation toward controlling tumor growth. Tumor vaccines that attempt to use tumor-specific antigens to activate the immune system to target tumor cells are a therapeutic approach that have long been under development. For TP53, it was demonstrated that vaccines could be produced against mutant TP53 and effectively inhibit tumor growth in vivo more than 15 years ago. The targeting of mutant TP53 epitopes or TP53 overexpression in tumors by vaccine has shown promise in a number of in vivo settings. Currently, TP53 vaccines such as p53-SLP are in clinical trials. The use of immunotherapy targeting TP53 in combination with cytotoxic chemotherapy is now under investigation (Table 6). More recently has been the development of therapeutics (anti-CTLA4 and anti-PD-1/PD-L1 antibodies), which are designed to inhibit immune blockade observed in tumors, thus enhancing the immune response. Several clinical trials utilizing this therapeutic approach are currently underway, and early results are promising. Clinical trials specific to lung cancers currently employ anti-CTLA4 antibodies (Ipilimumab) in combination with radiation (NCT02221739) or platinum-based therapies (NCT01331525). The anti-PD-1 antibodies, nivolumab (BMS-936558) or pembrolizumab (MK-3475), are being used in clinical trials alone (NCT02259621) or in combination with chemotherapy (NCT02039674) in lung cancer patients. However, as with other targeted therapies, biomarkers for these targets would be invaluable, allowing the tailoring of anti-CTLA4 or anti-PD-1/PD-L1 therapies toward patients who would benefit most.

As is the case with mutant KRAS tumors, a mechanistic understanding of mutant TP53 signaling is also being exploited in lung cancer therapeutics. TP53 is a significant player in the DNA damage response pathway in cells, suggesting that inhibition of the DNA damage response checkpoint proteins may have therapeutic value in mutant TP53 settings. The WEE1 inhibitor, AZD1775 (formerly MK1775), displays preferential effectiveness in mutant TP53 cell lines. A current clinical trial is recruiting ovarian cancer patients with mutated TP53 to be treated with AZD1775 and carboplatin (NCT01164995).

Serine/threonine kinase 11 (STK11)

STK11/LKB1 (serine/threonine kinase 11/Liver kinase B1) was originally identified as the causative gene mutated in the familial cancer disease, Peutz–Jeghers’ syndrome. Subsequent investigations for sporadic LKB1 mutations found a high prevalence for LKB1 inactivation in NSCLC relative to other solid tumors. More recent TCGA analyses of SCC and LAC tumors show that DNA mutations to LKB1 are primarily a characteristic of LAC. The detection of LKB1 inactivation has been difficult, with studies placing the frequency of LKB1 mutations between 15%–30% of LAC tumors and homozygous deletion, and the loss of heterozygosity of the LKB1 locus of chromosome 19p at 89%, suggesting that the true frequency of LKB1

Table 6 Selected clinical trials targeting TP53

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Note: *Identifier from ClinicalTrials.gov.

Abbreviations: TP53, tumor protein p53; NSCLC, non-small cell lung cancer.
inactivation in LAC is high. LKB1 inactivation commonly occurs in concert with activating KRAS mutations (mtKRAS) (10% of patients), and mtKRAS synergizes with the biallelic deletion of LKB1 in GEMMs to produce highly metastatic, aggressive tumors in the lung\(^\text{107}\) that are genetically distinct from other NSCLC tumors harboring mtKRAS alone.\(^\text{110}\)

Although the broad regulatory functions of LKB1 are still being elucidated,\(^\text{108}\) efforts have been made to define possible treatment options using a well-characterized GEMM of mtKRAS/LKB1-deficiency (mtKRAS/LKB1null).\(^\text{87,107,110–112}\)

Using gene expression analysis of GEMM mtKRAS/LKB1null NSCLC tumors, Carretero et al\(^\text{110}\) identified and demonstrated that the SRC kinase is a putative target in mtKRAS/LKB1null NSCLC and when combined with inhibitors to the RAS–MAPK and PI3K–AKT pathways, SRC inhibition induced significant tumor regression. Perhaps more interestingly, GEMM mtKRAS/LKB1null NSCLC tumors display increased activation of the PI3K pathway with reduced activation of the RAS–MAPK pathway, which is thought to manifest into resistance to MEK inhibition.\(^\text{87}\) Similarly, synthetic lethal RNA interference (RNAi) screens using tumor cell lines generated from GEMM mtKRAS/LKB1null NSCLC tumors identified Dymk, an enzyme responsible for dTTP biosynthesis, as a potential target in LKB1-deficient NSCLC.\(^\text{113}\)

Concurrent to these efforts are studies aimed toward taking advantage of hypersensitivity to stress present within LKB1-deficient cells. Substantial data indicate that inactivation of LKB1 renders cells unable to respond to stress resulting from a variety of sources.\(^\text{111,114,115}\) In particular, there is an appreciation that LKB1 is critical in mediating the effects of metformin and its analogs.\(^\text{105,116}\) Recruitment is underway to assess whether LKB1 gene status will determine the response to metformin plus standard therapy in LAC (clinical trial NCT01578551). Critically, treatment with phenformin, a more potent analog of metformin, significantly perturbs the growth of tumors and improves survival in the mtKRAS/LKB1null NSCLC GEMM, compared to GEMMs harboring mtKRAS alone or mtKRAS with TP53 deletion.\(^\text{112}\) In our laboratories, we found that mtKRAS/ LKB1null NSCLC cells are hypersensitive to the disruption of protein synthesis within the endoplasmic reticulum (ER), and subsequent activation of ER stress responses by an aggravator of ER stress can reduce NSCLC tumor growth in the mtKRAS/ LKB1null NSCLC GEMM.\(^\text{111}\) Given the potentially high incidence of LKB1 inactivation in LAC, the next step will be to develop and validate these approaches within the mtKRAS/ LKB1null NSCLC GEMM toward patient clinical trials.

Other molecular targets in LAC

While a significant amount of attention has been paid with respect to EGFR and ALK toward precision medicine strategies in LAC, other molecular targets are being explored. Figure 2 depicts selected molecular alterations in LAC with

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**Figure 2** Molecular alterations in lung adenocarcinomas with targeted therapeutic opportunities.

**Abbreviations:** RET, ret proto-oncogene; EGFR, epidermal growth factor receptor; mAbs, monoclonal antibodies; TKIs, tyrosine kinase inhibitor; ALK, anaplastic lymphoma receptor tyrosine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; PI3K, phosphatidylinositol 3-kinases; LKB1, serine/threonine kinase 11; p53, tumor protein p53; Hsp90, heat shock protein 90; MTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase.
targeted therapeutic opportunities. Mutations in *BRAF* occur in ~10% of adenocarcinoma patients according to TGCA.\textsuperscript{10,11} The prevalence of mutant *BRAF* in melanoma led to *BRAF* inhibitor development with clinical successes in *BRAF* mutant cases.\textsuperscript{117} LAC cases with mutant *BRAF* (V600E) have responded to the *BRAF* inhibitor, vemurafenib.\textsuperscript{118} Genomic alterations (mutations and amplifications) in hepatocyte growth factor receptor (*HGFR/MET*) occur in 12% of LAC cases.\textsuperscript{11} The amplification of *MET* is a known mechanism of EGFR TKI resistance, as well as a risk factor for metastasis.\textsuperscript{119,120} Inhibitors against *MET* are under clinical development toward the treatment of patients with aberrant *MET* expression.\textsuperscript{119,120} In particular are ongoing clinical trials to determine the safety of the *MET* inhibitor, RO5490258, also known as MET-Mab, in NSCLC (NCT01496742).

**Emerging molecular targets in SCC**

While FGFR and *PI3K* inhibitors are being explored clinically in squamous cell lung cancer, other molecular alterations may provide therapeutic opportunities. Figure 3 depicts frequent alterations in SCC with targeted therapeutic opportunities. The high prevalence of *TP53* mutations in this histologic subtype may dictate the use of *TP53* therapeutics, as has been discussed. The frequency of genomic alterations (mutation or amplification) in *EGFR, ERRB2, KRAS, BRAF*, and *MET* ranges between 3%–9% in squamous cell lung cancer.\textsuperscript{12} Though modest, these patient tumors could be treated with targeted therapeutic strategies discussed previously in this review. A more thorough understanding of the molecular drivers of squamous cell lung cancer is going to be necessary to improve precision medicine-based therapeutic strategies.

**Conclusion**

NSCLC remains a leading cause of cancer-related mortality in the US and throughout the world. New genomic technologies have begun to shed light on the genomic alterations and pathways that drive NSCLC and provide rationale for therapeutic intervention based on genomic aberrations. Despite demonstrated successes using genomic alterations to dictate therapy (such as *EGFR* mutations in NSCLC), there are significant challenges associated with the clinical use of precision medicine. First, while cost and time-to-results have improved, they are still hurdles to clinical adoption. Second, tumor heterogeneity presents a challenge to precision medicine, as the tumor piece providing the genomic alterations may not fully represent the bulk tumor. This has been cited as a potential concern for therapeutic resistance.\textsuperscript{121} Strategies for validation have been noted as a potential complication for the clinical adoption of NGS. Another challenge posed by the use of whole-genome sequencing in the clinical setting is providing genomic alterations (both somatic and germline)
that are not currently actionable targets, or deciding which of several alterations to target.

While success has been demonstrated in LAC patients with mutated EGFR or gene rearrangements in ALK, therapeutic resistance is pervasive in these subgroups. More importantly, the majority of NSCLC patients do not harbor either of these alterations, indicating a demonstrable need to develop novel therapies that address the bulk of NSCLC tumors. Novel therapeutic strategies are currently in preclinical and clinical development toward targeting recurrent alterations in NSCLC, such as TP53 and KRAS, as well as targeting specific resistance phenotypes. With improvements in our understanding of the molecular tumorigenesis in NSCLC, heavily influenced by next-generation sequence technologies and improved targeted therapeutics, the future of precision medicine in NSCLC should significantly impact NSCLC mortality.

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Disclosure
The authors report no conflicts of interest in this work.

References


## Supplementary material

### Table S1 HUGO approved name and symbol

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<tr>
<td>WEE1 G2 checkpoint kinase</td>
<td>WEE1</td>
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</tr>
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**Abbreviation:** HUGO, Human Genome Organisation.