New Strategies in Squamous Cell Carcinoma of the Lung: Identification of Tumor Drivers to Personalize Therapy

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ABSTRACT
Treatment for non-small cell lung cancer has been improving, with personalized treatment increasingly becoming a reality in the clinic. Unfortunately, these advances have largely been confined to the treatment of adenocarcinomas. Treatment options for squamous cell carcinoma of the lung have lagged behind, partly due to a lack of understanding of the oncogenes driving squamous cell carcinoma. Cytotoxic chemotherapy continues to be the only treatment option for many of our patients, and there are no genetic tests that are clinically useful for patients with squamous cell carcinoma. Recent basic science advances have identified mutations and alterations in protein expression frequently found in squamous cell carcinomas, and clinical trials are ongoing to target these changes.

BACKGROUND
Squamous cell carcinoma (SCC) accounts for about 30% of new cases of lung cancer in the United States. The patterns of incidence have changed over the decades as SCC has become less common, though it is still estimated to account for about 40,000 deaths yearly in the United States (1). This is thought to be due to decreased smoking rates as well as changes in the content of cigarettes. Until recently, SCC and adenocarcinoma had very similar overall survival; more recent analyses have shown that outcomes for metastatic adenocarcinoma are improved compared with patients with metastatic SCC (2), possibly due to new treatment options for adenocarcinoma.

For patients with localized or regional disease, treatment options differ very little between SCC and other subtypes of non-small cell lung cancer (NSCLC). Patients without involvement of mediastinal lymph nodes should undergo surgical evaluation, with consideration for adjuvant chemotherapy after resection (3). Patients with locally advanced disease, stage IIIA or IIIB, should be treated with multi-modality therapy, often incorporating chemotherapy and radiation with or without surgery. These decisions, for the most part, are made without regard to histology.

Unfortunately, many patients with NSCLC present with metastatic disease, or develop metastatic disease following local treatment. The past decade has seen a number of improvements in the treatment of metastatic NSCLC. For patients with epidermal growth factor receptor (\textit{EGFR}) mutations or anaplastic lymphoma kinase (\textit{ALK}) translocations, targeted therapies now represent standard front-line therapy (4, 5). A modern cytotoxic agent, pemetrexed, is used in the first line (6), second line (7), and maintenance (8) settings for patients with non-squamous NSCLC. Bevacizumab, a monoclonal antibody against the vascular endothelial growth factor receptor (VEGFR), can prolong survival when added to chemotherapy (9). Unfortunately, all of these advances are more effective for patients with non-squamous NSCLC. \textit{EGFR} mutations and \textit{ALK} translocations are not commonly found in patients with SCC, and bevacizumab is associated with an unacceptable risk of pulmonary hemorrhage (10). Pemetrexed is not effective in patients with SCC (6-8) and should not be used for these patients. Thus far, the only targeted agent approved for use in SCC of the lung is erlotinib, which has modest activity in patients with previously treated disease (11).

For patients with SCC, the standard of care is still cytotoxic chemotherapy. Standard front-line chemotherapy consists of a platinum-based doublet (12), and second line therapy is often docetaxel (13) or erlotinib (11). Recent data indicate that similarly to adenocarcinomas, SCCs are clinically, histologically and molecularly heterogeneous tumors. For patients with SCC, research is ongoing to...
identify driver mutations (see figure 1) as well as targeted agents, including profiling and sequencing studies conducted as part of The Cancer Genome Atlas project (TCGA, US National Cancer Institute). This article will discuss some of the recent discoveries into the genetics of SCC as well as current and future research direction.

ON THE HORIZON

Cytotoxic Chemotherapy

Though much ongoing clinical research in lung cancer focuses on targeted agents, cytotoxic chemotherapy may play an important role in improving treatment. Paclitaxel is a frequently used drug in NSCLC treatment, but difficulties with administration include poor solubility and frequent reactions to the solvent used (cremaphor). To avoid some of these issues, nanoparticle albumin-bound paclitaxel (nab-paclitaxel) was created. There is also some pre-clinical evidence that albumin may increase drug delivery to tumors, possibly by interacting with SPARC (secreted protein, acidic and rich in cysteine) (14, 15). In a randomized phase III trial in women with breast cancer, nab-paclitaxel showed higher response rates and longer time to progression than solvent-based paclitaxel (16). Phase II trials of nab-paclitaxel in lung cancer as monotherapy (17) and in combination with carboplatin (18) have shown response rates as high as 39%. A phase III trial randomizing patients with NSCLC to carboplatin/nab-paclitaxel or carboplatin/paclitaxel showed a significantly higher response rate in the nab-paclitaxel arm (33% vs 25%, p=0.005). Preliminary estimates of progression free survival were similar between the two arms (19). Several ongoing phase II trials combining carboplatin with nab-paclitaxel (NCT00729612 and NCT01236706) will study a variety of biomarkers, including SPARC, caveolin-1, and serum microRNAs.

Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is expressed only at low levels in normal lung tissue but is over-expressed in preneoplasia and in many SCCs (20). Erlotinib, a small molecule EGFR inhibitor, has been approved for use in NSCLC (including SCC) in maintenance therapy (21) and in previously treated disease(11), but benefits are modest and similar to those of cytotoxic therapy (22), with response rates less than 10%. Gefitinib, another EGFR tyrosine kinase inhibitor, appears to have similar activity to erlotinib, though it is not currently available in the United States (4, 22). EGFR mutations in the tyrosine kinase domain that confer sensitivity to gefitinib and erlotinib are found in a significant percentage of adenocarcinomas (23-25). These activating EGFR mutations have also been described in several patients with SCC (24, 26), but it has been hypothesized that these patients have incompletely sampled adenosquamous carcinoma rather than pure SCC (27). About 5% of SCCs have a deletion in the extracellular domain of EGFR (variant III EGFR mutation), but these mutations confer resistance to EGFR inhibitors in in vitro studies (28).

Cetuximab is a monoclonal antibody against EGFR, and has proven efficacy in squamous cell carcinoma of the head and neck (29, 30). In the FLEX study, patients with metastatic NSCLC received cisplatin and vinorelbine with or without cetuximab. Survival was significantly longer in the group receiving cetuximab (11.3 months vs 10.1 months, p=0.044) (31). This trial enrolled all histologies of NSCLC and, on subgroup analysis, both patients with SCC and adenocarcinoma experienced benefit from the addition of cetuximab. A Southwest Oncology Group Study, SWOG 0819, is currently accruing
patients to receive carboplatin and paclitaxel with or without cetuximab. Patients who are eligible (ie, non-squamous histologies) can also receive bevacizumab at the clinician’s discretion. Overall survival is a primary endpoint, and other objectives include the prospective study of EGFR copy number by fluorescent in situ hybridization (FISH) as a predictive marker for progression free survival, as well as a variety of other EGFR-related biomarkers, including KRAS and EGFR mutations.

*Insulin-like Growth Factor-1 Receptor (IGF-1R)*

IGF-1R is a cell-surface receptor with an intracellular tyrosine kinase domain. Binding of its ligands, IGF-1 or IGF-2, activates the kinase domain and stimulates downstream signaling, including the PI3K-Akt-MTor pathway and the RAF-MAPK pathway, promoting cell proliferation and survival (32). IGF-1R plays an important role in carcinogenesis, contributing to cell growth, cell division, and protection from apoptosis. Aberrant IGF-1R signaling has been seen in many tumor types (33), and alterations in this pathway predict poor prognosis in early stage lung cancers (34).

A randomized phase II trial randomizing patients to carboplatin and paclitaxel with or without figitumumab, an anti-IGF-1R monoclonal antibody had promising results, with response seen in 78% of the patients with SCC treated with figitumumab (Pfizer ™) (35). Unfortunately, a phase III registration trial was recently closed for futility and there was a concern for an increased risk of early death (36).

Small molecule IGF-1R inhibitors are also being actively studied. It is hoped that these agents have efficacy without some of the toxicities of anti-IGF-1R antibodies. In a phase II study (NCT01186861), erlotinib with or without OSI-906 (Astellas™), an inhibitor of IGF-1R and the insulin receptor, is being studied in the maintenance setting in all histologic types of NSCLC. The primary endpoint is progression free survival. Biomarker analyses, including analysis of epithelial marker E-cadherin, will also be performed.

*Fibroblast Growth Factor Receptor (FGFR)*

The four members of the FGFR family have important roles in the regulation of cell proliferation and survival (37). Mutations in these receptors have been described in other malignancies (38). In SCC, amplification of FGFR1 has been seen in over 20% of SCCs, but only rarely in adenocarcinoma (39, 40). In preclinical models, small molecule inhibitors of this receptor can cause decreased cell growth in both cell lines and xenograft models (39, 40). A phase III study (NCT00805194) combining docetaxel with BIBF 1120 (Boehringer Ingelheim™), a tyrosine kinase inhibitor with activity against FGFR, PDGFR, and VEGFR, recently completed accrual, and an ongoing European phase I/II study (NCT 01346540) is enrolling patients with squamous cell carcinoma to receive cisplatin and gemcitabine with or without BIBF 1120. Other inhibitors in this class are also in early phase development, including BGJ398 (Novartis™), AZD4547 (AstraZeneca™), and dovitinib (Novartis™).

*Discoid Domain Receptor 2 (DDR2) Kinase*

DDR2 is a kinase which interacts with collagen and has roles in cell adhesion and proliferation (41). Mutations have been described in lung cancer and can be found in about 4% of SCCs (42, 43). In cell line and xenograft models, growth of DDR2 mutant cells is inhibited by dasatinib, a tyrosine kinase inhibitor already in widespread use for the treatment of chronic myelogenous leukemia (42). In a clinical trial combining dasatinib with erlotinib, a patient with SCC who responded to treatment was
found to have a DDR2 mutation (42). An ongoing single arm phase II trial (NCT01491633) treats patients with SCC with dasatinib; DDR2 mutations are not required for enrollment but all patients will be tested. Another phase II trial which will open soon (NCT01514864) will enroll a molecularly selected group of patients, including patients with SCC and DDR2 mutations.

Phosphatidylinositol 3-Kinase (PI3K)

PI3Ks have crucial roles in cell survival, growth, and motility. This pathway is downstream of multiple other proteins involved in NSCLC: B-raf, K-ras, EGFR. The PI3KCA gene encodes the catalytic domain of one of these kinases, and is one of the most frequently mutated genes in human cancer, including frequent mutations in breast and gastric cancer (44). Mutations in PI3KCA have been described in about 3% of SCCs; copy number gains are present in about a third of tumors (45). These changes are found less frequently in adenocarcinomas. Knockdown of PIK3CA in cell lines with mutations or copy number gains inhibited growth (45). PI3K inhibitors have shown activity against lung cancer in vivo (46) and multiple agents targeting this kinase are in development. For patients with previously treated SCC, a phase II trial randomizing patients to docetaxel or PI3K inhibitor BKM120 (Novartis™) is currently accruing (NCT1297491); those with previously untreated SCC are eligible for a randomized phase II trial of carboplatin and paclitaxel with or without GDC-0941 (Genentech™), another PI3K inhibitor (NCT01493843). Other inhibitors of this pathway in development include BEZ-235 (Novartis™), PX-866 (Oncothyreon™), and BAY 80-6946 (Bayer™).

AKT1/Protein Kinase B

The AKT1 gene encodes protein kinase B, which helps to mediate PI3K signaling. The E17K missense mutation in this gene causes increased activation of the PI3K pathway (47, 48). These mutations are found in 1% to 7% of SCCs and are not found in adenocarcinomas (27, 47, 49). MK-2066 (Merck™), an Akt inhibitor, is currently being studied in several phase II trials. In one trial, patients with NSCLC and PIK3CA, AKT, or PTEN mutations will receive MK-2066 as a single agent (NCT01306045). In the BATTLE-2 trial (NCT01248247), patients with NSCLC will undergo biopsy prior to randomization to one of four targeted therapy arms. One arm includes MK-2066 in combination with erlotinib; another combines MK-2066 with AZD6244 (AstraZeneca™), a Mek inhibitor. This combination is also being studied in other malignancies including melanoma (NCT01519427).

Platelet-Derived Growth Factor (PDGF) Receptors

The PDGFs are a family of molecules which bind to PDGF receptors (PDGFRs) and have an important role in angiogenesis (50). Multiple studies have shown that higher levels of these growth factors in tumor cells predict negative outcomes in resected NSCLC, including SCC (51-53). A number of agents currently in clinic use for other diseases – sorafenib, sunitinib, and imatinib, for example – inhibit PDGFR. Sorafenib, a multi-tyrosine kinase inhibitor targeting PDGFR-β, VEGF, c-KIT, and B-raf, was studied in combination with carboplatin and paclitaxel in a placebo-controlled phase III trial. Unfortunately, patients with SCC had an increased risk of mortality when treated with sorafenib, carboplatin and paclitaxel compared to patients treated with carboplatin and paclitaxel alone (54). Another trial investigating a similar inhibitor, motesanib, in combination with chemotherapy, had to limit accrual to only patients with non-squamous histology, after an interim analysis revealed an
increased risk of hemoptysis in patients with SCC (55). Given the concern for increased toxicity with PDGFR inhibitors in SCC, further trials of these medications be viewed with caution. Two anti-PDGFR antibodies are currently in development for NSCLC. MEDI-575 (MedImmune™) is currently being studied in combination with carboplatin and paclitaxel in NSCLC in a phase I/II trial (NCT01268059). Another phase II trial (NCT00918203) combines IMC-3G3 (ImClone™) with carboplatin and paclitaxel. X-82 (Tyrogenex™), a VEGFR/PDGFR inhibitor, is currently in phase I testing (NCT01296581).

SOX2

SOX2 is a transcription factor that has important roles in embryogenesis and stem cell maintenance. The SOX2 gene is amplified in about 20% of SCCs, and amplification is associated with higher SOX2 expression (56-59). Amplification may be an early event in carcinogenesis (60). Amplification and overexpression are found much less frequently in adenocarcinomas (56, 58). Studies suggest that higher SOX2 expression is associated with better prognosis in SCC (58).

CONCLUSIONS

Squamous cell carcinoma of the lung is a growing area of interest in terms of molecular diagnostics and treatment options. Recent research has identified several potential driver oncogenes (see table 1 and figure 2). Though many of these mutations affect only a small portion of patients with SCC, identifying them and using appropriate targeted agents could lead to significantly improved outcomes, as has been shown in patients with adenocarcinoma and EGFR mutations or ALK rearrangements. For example, dasatinib, when used in an unselected group of patients with NSCLC, does not appear to be particularly active (61); however, in a group of patients with DDR2 mutations, this drug may prove to be more effective. Future clinical trials should incorporate biomarker analyses for all patients. Patients with certain molecular abnormalities, including DDR2 mutations, PI3KCA mutations, FGFR1 amplifications, and AKT1 mutations, should be enrolled onto appropriate clinical trials. Through this personalization of care, we hope to improve outcomes for patients with SCC.

Table 1: Targetable alterations in squamous cell carcinoma of the lung

<table>
<thead>
<tr>
<th>Target</th>
<th>Frequency (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR variant III mutation</td>
<td>5%</td>
<td>(28)</td>
</tr>
<tr>
<td>FGFR1 amplification</td>
<td>20%</td>
<td>(39)</td>
</tr>
<tr>
<td>DDR2 mutation</td>
<td>4%</td>
<td>(42)</td>
</tr>
<tr>
<td>PI3KCA mutation</td>
<td>3%</td>
<td>(45)</td>
</tr>
<tr>
<td>PI3KCA copy number gain</td>
<td>30%</td>
<td>(45)</td>
</tr>
<tr>
<td>AKT1 mutation</td>
<td>1%-7%</td>
<td>(27, 47, 49)</td>
</tr>
</tbody>
</table>

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FIGURE LEGENDS:
Figure 1: Subsets of non-small cell lung cancer and alterations in squamous cell carcinoma. Abbreviations: DDR2, discoid domain receptor-2 gene mutation; EGFR vIII, epidermal growth factor variant III mutation; FGFR, fibroblast growth factor receptor gene amplification; PI3KCA, PI 3-kinase CA gene mutation

Figure 2: Schematic figure showing altered pathways in squamous cell carcinoma of the lung and agents targeted against them. Abbreviations: DDR2, discoid domain receptor 2; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; IGF-1R, insulin-like growth factor-1 receptor; MAb, monoclonal antibody; PDGFR, platelet derived growth factor receptors; PI3K, phosphatidyl inositol-3 kinase; TKI, tyrosine kinase inhibitor
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