

Advances in molecular and immunologic targeted therapies for squamous cell carcinoma of the lung

Michael J. Leukam¹, Victoria M. Villafior²

¹Department of Medicine, University of Chicago, Chicago, USA; ²Department of Internal Medicine, Section of Hematology/Oncology, University of Chicago, Chicago USA

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Correspondence to: Victoria M. Villafior. Department of Medicine, Section of Hematology/Oncology, University of Chicago, 5841 S. Maryland Ave. MC2113, Chicago, IL 60637, USA. Email: vvillaff@medicine.bsd.uchicago.edu.

Abstract: While targeted therapy has revolutionized the treatment of some forms of non-small cell lung cancer (NSCLC) particularly adenocarcinoma of the lung, the standard treatments for advanced stage squamous cell carcinoma (SqCC) has remained platinum-doublet chemotherapy, and the prognosis remains poor. There remains a great need for breakthroughs in treatments for this common and deadly disease. Recently, a new understanding of the molecular and genetic makeup of SqCC, thanks to large-scale genetic and molecular assays, has resulted in a number of targeted therapies entering clinical investigation for use in SqCC. Treatments targeting common mutations in SqCC have been studied in patient populations not preselected for mutations or overexpression and have had some early success. For example, the vascular endothelial growth factor receptor (VEGFR) inhibitor ramucirumab has been approved by the United States Food and Drug Administration (FDA) for use in certain settings for patients not selected for genetic mutations with NSCLC, including SqCC. Other agents are being investigated in selected populations with demonstrated genetic mutations or amplifications in the targeted pathway based on preclinical and early clinical data suggesting enhanced benefit in those groups. Early results in targeted immunotherapy have been particularly successful in SqCC compared to other histologic subtypes of NSCLC, and the programmed cell death 1 (PD-1) immune checkpoint inhibitor nivolumab has now been approved for second-line therapy in SqCC by the FDA. In this review, several oncogenic signaling pathways will be examined. The recent preclinical and clinical literature establishing those pathways as potential treatment targets, as well as ongoing clinical studies focused on those pathways, will be discussed. Recent and ongoing studies in targeted immune checkpoint inhibition and vaccine immunotherapy will also be reviewed.

Keywords: Immunotherapy; molecular targeted therapy; non-small cell lung cancer (NSCLC); squamous cell carcinoma (SqCC)

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Introduction

Despite advances in risk reduction (primarily smoking cessation), surgical, chemotherapeutic and radiation treatments, lung cancer remains the largest cause of cancer mortality in the US by far (1). Lung cancer is typically divided by histology into small cell (15% of diagnoses) and non-small cell (85% of diagnoses) types, the latter comprising

of adenocarcinoma, squamous cell carcinoma (SqCC) and large cell carcinoma (2). From the 1950s until the 1980s, SqCC was the most common non-small cell lung cancer (NSCLC) and lung cancer overall (3). In the 1980s, the relative incidence of adenocarcinoma overtook the incidence of SqCC and now remains the most common subtype of lung cancer (4,5). However, the absolute incidence of SqCC has

been rising in women in recent years, and remains a common malignancy in both men and women (5).

Approximately 60% of all new diagnoses of NSCLC are advanced stage III or stage IV disease and mostly are considered unresectable. Patients with stage IV disease will typically undergo palliative chemotherapy and/or radiotherapy as the only treatment options (6). Until recently, all histologic subtypes of NSCLC were treated similarly with platinum-containing doublets with early data suggesting no differences in progression-free survival (PFS) and overall survival (OS) based on choice of regimen (7). More recent data supports treating histologic subtypes differently, with improvement in OS and PFS in SqCC using platinum and gemcitabine and inferior outcomes in SqCC compared to non-squamous histologies using platinum and pemetrexed (8). A number of specific mutations with approved targeted therapies in adenocarcinoma have further divided the treatment of NSCLC subtypes, including epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) for patients with known mutations in EGFR (9,10), and targeted TKI therapy for anaplastic large-cell lymphoma kinase (ALK) rearrangements (11). Currently there are few such targeted agents for SqCC of the lung, and platinum-containing doublet chemotherapy remains the standard of care.

The current lack of targeted molecular therapies for SqCC may not be long lived. Several whole-genome characterization studies of SqCC of the lung have identified potential actionable targets which differ from those identified in other histologic subtypes. Compared to adenocarcinoma, EGFR and Kirsten rat sarcoma viral oncogene homolog (KRAS) are much less common in SqCC of the lung (12-14). More frequent mutations or amplifications in SqCC are seen in tumor protein 53 (TP53), fibroblast growth factor receptors (FGFR), cyclin-dependent kinase (CDK), the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway, and inactivating mutations of human leukocyte antigen (HLA-A) (13,15). Many common mutations in SqCC of the lung are shared with other squamous-type malignancies, suggesting that treatments developed for squamous malignancies of other sites may have activity in SqCC of the lung (12). Additional immunohistochemical and targeted genetic expression studies have contributed to a growing understanding of the unique molecular landscape of SqCC of the lung—the state of which is reviewed herein—with the hope that breakthroughs in targeted therapies will improve the dismal outcomes in this common disease.

Targeted therapy

Epidermal growth factor receptor (EGFR)

EGFR is a cell-surface receptor tyrosine kinase (RTK) involved primarily with regulation of cell proliferation, as well as differentiation and apoptosis. Constitutively activating mutations of EGFR are associated with the development of malignancies, including NSCLC (16,17), though multiple studies have not identified similar EGFR mutations in the SqCC subtype specifically (14,18). The greatest effects of EGFR-targeted TKI therapy in NSCLC have been seen in patients with identified mutations, and EGFR inhibition with TKIs has now become a mainstay of treatment in lung adenocarcinoma in a population defined by identified EGFR mutations (19). Given the lack of such a population in SqCC, studies of small molecule inhibition with TKIs and targeted monoclonal antibody inhibition of EGFR have proceeded in non-selected populations only.

TKIs targeting EGFR that have a proven record of success in selected EGFR mutant adenocarcinoma patients have demonstrated a small benefit in non-selected SqCC populations. A phase III trial of erlotinib as a post-chemotherapeutic maintenance agent in advanced NSCLC showed a significant effect on PFS in EGFR wild-type patients regardless of histologic subtype (20). A recent meta-analysis of 8 randomized controlled of EGFR TKIs in non-selected patients with metastatic SqCC demonstrated a modest but significant benefit in OS and PFS (21).

Monoclonal antibodies specifically disrupting EGFR signaling have also been investigated in SqCC. A recent phase III trial in SqCC comparing cisplatin and gemcitabine alone to the same chemotherapeutic agents with the addition of necitumumab (an anti-EGFR antibody) as first-line therapy found a small but significant improvement in OS (22). Another phase III trial comparing first-line platinum-based chemotherapy to the same chemotherapy plus cetuximab (an anti-EGFR antibody) in NSCLC regardless of mutation status found a marginal benefit in the SqCC subgroup, but not in the other histologic subtypes (23). Similar benefits were not seen in a similar phase III trial in stage IV in non-squamous NSCLC treated with necitumumab along with platinum-based doublet as first-line therapy regardless of mutation status (24). Taken together, these data suggest that the marginal benefit of first-line EGFR inhibition in NSCLC with wild type EGFR is restricted to the SqCC subtype.

Active trials for EGFR inhibition in SqCC include a phase II trial of avelumab (a monoclonal antibody

targeting EGFR) in locally advanced or metastatic solid tumors including SqCC of the lung (Clinicaltrials.gov identifier: NCT01772004), as well as a phase II trial of chemotherapy and radiation therapy with or without panitumumab (a monoclonal antibody targeting EGFR) in stage IIIA NSCLC including SqCC (Clinicaltrials.gov identifier: CT00979212).

The overall benefit of EGFR inhibition with both TKIs and monoclonal antibodies in SqCC patients without an identified EGFR mutation is modest compared to the responses seen in EGFR mutation positive adenocarcinoma. There is moderate evidence in the form of the combined weight of multiple supporting studies that EGFR inhibition may be beneficial in some patients with SqCC and is currently a part of treatment options for SqCC in non-EGFR-mutant populations. A better understanding of non-genomic alterations in protein expression unique to SqCC (such as overexpression of wild-type EGFR) may identify the mechanism of the observed benefit, and suggest additional treatment targets not able to be identified through current genetic and molecular screening.

Fibroblast growth factor receptor (FGFR)

FGFRs are a large family of highly conserved receptors for polypeptide growth factors, with 22 members identified in humans. Four (FGFR1-4) are tyrosine kinase receptors, which act upstream from the ras/mitogen-activated protein kinase (MAPK) and PI3K/protein kinase B (AKT) pathways known to be involved in regulation of proliferation in lung cancers (25,26).

Circulating fibroblast growth factors have found to be elevated in multiple types of lung cancer, including SqCC (27). Multiple studies have additionally identified abnormalities in FGFR protein expression or genetic amplifications of FGFR in SqCC. A 2006 study of SqCC cell lines found a significant correlation between EGFR and FGFR3 overexpression (28). In a 2010 study, FGFR1 gene amplification was found in 22% of SqCC samples, and *in vitro*, knockdown of FGFR1 was associated in restriction of cell growth and increased apoptosis in the FGFR1-amplified cell lines only (29). Subsequent studies of FGFR1, summarized in a recent meta-analysis find *FGFR* gene amplifications in 19% of SqCC tumors overall, but do not find a prognostic value for such FGFR1 amplifications in OS or PFS (30).

Despite the presence of a subpopulation of SqCC tumors with known genetic amplifications in FGFR, there are several

ongoing trials investigating FGFR inhibition as a treatment mechanism in NSCLC in populations not pre-selected for FGFR amplification. Pazopanib (a multi-targeted TKI whose targets include FGFR1-4) is currently under investigation in non-selected NSCLC patients (including SqCC). An ongoing phase II/III trial is studying pazopanib as maintenance therapy in advanced disease (Clinicaltrials.gov identifier: NCT01208064). A phase II trial is studying pazopanib in combination with erlotinib as second or third line treatment in advanced disease (Clinicaltrials.gov identifier: NCT01027598), and a third phase II trial is investigating pazopanib as a first line therapy in combination with paclitaxel for advanced disease (Clinicaltrials.gov identifier NCT01179269). Nintedanib (another multi-targeted TKI initially developed for ILD and NSCLC) is currently being investigated in a phase I/II trial evaluating effectiveness as first line therapy with standard chemotherapy specifically in patients with SqCC regardless of FGFR amplification status (Clinicaltrials.gov identifier NCT01346540). If successes are seen in studies of non-selected populations, subgroup analysis may reveal if the FGFR-amplification-positive group is disproportionately responding, or, as in the EGFR inhibition studies, there is a benefit in FGFR inhibition even the absence of identified amplifications.

Early clinical data is available from studies in groups selected for FGFR amplification. Two studies were completed with pan-FGFR inhibitor experimental medications AZD4547 and BGJ398 in patients with SqCC that had documented amplification of FGFR1. In a phase Ib study of AZD4547 as second-line therapy, an effect was seen (ORR 8%), but the efficacy rate for continuation of the study was not met (31). BGJ398 was studied as second-line therapy in a phase I trial and demonstrated an ORR of 16% (32). Further studies of BGJ398 are planned. Though the early data demonstrate mixed success, given the relatively high frequency of FGFR gene amplifications in SqCC, a successful targeted therapy could represent a major treatment breakthrough. Ongoing studies of FGFR inhibition in FGFR1 amplification positive squamous tumors include a phase II trial of ponatinib—a multi-targeted TKI with activity against FGFR—as second-line therapy in both squamous head and neck cancers and SqCC of the lung with documented FGFR kinase alterations (Clinicaltrials.gov identifier NCT01761747).

Phosphoinositide 3-kinase (PI3K)

The PI3K/AKT signaling pathway is downstream of

many cell-surface RTKs and is involved with regulation of cell survival, proliferation and metabolism, among other processes. Mutations in this pathway were commonly identified in large-scale genetic screens of NSCLC (nearly half of specimens analyzed), and are particularly associated with the squamous subtype, with a higher frequency of mutations than in adenocarcinoma (14,15,33,34). Specifically, mutations in PI3CA were found in approximately 10% of SqCC in a large genomic analysis, with amplification being the most common alteration (making up approximately 40% of mutations found) (34,35).

Rational therapeutic design focused on this pathway is supported by the finding that inactivation of downstream targets of the PI3K/AKT pathway in mouse models leads to the development of SqCC of the lung (36). In addition, mouse models with SqCC harboring PI3CA mutations have shown response to targeted therapy with PI3K inhibition in preclinical studies (37,38). Based on these early data, trials in human subjects have been launched. Buparlisib (also known as BKM120) is a small-molecule PI3K inhibitor being studied in pretreated metastatic SqCC and nonsquamous patients in a comparative two-stage phase II trial (Clinicaltrials.gov identifier: NCT01297491), and LY3023414, a small molecule inhibitor of PI3K and a downstream target of PI3K [mammalian target of rapamycin (mTor)] is being studied in a phase I trial in patients with advanced malignancies including NSCLC (Clinicaltrials.gov identifier NCT01655225). Effectiveness data in human subjects research has not yet been reported.

Vascular endothelial growth factor (VEGF)

One of the hallmarks of cancer is the ability of a tumor to develop its own blood supply to support further growth (39). Important mediators of angiogenesis include vascular endothelial growth factor and its receptor (VEGF and VEGFR). Bevacizumab is a monoclonal antibody against VEGF that is approved for use in several malignancies, including non-squamous NSCLC, but has been associated with increased hemoptysis in SqCC, and its use is currently contraindicated in that histologic subtype (40-42). Ramucirumab is another monoclonal anti-VEGF antibody, but it has not been associated with the same bleeding risk. In a phase III study of docetaxel with or without ramucirumab for second-line therapy in NSCLC (including SqCC), marginal improvements in OS (10.5 vs. 9.1 months) and in PFS (4.5 vs. 3.0 months) were seen in patients who received ramucirumab (43). Based on these data, ramucirumab with docetaxel is now FDA approved for second-line therapy for

NSCLC, including SqCC subtypes (44).

Ongoing clinical trials involving small molecule inhibitors include a phase III study of vandetanib (an inhibitor of both EGFR and VEGFR) with docetaxel as second-line therapy for NSCLC including SqCC not selected for VEGF or VEGFR mutations (Clinicaltrials.gov identifier: NCT00312377) and a phase II study of lucitanib (an inhibitor of FGFR 1-3, VEGFR 1-3, and PDGFR α/β) as second-line monotherapy in patients with advanced/metastatic disease which has a known amplification or activating mutation in FGFR1, FGFR2, FGFR3, VEGFA, or PDGFR α (Clinicaltrials.gov identifier NCT02109016).

Discoidin domain receptor (DDR)

Discoidin domain receptors 1 and 2 (DDR1 and DDR2) are cell-surface protein RTKs that bind to type I collagen and interact with downstream signaling targets that regulate cell proliferation and survival, including PI3K (45). Mutations in DDR2 were identified in approximately 4% of SqCC samples in one study, and activation of DDR1 was noted in a large survey of oncogenic kinase signaling in NSCLC (46).

Preclinical studies using xenograft mouse models with tumors made up of SqCC cells containing gain of function mutations in DDR2 demonstrated a strong response to dasatinib, a TKI with multiple targets including DDR1 and DDR2 (47). However, both a phase II trial studying dasatinib as first line therapy in advanced NSCLC (not selected to DDR2 mutations) and a phase I/II trial in advanced NSCLC (not selected for DDR2 mutation) with a combination of erlotinib and dasatinib failed to demonstrate a significant clinical benefit (48,49). Another phase II trial using dasatinib in advanced SqCC, with plans to correlate response rates to DDR2 mutation status, was halted early due to excess toxicity (50). Finally, a phase II trial studying dasatinib in patients with SqCC and known DDR2 mutations was halted due to slow accrual (Clinicaltrials.gov identifier NCT01514864). While patients with DDR2-mutant SqCC may yet be shown to benefit from inhibition of the DDR2 pathway, the low numbers of DDR2 mutations in the population may make the effect difficult to study. Studies with access to large populations will likely be required to amass enough DDR-mutation positive patients to be adequately powered.

Cyclins and CDKs

Cell division is a highly regulated process that includes several checkpoints (notably the G1-S checkpoint between

cell growth and DNA replication), which are tightly regulated in part by interactions between checkpoint inhibitors (e.g., retinoblastoma and P53), and checkpoint activators such as complexed cyclins and CDKs (51,52). In a large genetic screening study of NSCLC, cyclins, CDKs, and their regulatory pathways were found to harbor mutations, specifically CCND1 (amplified in 13% of cases), CDK6 (amplified in 4%), and the gene for p16 (which inhibits CDK4 and CDK6), which was mutated or deleted in 45% of tumors (35). CDKN2A and CCND1 are found to be enriched specifically in SqCC, and in one screening study, a subpopulation of tumors containing both a high level of cyclin pathway mutations and a low level of PI3K mutations was identified, suggesting that there are tumors in which cell-cycle directed therapy might be particularly effective (12,15).

Preclinical data gathered involving cell-cycle regulation include the development of two different inhibitors of CDK4/6 (LY2835219 and PD 0332991) that are active against xenograft tumors formed from human cancer cell lines in mice (53,54). Clinical data gathered to date is limited. A phase I study of flavopiridol (a pan-selective CDK inhibitor) in combination with standard chemotherapy as first line therapy was done in 12 patients with NSCLC, in which the drug was well tolerated, and partial responses were seen in 8 patients (55). Further studies will be required to assess the effectiveness of cell cycle targeted therapies in both non-selected and in known CDKN2A or CCND1 mutation positive populations.

Mesenchymal epithelial transition factor and hepatocyte growth factor (HGF)

Hepatocyte growth factor (HGF) and its RTK mesenchymal epithelial transition factor (c-MET) normally function upstream of multiple pathways involved in proliferation, angiogenesis, survival and migration, and is normally active in adults in times of tissue injury and repair (56). MET receptor amplification has been identified in up to 40% of lung cancer tissues, and both elevation in detectable levels of HGF and overexpression of c-MET are associated with a poor prognosis in NSCLC (57-59). Overexpression of HGF/c-MET has also been linked specifically with progression in NSCLC (60).

Preclinical data supported inhibition of the HGF/c-MET pathway with rilotumumab, an anti-HGF antibody that blocks interaction with the c-MET receptor; in mice with allograft tumors, rilotumumab enhanced the efficacy of

both docetaxel and temozolamide (61). Rilotumumab was well tolerated in a phase I study in patients with a variety of solid tumors (62).

Antibodies against c-MET have also been studied in NSCLC populations (including SqCC), such as onartuzumab (anti c-MET monoclonal antibody). In a recent phase II trial of recurrent NSCLC patients regardless of MET expression level status, the intention to treat group demonstrated no PFS or OS advantage, but the subgroup with tumors that overexpressed MET showed an advantage in both PFS and OS, while the subgroup without MET expression showed a decreased OS compared to placebo (63). Based on these results, a phase III trial was begun in NSCLC patients with advanced disease (including squamous histology) whose tumors overexpress MET by immunohistochemistry comparing erlotinib alone to erlotinib with onartuzumab (64). Surprisingly, given the promising phase II data, the METLung phase III trial was halted due to futility given lack of difference in response and progression free survival with the addition of onartuzumab to erlotinib at planned interim analysis (65).

Ongoing studies of c-MET inhibition in NSCLC include a phase II study of an experimental c-MET inhibitor capmatinib as second-line therapy in advanced NSCLC (including SqCC) not selected for c-MET expression level (Clinicaltrials.gov identifier NCT02414139), and a phase I study of experimental c-MET inhibitor PF-02341066 in NSCLC (including SqCC) patients with identified c-MET amplification, proto-oncogene tyrosine-protein kinase ROS (ROS1) mutation or anaplastic lymphoma kinase (ALK) rearrangements (PROFILE 1001, Clinicaltrials.gov identifier NCT00585195)

Immunotherapy

Programmed death receptor and ligands

One mechanism of immune suppression in SqCC is suggested by the relatively high levels of expression of programmed death receptor ligands (PDL) 1 and 2 in SqCC of the lung, which are expressed at levels significantly higher than adenocarcinoma (66,67). Data are mixed on the prognostic significance of elevated PDL1 in NSCLC. A recent meta-analysis found overall decreased OS with increased PDL1 expression (68), though a single study found increased OS in early stage disease only (69). The significance of elevated PDL1 expression is illuminated by its function as part of an immune checkpoint. Evasion of

immune surveillance or suppression of immune response is considered to be a hallmark of cancer (39), allowing abnormal cells to proliferate without a response from cytotoxic defense mechanisms. When PDL1 and PDL2 bind to the programmed death receptor (PD1) on cytotoxic CD8+ T-cells, activation of PD1 causes anergy and prevents the secretion of pro-inflammatory cytokines (70). PD1 activation on CD4+ T-cells, in part, drives a transformation into immune-suppressing T-regulatory cells (71). These functions normally serve to dampen inappropriate immune responses, but in the case of SqCC, may assist in evasion of the appropriate immune response.

Disrupting the PD1/PDL1 interaction is believed to allow for removal of the immune inhibition of the surrounding T cells, increasing immune anti-tumor activity. Promising results were first seen in hematologic malignancies (pamidrolumab, anti-PD1 antibody), followed by melanoma (pembrolizumab, anti-PD1 antibody) (71). Trials of PD1/PDL-1 inhibition have been promising in NSCLC, and the SqCC subtype seems uniquely sensitive to these inhibitors. Early results from a phase I/II clinical trial of MEDI4736, (anti-PDL1 antibody), demonstrated an overall response rate of 21% in SqCC compared to 10% in adenocarcinoma (72). Early results from an ongoing phase I trial of another anti PDL1 antibody (MPDL3280A) evaluated response rates of NSCLC with intensity of pre-treatment infiltrating lymphocyte PDL1 expression and found higher expression correlated with a higher likelihood of response (73). These data suggest it may be possible to identify those patients most likely to benefit from PD1/PDL1 checkpoint inhibition prior to initiating treatment (74).

Clinical trials investigating the effectiveness of nivolumab (an antibody against PD1) in SqCC have demonstrated significant early successes. The Checkmate 017 trial was a phase II study that investigated nivolumab as a salvage therapy in heavily pretreated patients, demonstrating an ORR of 15%, an OS of 8.2 months and a 1-year survival of 41% (75). Nivolumab was also compared to docetaxel in advanced or metastatic in Checkmate 063, a phase III study that was halted early after meeting its primary endpoint of significantly improved OS (9.2 vs. 6.0 months) (76). Due to these results, the Food and Drug Administration (FDA) has approved nivolumab in the treatment of SqCC with progression on or after standard chemotherapy (77).

The many ongoing studies of PDL1/PD1 inhibition in NSCLC include a phase III trial of pembrolizumab, an antibody against PD1, versus placebo with or without standard adjuvant chemotherapy for resected stage IB-III

NSCLC, including SqCC (Clinicaltrials.gov identifier: NCT02504372) and a phase II trial of nivolumab as second-line therapy specifically in advanced-stage SqCC (Checkmate 171, Clinicaltrials.gov identifier: NCT02409368). A phase III trial of nivolumab as first-line therapy for NSCLC compared to platinum-doublet chemotherapy is now recruiting, and will include an arm specific to SqCC (Checkmate 227, Clinicaltrials.gov identifier: NCT02477826). A large, multi-arm phase I study of nivolumab in advanced NSCLC as monotherapy or in combination with either cytotoxic chemotherapy or with small molecule inhibitors such as bevacizumab and erlotinib is currently underway, and will include separate arms for squamous and non-squamous histologic subtypes (Checkmate 012, Clinicaltrials.gov identifier NCT01454102). Several other early phase trials are underway investigating nivolumab in NSCLC in the maintenance (Clinicaltrials.gov identifier: NCT02434081) and neoadjuvant (Clinicaltrials.gov identifier: NCT02259621) settings, and in combination with c-MET inhibitors (Clinicaltrials.gov identifier: NCT02323126).

Cytotoxic lymphocyte antigen

Cytotoxic T lymphocyte antigen-4 (CTLA4) is expressed on the surface of cytotoxic T-cells, and forms part of a different immune checkpoint by competing with the T-cell costimulatory molecule for their shared ligands CD80 or CD86 (78). T-cell CTLA4 expression is higher in patients with NSCLC, and higher yet in metastatic disease, though the mechanism is unknown (79). Higher levels of expression are found in SqCC compared to adenocarcinoma, and within the patient population with SqCC, higher CTLA4 levels are associated with decreased survival (67).

CTLA4 inhibition is being studied in a range of cancers based on a similar rationale as PD1/PDL1, namely that blocking an immune checkpoint will allow for increased antitumor immune activity. Ipilimumab is an anti-CTLA4 antibody that was studied in a phase II trial of first line therapy of chemotherapy with and without ipilimumab, finding a small but statistically significant improvement in PFS, which was greater in SqCC than in non-squamous subtypes (80). Based on these results, a phase III trial is underway focusing specifically on ipilimumab in SqCC (Clinicaltrials.gov identifier: NCT01285609).

Vaccines

The use of vaccines directed towards malignant cells has

long been area of active investigation in cancer treatment, with some successes in melanoma and prostate cancer. One early target for vaccine therapy was melanoma-associated antigen A3 (MAGE-A3), a tumor antigen not expressed on noncancer cells but found on approximately 30% of NSCLC tumors. Unfortunately, a large phase III trial of a MAGE-A3 vaccine failed to meet its primary endpoint of increased DFS in NSCLC patients and further investigations of the vaccine in NSCLC are not planned at this time (81). Another potential vaccine target that was considered was mucin-1 glycoprotein (MUC1) which is overexpressed and abnormally glycosylated in NSCLC cells (82). However, in a phase III trial of the anti MUC1 vaccine tecemotide as maintenance therapy after chemoradiation for NSCLC, no difference was found compared to placebo (83).

While tumor associated antigen vaccines for NSCLC have not yet shown hopeful results, there have been some mixed data for whole cell vaccines. Belagenpumatucel-L is a whole-cell vaccine made up of NSCLC cell lines (adenocarcinoma, SqCC, and large cell carcinoma) that were transfected with an antisense plasmid for transforming growth factor beta-2 (TGF β 2) (84). TGF β 2 is a cytokine that suppresses immune cytotoxic function and enhances the development of immune-suppressing T-regulatory cells (85,86). Preclinical studies supported the effectiveness of TGF β 2 antisense oligonucleotides in suppressing or reversing multiple tumor types in animal models (87-89). Despite hopeful results in phase I and II trials, a phase III trial with belagenpumatucel-L in patients with stage IIIB and IV NSCLC did not meet its primary endpoint of improved OS (90,91). However, subgroup analysis found that patients randomized within 12 weeks of completion of chemotherapy had significantly improved OS, particularly noted in patients randomized within 12 weeks with non-adenocarcinoma histology (OS of 19.9 months with belagenpumatucel-L *vs.* 12.3 month with placebo) (91). Based on these subgroup analyses and the overall safety profile, the FDA has supported continued study of belagenpumatucel-L (92).

Lung-MAP

Based on the early data for several molecular targeted therapies and immunotherapies in SqCC of the lung as outlined above, a large, multi-arm phase II/III trial has been developed by the Southwest Oncology Group called Lung-MAP (Clinicaltrials.gov identifier NCT02154490)

which will investigate several targeted therapies as second-line therapies simultaneously. In this ambitious study, patients with recurrent stage IIIB/IV SqCC will be tested for a variety of biomarkers and assigned to a targeted arm based on the mutation or amplification their tumor harbors. If none of the study targets are identified in the sample, the patient will be assigned to an immunotherapy arm comparing anti-B7H1 monoclonal antibody MEDI4736 with docetaxel to docetaxel alone. Patients with tumors positive for PI3KCA mutations will be assigned to an arm comparing PI3 kinase inhibitor GDC-0032 with docetaxel to docetaxel alone. Patients with tumors positive for CDK4/6, CCND1, CCND2, and CCND3 will be assigned to an arm comparing palbociclib (a selective small molecule inhibitor of CDK4/6) with docetaxel to docetaxel alone. Patients with tumors positive for FGFR1, FGFR2, and FGFR3 will be assigned to an arm comparing FGFR inhibitor AZD4547 with docetaxel to docetaxel alone. Finally, patients with tumors overexpressing HGF/c-MET will be assigned to an arm investigating anti-HGF antibody rilotumumab in combination with erlotinib versus erlotinib alone. Primary outcome measures are OS and PFS.

Conclusions

Compared to the growing options for targeted therapy in adenocarcinoma, SqCC of the lung continues to rely largely on standard platinum based chemotherapy. The notion of treating SqCC differently than other histologic subtypes has recently been advanced with data supporting superiority of a platinum-based regimen containing gemcitabine compared to other subtypes (8). Many new molecular targets for therapy have been suggested by large-scale genome and phosphorylation studies of SqCC that have identified a molecular fingerprint that is unique among the family of NSCLC.

Some of the molecularly targeted therapies under investigation for SqCC have demonstrated small clinical success when applied to non-selected populations. For example, marginal benefits in OS with the VEGF-inhibitor ramucirumab were demonstrated in a population not selected by genetic mutation or overexpression. These modest benefits were enough for FDA approval for ramucirumab with docetaxel as a second-line therapy for all NSCLC, including SqCC. Additionally, multiple early phase trials of EGFR therapy (both monoclonal antibodies and TKIs) have shown small, but significant, clinical responses in a non-selected population of SqCC, despite

the rarity of EGFR mutations this histologic subtype. The broad effect of these targeted therapies may reflect a subpopulation that has yet to be identified with dependence on a specific oncogene in the targeted pathway, or may reflect a general principle SqCC proliferation using wild-type signaling pathways. Careful subgroup analysis in clinical studies and advances in the basic molecular science of SqCC may help clarify which patients may realize the greatest benefit for VEGF- and EGFR-targeted therapies. Other targeted therapies have shown their greatest benefit in preclinical and early clinical studies in populations with known amplifications or mutations in the targeted pathway (FGFR, PI3K, DDR2, CDK4/6, and HGF/c-MET). None have yet demonstrated clinical success in a defined subpopulation, but there are many ongoing trials investigating various small molecule inhibitors and monoclonal blocking antibodies. Several targeted therapies are being investigated simultaneously in SqCC subgroups defined by an activating mutation in the phase II/III Lung-MAP trial.

The most exciting recent data has been in the realm of immunotherapy. While vaccine therapy for SqCC has not been proven effective in several phase III trials, encouraging results have been seen in studies of targeted immune checkpoint inhibitors. Both PD1/PDL1 and CTLA4 inhibition have shown greater clinical response rates in SqCC as compared to other histologic NSCLC subtypes, and have demonstrated a favorable safety profile in early phase studies. Based on the clinical response rate and the occurrence of some unusually durable responses in a phase III trial, the targeted PD1 immune checkpoint inhibitor nivolumab was approved as second-line therapy for NSCLC, specifically SqCC (77). Many studies of immune checkpoint inhibition as single-agent and combination therapy in various roles (including first-line therapy) are now underway, and have the potential to rapidly alter the treatment landscape for SqCC.

Even in the midst of a flowering of research in targeted therapies for SqCC, much remains to be learned about the biology and treatment of this difficult disease. Large-scale genomic studies have provided many possible targets for treatment, though the relative importance of each identified mutation to tumorigenesis and the usefulness of each as a treatment target remain largely unknown. Preclinical studies and clinical trials are still working through the many targets identified in screening studies, though high profile failures in targeted therapies such as c-MET inhibition—despite biological plausibility and encouraging early

clinical data—suggest that much remains unknown about the signaling interactions upon which SqCC depends to grow and spread. As the science of molecular biology advances alongside clinical medicine, a new generation of basic studies of genetic expression and protein signaling interactions in SqCC over time may be necessary to develop enough treatment targets to control and eventually defeat this dreaded disease.

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