Predictive biomarkers for response to EGFR-directed monoclonal antibodies for advanced squamous cell lung cancer

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Background: Upregulated expression and aberrant activation of the epidermal growth-factor receptor (EGFR) are found in lung cancer, making EGFR a relevant target for non-small-cell lung cancer (NSCLC). Treatment with anti-EGFR monoclonal antibodies (mAbs) is associated with modest improvement in overall survival in patients with squamous cell lung cancer (SqCLC) who have a significant unmet need for effective treatment options. While there is evidence that using *EGFR* gene copy number, *EGFR* mutation, and EGFR protein expression as biomarkers can help select patients who respond to treatment, it is important to consider biomarkers for response in patients treated with combination therapies that include EGFR mAbs.

Design: Randomized trials of EGFR-directed mAbs cetuximab and necitumumab in combination with chemotherapy, immunotherapy, or antiangiogenic therapy in patients with advanced NSCLC, including SqCLC, were searched in the literature. Results of associations of potential biomarkers and outcomes were summarized.

Results: Data from phase III clinical trials indicate that patients with NSCLC, including SqCLC, whose tumors express high levels of EGFR protein (*H*-score of $\geq$200) and/or gene copy numbers of *EGFR* (e.g. $\geq$40% cells with $\geq$4 *EGFR* copies as detected by fluorescence *in situ* hybridization; gene amplification in $\geq$10% of analyzed cells) derive greater therapeutic benefits from EGFR-directed mAbs. Biomarker data are limited for EGFR mAbs used in combination with immunotherapy and are absent when used in combination with antiangiogenic agents.

Conclusions: Therapy with EGFR-directed mAbs in combination with chemotherapy is associated with greater clinical benefits in patients with NSCLC, including SqCLC, whose tumors express high levels of EGFR protein and/or have increased *EGFR* gene copy number. These data support validating the role of these as biomarkers to identify those patients who derive the greatest clinical benefit from EGFR mAb therapy. However, data on biomarkers for EGFR-directed mAbs combined with immunotherapy or antiangiogenic agents remain limited.

Key words: non-small-cell lung cancer, squamous cell lung cancer, EGFR-directed monoclonal antibodies

Introduction

Non-small-cell lung cancer (NSCLC) is a heterogeneous disease that accounts for ∼85% of lung cancer diagnoses [1]. The different subtypes of NSCLC, which include adenocarcinoma and squamous cell lung cancer (SqCLC), are histopathologically distinct and can exhibit differential treatment responses, including in overall survival (OS) and toxicity [2–6]. SqCLC is associated with a significant unmet need; it can be very aggressive; patients tend to be older, present at a later stage, and have a high incidence of comorbidities [7, 8], all of which can reduce the effectiveness of treatment and increase toxicity [9]. This is exemplified by the currently available therapies of bevacizumab, nintedanib, and pemetrexed, which are available for the treatment of patients with NSCLC, but excluded for the treatment of patients with SqCLC due to unacceptably low levels of efficacy and/or high toxicity [3, 10]. Upregulated expression and aberrant activation of epidermal growth-factor receptor (EGFR) have been shown to play a role in lung cancer, making EGFR a relevant target for NSCLC [11–16]. Therefore, it is important to review progress in targeting EGFR in patients with SqCLC.

Current therapies directed against EGFR include tyrosine kinase inhibitors (TKIs) such as erlotinib, gefitinib, afatinib, and osimertinib and EGFR-directed monoclonal antibodies (mAbs) such as cetuximab, panitumumab (not indicated for NSCLC), and necitumumab [3]. EGFR TKIs bind to EGFR and downregulate signaling downstream of EGFR by inhibiting receptor tyrosine kinase autophosphorylation [17]. For NSCLC, treatment with EGFR TKIs in the first-line treatment setting should be limited to patients whose tumors harbor EGFR mutations [18]. However, activating EGFR mutations are very rare in SqCLC, occurring in <4% of patients, which makes therapy with EGFR TKIs unsuitable for the vast majority of this patient population. Consequently, molecular testing for activating EGFR mutations is seldom carried out for patients with SqCLC [19–21]. As such, EGFR TKIs have a minor role in the first-line treatment of SqCLC, while afatinib has demonstrated a minimal OS benefit in second-line treatment versus erlotinib in unselected patients [22].

An alternative strategy is to use EGFR-directed mAbs, such as cetuximab and necitumumab, which function by inducing internalization of the antibody-receptor complex and downregulation of the receptor after binding to the extracellular portion of EGFR [23]. EGFR protein expression is detected in a high proportion of patients with NSCLC and is associated with poor prognosis [24]. In contrast to EGFR TKIs, there are modestly positive OS data with EGFR mAbs in first-line treatment of patients with SqCLC [23, 25]. Recent advances in the development of EGFR-directed mAbs for the treatment of patients with SqCLC [23, 25, 26] confirm the need for identifying the optimal predictive biomarkers that could assist clinicians in the selection of patients who will benefit the most from this targeted therapy.

In this review, we discuss the evidence for the potential impact of predictive biomarkers on identifying patients with SqCLC who are most likely to have a significant clinical benefit from treatment with EGFR mAbs used in combination with chemotherapy, immunotherapy, or antiangiogenic agents.

Predictive biomarkers for EGFR mAbs in combination with chemotherapy

Several potential predictive biomarkers for response to EGFR-directed mAbs have been investigated. These include EGFR protein expression as measured by immunohistochemistry (IHC), *EGFR* gene copy numbers as measured by fluorescence *in situ* hybridization (FISH), and mutations in the *EGFR* and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) genes.

EGFR protein expression

EGFR protein expression level has been assessed as a predictive biomarker for response to treatment with EGFR-directed mAbs in patients with NSCLC, including SqCLC.

FLEX clinical trial. The phase III FLEX clinical trial (NCT00148798) assessed the efficacy of cetuximab, an EGFR-directed mAb for the treatment of patients with advanced NSCLC, including those with SqCLC histology [23]. This trial compared cetuximab plus cisplatin–vinorelbine chemotherapy versus cisplatin–vinorelbine chemotherapy alone for the treatment of chemotherapy-naïve patients with advanced NSCLC that express EGFR in ≥1 positively immunostained tumor cell. Median OS was significantly increased by 1 month in patients treated with cetuximab plus chemotherapy compared with those who received chemotherapy alone [hazard ratio (HR)=0.87; 95% confidence interval (CI) 0.76–1.00; $P = 0.04$] (Figure 1A). Similar results were reported in the subset of patients with SqCLC (34%) (HR = 0.80; 95% CI 0.64–1.00) (Figure 1A) [23].

In a retrospective analysis of FLEX, the IHC *H*-score cut-off was used to assess EGFR expression as a predictor of response to cetuximab [26]. For patients with NSCLC in the EGFR high-expression group [*H*-score ≥200; $n = 345$ (31%)], median OS was significantly increased by >2 months for those treated with cetuximab plus cisplatin–vinorelbine chemotherapy compared with cisplatin–vinorelbine chemotherapy alone (HR = 0.73; 95% CI 0.58–0.93; $P = 0.011$) (Figure 1A). Furthermore, no significant differences in OS were observed between treatments for patients in the EGFR low-expression group ($n = 776$; 69%; HR = 0.99). Similarly, median OS was significantly increased by >2 months in patients with SqCLC in the EGFR-high group treated with cetuximab plus cisplatin–vinorelbine chemotherapy compared with cisplatin–vinorelbine chemotherapy alone (HR = 0.62; 95% CI 0.43–0.88) (Figure 1A). Contrary to the findings from the retrospective FLEX analysis, however, a phase III study of patients with NSCLC treated with docetaxel or pemetrexed with or without cetuximab did not show an interaction between *H*-score (200 cut-off) and OS ($P = 0.35$) or progression-free survival (PFS) ($P = 0.71$), although other cut-offs were not evaluated and the evaluators differed from those who developed the classification [27].

BMS099 clinical trial. In the phase III BMS099 clinical trial (NCT00112294), an accompanying trial to the FLEX trial that did not include restrictions on EGFR expression or histological subtypes, the addition of cetuximab to taxane–carboplatin chemotherapy
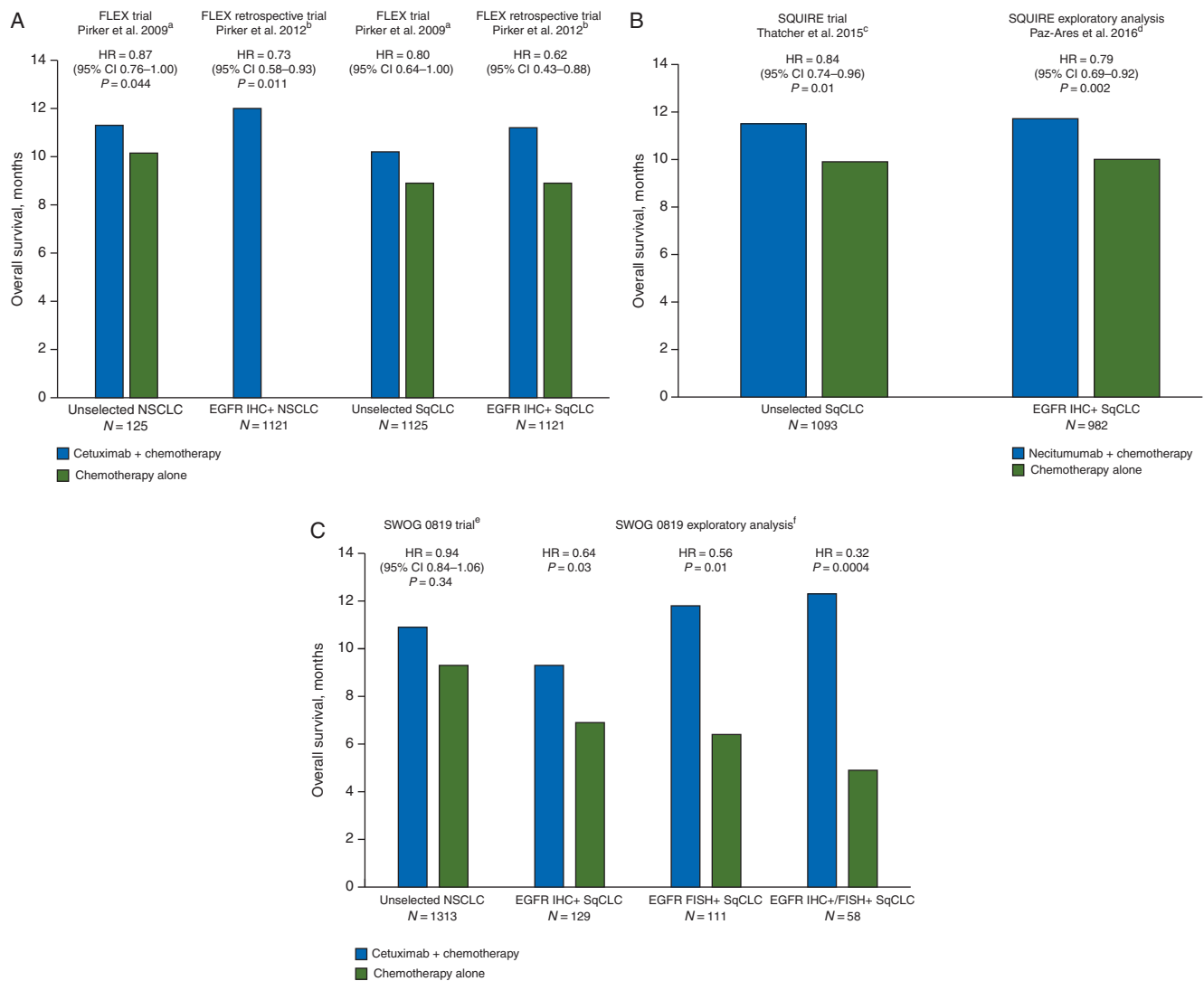
Figure 1. (A) Overall survival of patients with NSCLC treated with cetuximab plus chemotherapy, necitumumab plus chemotherapy, or chemotherapy alone. Patients with NSCLC and SqCLC (unselected and high EGFR expressing) treated with cetuximab plus chemotherapy or chemotherapy alone. (B) Patients with SqCLC (unselected and high EGFR expressing) treated with necitumumab plus chemotherapy or chemotherapy alone. (C) Patients with NSCLC (unselected, high EGFR expressing, EGFR FISH+, and high EGFR expressing/EGFR FISH+) treated with cetuximab plus chemotherapy or chemotherapy alone. CI, confidence interval; EGFR, epidermal growth-factor receptor; FISH, fluorescence *in situ* hybridization; HR, hazard ratio; IHC, immunohistochemistry; NSCLC, non-small-cell lung cancer; SqCLC, squamous non-small-cell lung cancer. [a]Pirker et al. [23]; [b]Pirker et al. [26]; [c]Thatcher et al. [25]; [d]Paz-Ares et al. [30]; [e]Herbst et al. [36]; [f]Hirsch et al. [37].

significantly improved the overall response rate (ORR) compared with chemotherapy alone in patients with advanced NSCLC (25.7% versus 17.2%, respectively; $P = 0.007$) [28]. Median OS was also improved, although this did not reach statistical significance [9.69 months with cetuximab versus 8.38 months in the taxane–carboplatin arm (HR = 0.89; 95% CI 0.75–1.05; $P < 0.1685$)]. Improvement in PFS with the addition of cetuximab was only shown in a post hoc analysis of the SqCLC patient population (HR = 0.70; 95% CI 0.47–1.05). The contrasts in clinical benefit between the FLEX and BMS099 clinical trials supported the need for a biomarker for the selection of patients with NSCLC who would benefit from therapy with EGFR-directed mAbs.

SQUIRE clinical trial. The phase III SQUIRE trial (NCT00981058) compared necitumumab plus gemcitabine and cisplatin chemotherapy versus chemotherapy alone in chemotherapy-naïve patients with advanced SqCLC [25]. The primary end point of OS was significantly increased by >1 month in patients treated with necitumumab plus chemotherapy compared with chemotherapy alone (HR = 0.84; 95% CI 0.74–0.96; $P = 0.01$) (Figure 1B). Based on these results, necitumumab, in combination with cisplatin and gemcitabine, was granted approval by the United States Food and Drug Administration (FDA) for the front-line treatment of patients with metastatic SqCLC [29].

In a pre-specified exploratory analysis of SQUIRE that used the *H*-score to define EGFR high expression, the OS hazard ratio for treatment with necitumumab plus cisplatin–gemcitabine versus cisplatin–gemcitabine alone favored patients in the EGFR-high group (*H*-score ≥200; HR = 0.75; 95% CI 0.60–0.94) compared

with those in the EGFR-low group (*H*-score <200; HR = 0.90; 95% CI 0.75–1.07) [25]. Median OS was later shown to be significantly increased by >1.5 months for patients with EGFR-positive tumors (EGFR > 0) treated with necitumumab plus chemotherapy compared with chemotherapy alone (HR = 0.79; 95% CI 0.69–0.92; *P* = 0.002) (Figure 1B). Importantly, OS was not found to be longer in the 5% of patients with EGFR-negative tumors (HR = 1.52) [30]. Based on these results, necitumumab in combination with cisplatin and gemcitabine was approved by the European Medicines Agency (EMA) as a first-line treatment option for patients with advanced SqCLC expressing EGFR by IHC [31]. However, given that the subgroup of patients with EGFR-negative tumors only comprised ∼5% of the study population, many physicians have questioned the need to assess EGFR expression before instituting necitumumab in clinical practice.

INSPIRE clinical trial. The INSPIRE trial (NCT00982111) further assessed the role of histology on the efficacy of necitumumab by comparing first-line necitumumab plus pemetrexed and cisplatin chemotherapy versus pemetrexed and cisplatin chemotherapy alone for the treatment of patients with advanced non-squamous NSCLC (i.e. adenocarcinoma, large-cell carcinoma, and other non-squamous histology) [32]. In contrast to the findings from SQUIRE, no significant differences were observed in OS between the two cohorts in the INSPIRE clinical setting. Median OS was 11.3 months in the necitumumab plus pemetrexed and cisplatin group versus 11.5 months in the pemetrexed and cisplatin group [HR = 1.01 (95% CI 0.84–1.21); *P* = 0.96]. In addition, there were no significant differences in OS between treatment groups in high and low EGFR protein expression groups (*H*-score ≥200 and <200, respectively).

The limited efficacy of necitumumab in patients with advanced non-squamous NSCLC (INSPIRE) compared with patients with SqCLC (SQUIRE) may be due to the lower frequency with which increases in *EGFR* gene copy number and protein levels are observed in tumors from patients with non-squamous NSCLC [11].

Meta-analysis of two necitumumab and five cetuximab clinical trials. A recent meta-analysis of seven phase III clinical trials of EGFR-directed mAbs (necitumumab and cetuximab) systematically reviewed available data to evaluate the efficacy and toxicity of this therapy plus chemotherapy versus chemotherapy alone for the treatment of patients with advanced NSCLC [33]. Treatment with EGFR-directed monotherapy plus chemotherapy significantly increased OS (HR = 0.90; 95% CI 0.84–0.95), PFS (HR = 0.93; 95% CI 0.87–0.98), and ORR (OR = 1.27; 95% CI 1.06–1.51) in patients with NSCLC compared with chemotherapy alone. In subgroup analyses, treatment with EGFR-directed mAbs in combination with chemotherapy was associated with improved OS in patients with SqCLC (HR = 0.84; 95% CI 0.76–0.92), in patients with NSCLC whose tumors had high EGFR expression, defined as *H*-score ≥200 (HR = 0.83; 95% CI 0.70–0.98), and in smokers (HR = 0.87; 95% CI 0.79–0.96). Furthermore, the association between treatment with EGFR-directed mAbs and OS, PFS, and ORR was highest among patients with SqCLC whose tumors had high EGFR expression (HR = 0.71; 95% CI 0.59–0.86).

EGFR gene copy number and mutation

BMS099 clinical trial. A retrospective, correlative analysis of data from the BMS099 clinical trial aimed to identify biomarkers for the selection of patients with advanced NSCLC who would most likely benefit from treatment with cetuximab [34]. Biomarkers analyzed included *KRAS* and *EGFR* mutations, EGFR protein expression, and *EGFR* gene copy number. Mutations in *KRAS* and *EGFR* were found in 17% (35 of 202) and 10% (17 of 166) of patients, respectively. EGFR protein expression was detected in 89% of patients (131 of 148), and FISH+ (FISH+ defined as ≥40% cells with ≥4 *EGFR* copies and gene amplification in ≥10% of analyzed cells) was detected in 52% of patients (54 of 104). However, there was no significant association between response to treatment and *EGFR* expression, mutation, or copy number. Similar results for *KRAS* and *EGFR* mutations and *EGFR* gene copy numbers were reported in a retrospective analysis of the FLEX trial [35].

SWOG 0819 clinical trial. The phase III SWOG 0819 trial (NCT00946712) compared cetuximab with carboplatin–paclitaxel chemotherapy versus carboplatin–paclitaxel chemotherapy alone in chemotherapy-naïve patients with advanced NSCLC [36]. Bevacizumab was allowed in either arm of the study if there were no contraindications, such as SqCLC. No significant differences were observed in PFS or OS among unselected patients (Figure 1C). However, the data suggested that patients with *EGFR* FISH+ tumors may have experienced a statistically insignificant trend toward a benefit in PFS (HR = 0.91; 95% CI 0.74–1.12) and OS (HR = 0.83; 95% CI 0.67–1.04).

In an exploratory analysis of the SWOG 0819 clinical trial that assessed EGFR-expression levels as a predictive biomarker for clinical response to therapy with cetuximab, tumors from patients with advanced SqCLC were characterized as FISH+ (defined as *EGFR*/centromeric region of chromosome ≥2 or ≥10% of cells with ≥15 *EGFR* copies and ≥40% of cells with four *EGFR* copies) or FISH− and as having high or low EGFR-expressing tumors, as assessed by IHC [37]. Patients with FISH+ SqCLC who were treated with cetuximab plus carboplatin–paclitaxel (*n* = 55; 17.1%) showed improved median OS of ∼ 5 months compared with chemotherapy alone (*n* = 56; 17.4%; HR = 0.56; *P* = 0.01) (Figure 1C). Furthermore, patients with FISH+, high EGFR-expressing SqCLC who were treated with cetuximab plus carboplatin–paclitaxel (*n* = 30; 9.3%) showed improved median OS of >7 months compared with chemotherapy alone (*n* = 28; 8.7%; HR = 0.32; *P* = 0.0004) (Figure 1C). Similarly, patients with high (*H*-score ≥200) EGFR-expressing SqCLC who were treated with cetuximab plus carboplatin–paclitaxel experienced improved median OS of ∼ 3 months compared with chemotherapy alone (HR = 0.64; *P* = 0.03) (Figure 1C). No significant differences in OS were observed for the unselected and adenocarcinoma patient populations.

SQUIRE clinical trial (NCT00981058). In a pre-specified exploratory analysis of the phase III SQUIRE clinical trial that used FISH to assess *EGFR* gene expression, treatment with necitumumab plus cisplatin–gemcitabine versus cisplatin–gemcitabine alone was favored in patients in the *EGFR* FISH+ group (median OS 12.6 versus 9.2 months, respectively; HR = 0.70; 95% CI

Table 1. Ongoing studies of immunotherapy agents in first-line treatment of NSCLC

Study	Phase	Drug	Treatment cohorts	Patient population
NCT02367794	III	Atezolizumab	Atezolizumab with carboplatin and paclitaxel or carboplatin and nab-paclitaxel versus carboplatin and nab-paclitaxel	Stage IV SqCLC
NCT02409342	III	Atezolizumab	Atezolizumab versus cisplatin or carboplatin and pemetrexed or gemcitabine	Stage IV NSCLC
NCT02576574	III	Avelumab	Avelumab versus platinum-based doublet	Stage IV PD-L1+ NSCLC
NCT02542293	III	Durvalumab	Durvalumab with tremelimumab versus standard of care	Advanced or metastatic NSCLC
NCT02434081	II	Nivolumab	Nivolumab with standard first-line chemotherapy and radiotherapy	Locally advanced stage IIIa/b NSCLC
NCT02477826	III	Nivolumab	Nivolumab or nivolumab with ipilimumab or nivolumab with platinum-doublet chemotherapy versus platinum-doublet chemotherapy	Stage IV or recurrent NSCLC
NCT02591615	II	Pembrolizumab	Pembrolizumab followed by carboplatin and paclitaxel or pemetrexed	Chemotherapy-naïve stage IV NSCLC
NCT03322566	III	Pembrolizumab	Pembrolizumab with epacadostat alone or with platinum-based chemotherapy versus pembrolizumab with platinum-based chemotherapy plus placebo	Metastatic NSCLC
NCT02220894	III	Pembrolizumab	Pembrolizumab versus platinum-based chemotherapy	Advanced or metastatic NSCLC
NCT02775435	III	Pembrolizumab	Carboplatin–paclitaxel/nab-paclitaxel with or without pembrolizumab	Metastatic SqCLC
NCT03134872	III	SHR-1210	SHR-1210 with pemetrexed and carboplatin	Chemotherapy-naïve stage IIIb/IV non-squamous NSCLC

NSCLC, non-small-cell lung cancer; PD-L1, programmed death ligand-1; SqCLC, squamous non-small-cell lung cancer.

0.52–0.96), but was not favored in those in the *EGFR* FISH– group (11.1 versus 10.7 months, respectively; HR = 1.02; 95% CI 0.80–1.29) [30].

Taken together, results from subgroup analyses of phase III clinical trials support the use of EGFR expression and *EGFR* FISH+ as predictive biomarkers to aid in the selection of patients with advanced NSCLC, including SqCLC, who would derive the most benefit from clinical therapy with EGFR-directed mAbs cetuximab and necitumumab. Currently, IHC for EGFR expression/*H*-score and *EGFR* FISH analyses are not being routinely carried out on SqCLC specimens. The results described suggest that incorporating these analyses would identify patients who have an opportunity to benefit from anti-EGFR mAbs.

EGFR-directed mAbs in combination with immunotherapy

Immunotherapy agents, or immune checkpoint inhibitors, are increasingly being used to treat patients with NSCLC, including SqCLC, and their use in combination with EGFR mAbs should be an important development for these patients. The identification of biomarkers to target the combinations to patients who will derive benefit is, therefore, an important step. Antibodies targeting the programmed death-1 (PD-1) receptor and its ligand, PD-L1, are among the currently approved immunotherapies for the treatment of patients with NSCLC, and several studies of immunotherapy agents for first-line treatment of patients with

NSCLC are currently ongoing (Table 1). Pembrolizumab, a PD-1 inhibitor, is the standard first-line treatment in patients with NSCLC with PD-L1 expression levels ≥50%, and it is also indicated for second-line treatment in patients with NSCLC whose tumors express PD-L1 in ≥1% of tumor cells [38]. Nivolumab and atezolizumab, PD-1 and PD-L1 inhibitors, respectively, are recommended as preferred second-line therapy for patients with NSCLC who have not previously received treatment with pembrolizumab [3, 39, 40]. Durvalumab is a PD-L1 inhibitor indicated for second-line therapy of patients with advanced urothelial cancer. Recent data suggest that it is likely to become the standard of care in patients with stage III NSCLC with no disease progression after platinum-based chemoradiation [41, 42].

Results from randomized phase III trials demonstrated that second-line treatment of patients with advanced NSCLC with pembrolizumab, nivolumab, and atezolizumab was superior to docetaxel with respect to OS after first-line treatment with platinum doublet chemotherapy [43–46]. Furthermore, the benefit of immunotherapy over chemotherapy was shown to increase with higher PD-L1 levels. Based on these findings, guidelines now recommend that patients with advanced metastatic disease be tested for PD-L1 expression once diagnosed [3]. However, routine implementation of PD-L1 testing in the clinical setting has been adversely affected by the different companion/complementary PD-L1 IHC assays that have been specifically developed for each of the approved anti-PD-1/anti-PD-L1 immunotherapies [47].

A phase Ib study (NCT02451930) has recently completed assessing the efficacy and safety of pembrolizumab and

Table 2. Biomarkers for use with mAb therapy directed against EGFR, PD-1/PD-L1, and VEGF/VEGFR in NSCLC

mAb	Biomarker	Evidence
EGFR	EGFR protein expression	Significant increase in OS for cetuximab plus chemotherapy versus cisplatin–vinorelbine chemotherapy in patients with NSCLC whose tumors have high EGFR expression [26] and favored OS hazard ratio for necitumumab plus cisplatin–gemcitabine versus cisplatin–gemcitabine in patients with SqCLC whose tumors express high levels of EGFR [25]
	EGFR gene copy number	Improved PFS and OS for treatment with cetuximab plus carboplatin–paclitaxel chemotherapy in patients with NSCLC, including SqCLC, whose tumors are *EGFR* FISH+ [36]
	EGFR mutation	No significant association between mutations and response to cetuximab with chemotherapy in patients with NSCLC, including SqCLC [34]
PD-1/PD-L1	PD-L1 expression	Increased clinical benefit from second-line pembrolizumab, nivolumab, and atezolizumab versus chemotherapy in patients with NSCLC whose tumors express higher PD-L1 protein levels [44–46]
	Tumor mutational burden	Increased clinical benefit from pembrolizumab, nivolumab, and atezolizumab in patients with NSCLC whose tumors have a high tumor mutational burden versus patients whose tumors have a lower mutation load [49–52]
VEGF/VEGFR	Biomarkers are not currently available	

EGFR, epidermal growth-factor receptor; FISH, fluorescence *in situ* hybridization; mAB, monoclonal antibody; NSCLC, non-small-cell lung cancer; OS, overall survival; PD-1, programmed death-1; PD-L1, programmed death ligand-1; PFS, progression-free survival; SqCLC, squamous cell lung cancer; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

necitumumab combination therapy for second-line treatment of PD-L1-selected patients with stage IV NSCLC [48]. Escalating doses of necitumumab (600–800 mg) with pembrolizumab (200 mg) were administered on day 1 and every 3 weeks. The results suggest modest activity for the combination in a population with a high proportion of patients with PD-L1-negative tumors: median (95% CI) PFS was 4.1 (2.4–6.9) months and the 6-month OS (95% CI) rate was 74.7% (61.5–83.9). No additional studies of EGFR mAbs in combination with immunotherapy could be identified in patients with advanced NSCLC.

Tumor-infiltrating lymphocytes and tumor mutational burden have recently emerged as potential biomarkers for assessing the likelihood of benefit from immunotherapy (Table 2). A role for tumor mutational burden as a biomarker is supported by the increased clinical benefit from pembrolizumab, nivolumab, and atezolizumab experienced by patients with NSCLC whose tumors have a high tumor mutational burden, as exemplified by a longer PFS for patients with NSCLC with a high mutational burden treated with pembrolizumab compared with those with a low tumor mutational burden (median 14.5 versus 3.7 months; HR = 0.19; 95% CI 0.05–0.70, respectively) [49–52]. However, clinical data for these biomarkers in NSCLC are currently limited, and they are, therefore, not currently implemented in clinical practice.

EGFR-directed mAbs in combination with antiangiogenic agents

Similar to EGFR signaling, angiogenesis has been showed to play an important role in tumor growth and survival. Therefore, agents targeting this pathway (such as bevacizumab and ramucirumab) have been proved to play a role in advancing the treatment of patients with NSCLC. Bevacizumab, a humanized mAb

directed against vascular endothelial growth factor (VEGF), was the first angiogenesis inhibitor approved for first-line treatment of patients with non-squamous NSCLC based on data from two studies that demonstrated ∼ 2 months of improvements in PFS and an improvement in OS, with results later replicated in Chinese patients with non-squamous NSCLC [53–55]. In a randomized phase II trial, cetuximab plus bevacizumab for the treatment of chemotherapy-naïve patients with advanced non-squamous NSCLC showed a prolonged median PFS of 6.05 months when combined with six cycles of chemotherapy [56]. It is important to note, however, that bevacizumab is contraindicated for treatment of patients with SqCLC due to a heightened risk of life-threatening pulmonary hemorrhage in this patient population [3, 57].

Ramucirumab, a mAb directed against VEGF receptor 2, has subsequently been approved for use in combination with docetaxel for the treatment of patients with metastatic platinum-resistant NSCLC, including SqCLC. Ramucirumab has been shown to be effective as second-line therapy, improving both OS and PFS. In the phase III REVEL study (NCT01168973), ramucirumab 10 mg/kg+docetaxel 75 mg/m^2 every 3 weeks resulted in a median (95% CI) OS of 10.5 (9.5–11.2) months compared with 9.1 (8.4–10.0) months with placebo+docetaxel [58]. No clinical trials combining ramucirumab therapy with EGFR-directed mAbs are currently ongoing in patients with NSCLC.

Currently, there is no clear consensus on which specific patient groups may derive benefit from combined therapy with EGFR and VEGF receptor mAbs, particularly in patients receiving concurrent EGFR mAbs, which supports the need to establish predictive biomarkers in this setting. Unfortunately there are no clinically validated biomarkers that are predictive of antiangiogenic effectiveness in NSCLC [59], and further clinical trials are needed to establish robust biomarker data.

Discussion

Conclusions

SqCLC is associated with a significant unmet need for additional therapeutic options. EGFR-directed mAbs necitumumab and cetuximab have been investigated for the treatment of patients with advanced NSCLC, including SqCLC, in several clinical settings. Treatment with EGFR mAbs combined with chemotherapy has been shown to significantly increase response rates and OS in patients with NSCLC, including SqCLC, although results may be considered clinically modest in the era of immunotherapy. These data strongly suggest a greater clinical benefit in patients with NSCLC, including SqCLC, whose tumors exhibit a high level of *EGFR* expression or gene copy number.

With multiple recent positive immunotherapy trials across different lines of treatment and different disease stages, the treatment landscape in NSCLC is rapidly changing. Two recent studies have shown superior PFS in patients with NSCLC treated with first-line platinum chemotherapy combined with a PD-1/PD-L1 mAb [60, 61]. Anticipating similar positive results in some of the ongoing trials assessing the efficacy of first-line platinum doublets combined with PD-1/PD-L1 mAbs in patients with advanced SqCLC (Table 1), it is reasonable to consider incorporating EGFR mAbs into chemo-immunotherapy regimens in biomarker-selected SqCLC patients. Similarly, biomarker studies in ongoing phase I/II studies evaluating PD-1/PD-L1 mAbs combined with EGFR mAbs may identify patients most likely to benefit from combined EGFR and PD-1/PD-L1 mAb treatment strategies in the first- or second-line settings.

Disclosure

PDB has acted as a consultant/advisor to Lilly and has received honoraria from, and acted as a consultant/advisor to, Biodesix, Bristol-Myers Squibb, Helsinn, and Merck and received honoraria from Celgene, Pfizer, and Roche/Genentech for safety/data monitoring committees. DG has acted as a consultant/advisor to Lilly and Merck. FRH has acted as a consultant/advisor to Bristol-Myers Squibb, Lilly, Pfizer, Merck, Ventana, and Roche/Genentech, and has received research funding from Bristol-Myers Squibb, Amgen, and Lilly/ImClone Systems. KMK has lecture fees and/or consultancy fees from AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Lilly, Merck Sharp & Dohme, Merck Serono, Novartis, Pfizer, Roche, and Roche Diagnostics. CO is an employee of Lilly. LP-A has received personal fees from AstraZeneca, Bristol-Myers Squibb, Clovis, Lilly, Roche, Novartis, Merck, Clovis Oncology, Amgen, and Pfizer. CB has nothing to disclose. JDB has received grants from ViewRay and Mevion Medical Systems, has acted as an advisor to ViewRay and Varian, and has received honoraria from AstraZeneca. PAB has acted as a consultant for AstraZeneca, Lilly, and Genentech and has received honoraria for data safety monitoring committees from Merck, Genentech, and Merck Serono. MC has nothing to disclose. JRJ is chief medical officer of Oncimmune. ESK has nothing to disclose. CJL has acted as a consultant/advisor to Lilly, AstraZeneca, Bristol-Myers Squibb, Merck, Roche/Genentech, Celgene, Takeda, Stemcentrx, Abbott, and Teva and has served on the data safety monitoring committees of Incyte, Peregrine, and Amgen. RBN has nothing to disclose. SN has participated in speaker bureaus for Boehringer Ingelheim, Merck, AstraZeneca, Bristol-Myers Squibb, and Roche. MP has acted as an advisor to Lilly, Merck, Bristol-Myers Squibb, AstraZeneca, and Roche/Genentech. SSR has acted as a consultant/advisor to Amgen, AbbVie, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Genentech, Lilly, and Novartis. MR has acted as a consultant/advisor to, and has participated in speaker bureaus for, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Lilly, Merck, Merck Sharp & Dohme, Pfizer, F. Hoffmann-La Roche, and Celgene. CHR has received personal fees from Lilly, Genentech, Boehringer Ingelheim, AstraZeneca, and Celgene. EFS has received research grants from AstraZeneca, Bristol-Myers Squibb, Roche, and Genentech and has served as a consultant/advisor for Lilly, AstraZeneca, Bristol-Myers Squibb, Bayer, Merck Sharp & Dohme, Pfizer, Novartis, and Roche. MAS has acted as a consultant/advisor to Lilly. DRS has nothing to disclose. JFV has received research funding from AstraZeneca and Merck Sharp & Dohme, has acted as an advisor to Apotex, AstraZeneca, Boehringer Ingelheim, Lilly, Merck Sharp & Dohme, and Novartis and has been a lecturer for AstraZeneca, Lilly, Merck Sharp & Dohme, and Novartis. HW has been an unpaid advisor to Roche/Genentech, Merck, and Clovis, has received research funding paid to her institution from Lilly, Roche/Genentech, ACEA, Celgene, Bristol-Myers Squibb, Clovis, Gilead, MedImmune/AstraZeneca, Pharmacyclics, Pfizer, Exelixis, and Xcovery, has received honoraria from Novartis for a lecture, and has served on a data safety monitoring committee for Peregrine. NT has received personal fees from Amgen, Celgene, OncoGenex, AstraZeneca, Roche/Genentech, and Otsuka and has acted as a consultant/advisor to Lilly.

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