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Chapter 11

EGFR-RELATED TARGETED THERAPY IN ESOPHAGEAL CANCER

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ABSTRACT

Esophageal cancer represents a significant global health problem. Esophageal squamous-cell carcinoma is the predominant type worldwide, accounting for about 90% of all cases. Due to late-stage detection and ineffective therapies, 5-year survival rates are approximately 15-25%. Overexpression of EGFR confers a poor prognosis in esophageal carcinoma, potentially providing an important therapeutic target for selected patients. Inhibition of EGFR pathway with monoclonal antibody or tyrosine kinase inhibitors is effective in other cancers (colon cancer and non-small cell lung cancer) and has been widely used in clinical practice. The strategy of anti-EGFR therapies is also currently under exploration in patients with esophageal cancer. Here, we discuss the preclinical rationale for targeting human EGFR and recent clinical reports of targeted agents in esophageal cancer.

INTRODUCTION

Esophageal cancer is a significant global health problem. An estimated 455,800 new esophageal cancer cases and 400,200 deaths occurred worldwide in 2012. Esophageal cancer includes two main subtypes, squamous-cell carcinoma (SCC) and adenocarcinoma (AC). Although SCC accounts for about 90% of cases of esophageal cancer globally, the incidence and mortality rates of esophageal adenocarcinoma have been progressively increasing in several regions of North America and Europe [1]. Management is generally similar for the two types. In the localized-disease setting, fewer than half of the patients are cured by surgery, even with the addition of perioperative chemotherapy or chemoradiotherapy [2]. For

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patients whose condition is declining or who are not healthy enough to undergo esophagectomy, definitive concurrent chemoradiotherapy has been widely recognized as a viable option; in the Radiation Therapy Oncology Group (RTOG) 8501 trial, a long-term survival rate of approximately 25% was reported using concurrent chemotherapy with cisplatin and fluorouracil [3]. In the metastatic-disease setting, palliative chemotherapy improves survival compared with supportive care alone; however, these treatments only result in a median survival of 9-12 months in randomized controlled trials [4]. To improve the outcome of patients with esophageal cancer it is imperative that more effective treatment strategies be developed.

GENETIC STATUS OF EGFR

The epidermal growth factor receptor (EGFR) is a commonly expressed trans-membrane glycoprotein of the tyrosine kinase growth factor receptor family, the principal ligands for which are the epidermal growth factor (EGF), transforming growth factor α (TGF-α), and amphiregulin. When a ligand binds to its extracellular domain, the EGFR is activated, and results in autophosphorylation and activation of downstream molecules such as Ras, ErK, PI3K, and Akt. In tumorigenesis, EGFR plays an important role in that it promotes growth of cells and is highly expressed in a variety of solid tumors, with overexpression observed in 50% to 70% of esophageal cancers, which may be correlated with poor prognosis and inferior response to therapy [5-7].

It is known that esophageal cancer predominantly shows EGFR gene copy number alterations and protein overexpression, with relatively uncommon EGFR mutation. EGFR amplification seems to be more frequent in esophageal cancer, having been identified in 21% of adenocarcinomas and 12-28% of esophageal SCCs [8, 9]. EGFR overexpression is seen in approximately one third to half of all operable esophageal adenocarcinomas [10]. A higher frequency of overexpression (71%) has been reported in resected esophageal SCC [11]. Additionally, activating mutations in exons 18–21 of EGFR have been found in 11.7% (2/17) of esophageal cancer cases [12].

The presence of EGFR alteration in esophageal cancer provided the rationale for investigation of the role of target-directed therapy as monotherapy or in the setting of a combination of chemotherapy and chemoradiation. The activities of two types of agents targeting the EGFR signaling pathway are currently being explored in prospective clinical trials: the anti-EGFR monoclonal antibodies cetuximab, panitumumab and nimotuzumab, and the small molecule tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib.

ANTI-EGFR IN ADVANCED DISEASE

Second Line Therapy

Oral TKIs compete with adenosine triphosphate for binding to the receptors of tyrosine kinase domain, inhibit the enzyme's ability to autophosphorylate, and block the receptor-

dependent signaling cascade. Both erlotinib and gefitinib have been evaluated as monotherapy in second-line therapy in esophageal cancer.

In a phase II trial, 36 patients who had failed prior chemotherapy received gefitinib of 500 mg/d, and response was evaluated every 8 weeks. One patient (2.8%) achieved a partial response, 10 (27.8%) had stable disease, and the progression-free survival was 2 months with overall survival of 5.5 months. In a subgroup analysis, a higher disease control rate (response plus stable disease) was observed in females (P = 0.038) and in patients with SCC (P = 0.013) or high EGFR expression (P = 0.002) [13]. Adelstein et al. reported their results in patients with AC or SCC from a phase II trial of gefitinib. Fifty-eight patients (including 18 who were chemotherapy-naïve) received gefitinib 250mg daily for a minimum of 8 weeks. So far, four patients (7%) have a PR and ten patients (17%) have stable disease, resulting in 24% disease control with a mean duration of 6.1 months. The overall survival for all patients was 5.5 months [14]. Although gefitinib has a modest activity in second-line treatment of advanced esophageal cancer, the small number of patients precludes any definitive conclusion from these studies.

A phase III study (COG) randomizing patients with ESCC and adenocarcinoma to gefitinib versus placebo after one or two lines of chemotherapy has recently been carried out, with 224 patients allocated gefitinib and 225 allocated placebo included in the final analyses [15]. Overall survival did not differ between groups (median 3.73 months for gefitinib vs 3.67 months for placebo, p=0.29). Median progression-free survival was marginally longer with gefitinib than it was with placebo (1.57 months vs 1.17 months, p=0.020). Odynophagia was significantly better in the gefitinib group (p=0.004). Although there was no significant difference between gefitinib and placebo for the primary outcome, in respect of overall survival, gefitinib was well-tolerated and improved progression-free survival, disease control, and patient-reported outcomes. The data suggested that a subgroup of patients might gain clinically significant benefits from this treatment, and identification of a predictive biomarker for this gefitinib-responsive subgroup of patients is important. Subsequent translational research on tumor specimens from COG to identify predictive biomarkers for gefitinib (TRANSCOG study) is ongoing.

A phase II study of erlotinib monotherapy in previously treated esophageal cancer was also reported. Of the 30 patients included in the trial, six (20%) with EGFR-negative tumors and 24 (80%) with EGFR overexpressing tumors were treated. Two partial responses were observed in the EGFR-positive cohort (8%), and no responses were observed in the EGFR-negative cohort. No correlation between EGFR status and degree of expression with erlotinib efficacy could be established, possibly due to the small number of patients. Similarly to gefitinib, erlotinib shows more activity in SCC. Two patients with SCC obtained a response, which lasted for 5.5 and 7 months. The median time to disease progression in the study was 3.3 months in SCC and 1.6 months in adenocarcinomas [16]. Actually, a number of different characteristics have been confirmed between esophageal pathological phenotypes in comparative analysis [17]. This should be considered in the future trial before the presence of specific predictive molecular characteristics in esophageal cancer.

Cetuximab, a chimerized antibody of the IgG1 subclass, was originally derived from a mouse myeloma cell line. Cetuximab blocks binding of EGF and TGFα to EGFR and inhibits ligand-induced activation of this tyrosine kinase receptor. Cetuximab also stimulates EGFR internalization, effectively removing the receptor from the cell surface for interaction with ligand.

In a phase II trial, cetuximab was investigated as second-line therapy in patients with metastatic esophageal adenocarcinoma. Patients received cetuximab 400mg/m2 IV on week one and 250 mg/m2 IV weekly thereafter. A total of 55 patients enrolled on this trial achieved an overall 6-month survival probability of 36% (thus failing to reach the trial's objective of 42%). Overall survival was only 4.0 months, and progression-free survival was 1.8 months. However, for the nine patients with either a partial response or stable disease, the median overall survival was 8.3 months, and the median progression-free survival was 4.0 months [18].

Overall, interesting activity with acceptable toxicity of anti-EGFR agents is seen in these initial studies. More definitive results from larger trials are expected. Well-validated biomarkers will certainly help to define subgroups of patients that will benefit most.

First Line Therapy

In the first-line therapy for metastatic esophageal cancer, monotherapy with oral TKIs or antibodies has seldom been conceived. This may be due to the unsatisfactory performance of anti-EGFR therapy alone in the second-line setting. TKIs have been extensively evaluated in combination with standard chemotherapy regimens in chemotherapy-naive patients with advanced non-small cell lung cancer with no benefit in the response rate, progression-free survival, or overall survival [19, 20]. One possible explanation is that TKIs induce cell cycle arrest in the G1 phase, which makes the cells less sensitive to cytotoxic agents. However, positive results have been found when cetuximab was combined with chemotherapy in other tumors, e.g., advanced colorectal adenocarcinomas and head and neck SCC, and this approach has also been investigated in metastatic esophageal cancer.

In a randomized phase II study, the activity of cetuximab in combination with cisplatin and 5-fluorouracil (CF) chemotherapy was assessed in advanced esophageal SCC [21]. Sixty-two eligible patients were included, 32 receiving cetuximab plus CF and 30 CF. The primary endpoint of overall response rate was 19% and 13%, and the disease control rate 75% and 57% for the two arms respectively. A trend toward longer progression-free survival (5.9 mo vs 3.6 mo) and overall survival (9.5 mo vs 5.5 mo) was noted in the cetuximab arm. Interestingly, cetuximab did not exacerbate grade 3 or 4 toxicities, except for rash and diarrhea. However, the progression-free and overall survivals in the standard arm were much lower than expected in this setting, and the study was not powered to detect a survival difference.

In another randomized phase II study (CALGB 80403/ECOG 1206), three chemotherapy regimens were evaluated to determine what chemotherapy is best in combination with cetuximab in metastatic esophageal and gastroesophageal junction cancer [22]. Patients were randomized to epirubicin, 5-FU, cisplatin or 5-FU, folinic acid, oxaliplatin (FOLFOX) or irinotecan/cisplatin. All three arms received concomitant cetuximab. The majority of patients (approximately 90%) had esophageal adenocarcinoma. Results have been presented so far in an abstract form for the adenocarcinoma patients. The two first arms were more effective (response rates greater than 50%) and the FOLFOX arm less toxic. Conclusions regarding the clinical utility of adding cetuximab to first-line chemotherapy are awaiting information from ongoing randomized phase III trials.

The other clinically available anti-EGFR monoclonal antibody, panitumumab, has been investigated in a phase I dose-finding study examining its use in combination with epirubicin, oxaliplatin, and capecitabine (EOC) chemotherapy in advanced esophagogastric cancer [23]. Because of the excessive toxicity (primarily diarrhea), the recommended dose for EOC plus panitumumab was modified to epirubicin 50 mg/m2, oxaliplatin 100 mg/m2, capecitabine 1000 mg/m2/d, and panitumumab 9 mg/kg every 3 weeks. This dose has been selected for the subsequent phase II/III REAL3 study. The UK National Cancer Research Institute REAL3 study evaluated the effect on overall survival with the addition of panitumumab to EOC in patients with advanced-stage esophagogastric cancer [24]. Between 2008 and 2011, the study enrolled 553 eligible patients. Median overall survival in 278 patients allocated panitumumab group was 8.8 months compared with 11.3 months in 275 patients allocated to the control group (p=0.013). The addition of panitumumab was associated with increased incidence of grade 3-4 diarrhea (17%), rash (11%), mucositis (5%), and hypomagnesaemia (5%). In the prespecified subgroup analysis of patients with esophageal cancer, a similar effect disfavoring panitumumab was also noted (n = 217 patients, HR 1.32, 95%CI 0.90-1.94). Based on the findings of REAL3, use of panitumumab in combination with EOC cannot be recommended in an unselected population with advanced esophagogastric adenocarcinoma, and was associated with inferior overall survival. The possible explanations for the negative results need to be considered, including the reduced dose intensity of chemotherapy, the potential negative interaction between panitumumab and one or more of the EOC components, and the molecularly unselected population.

A third investigational anti-EGFR antibody, nimotuzumab, has been studied in patients with ESCC in the first line metastatic setting. Nimotuzumab in combination with paclitaxel and cisplatin was tested in a phase II study. The preliminary results showed a 63.6% partial response rate and 31.8% stable disease in 25 patients [25]. In another phase II trial, the short-term effect and adverse reaction of nimotuzumab in combination with 5-FU and cisplatin on advanced ESCC was also investigated. Sixteen out of a total of 19 patients can be evaluated; partial response of 42.1% and disease control rate of 68.4% were reported [26].

Matuzumab (EMD72000) is also an anti-EGFR monoclonal antibody. In the randomized phase II MATRIX trial, the efficacy of matuzumab was evaluated in combination with epirubicin, cisplatin, and capecitabine (ECX) versus ECX alone. Thirty-five patients received ECX/matuzumab and 36 patients ECX. Addition of matuzumab to ECX did not improve objective response, with a trend towards inferior outcome in terms of median PFS (4.8 months versus 7.1 months) and overall survival (9.4 months versus 12.2 months). The lack of benefit may have been related to the relatively low maximum tolerated dose of matuzumab (800 mg weekly), which may not have saturated EGFR occupancy [27].

Collectively, these findings suggest that the EGFR pathway does not represent a good therapeutic target in unselected patients with esophageal adenocarcinoma or ESCC. However, ongoing tissue analyses might identify a biomarker-defined subgroup of patients who could derive benefit from this treatment strategy.

Anti-EGFR in Local-Advanced Disease

Definitive chemoradiation is an effective treatment modality for esophageal cancer, with clear evidence that chemoradiation can improve survival and cure patients. Although cure

rates have improved, further improvements are greatly needed. The development of targeted therapies in esophageal cancer has primarily been in the metastatic setting, followed by introduction into chemoradiation. Anti-EGFR therapy has shown some activities in metastatic esophageal cancer, including adenocarcinoma and SCC, due to the high expression of EGFR. This approach was then moved to and explored in the locally advanced setting.

In the development of tolerance to irradiation, an intracellular phosphorylation cascade starting from autophosphorylation of EGFR has been recognized as an important signaling pathway associated with cell survival [28]. Low-dose irradiation has been shown to lead to autophosphorylation of EGFR and activate mitogen-activated protein kinase (MAPK), resulting in an increased tumor cell proliferation response [29]. The phosphoinositide-3 kinase (PI3K)-Akt is another signaling pathway downstream of EGFR, associated with anti-apoptosis effect [30]. Given that the MAPK and PI3K-Akt pathways lead to radiation resistance through activation in an EGFR-dependent manner, EGFR inhibitors may be promising radio-sensitizing agents. In one preclinical report, a synergistic antitumor effect from the combination of gefitinib with radiotherapy has been identified in esophageal SCC cell lines [31]. Further work in cell culture and xenograft models has shown that radiation-induced EGFR phosphorylation and tumor proliferation could be effectively blocked by the addition of an EGFR-signaling inhibitor. Notably, preclinical studies have also shown that cetuximab can reverse resistance to radiation in established radioresistant esophageal carcinoma cell; the mechanisms may include cell cycle perturbation and enhancement of radiation-induced apoptosis [32]. The results suggest that the inhibition of EGFR pathways as a radiosensitizing strategy in cancer management may have potential value in clinical practice.

Bonner et al. demonstrated that radiotherapy plus cetuximab improved median and overall survival and significantly enhanced local tumor control compared to radiotherapy alone in SCC of head and neck cancer [33]. This approach was investigated in a group of esophageal SCC which presented intolerance to chemoradiotherapy because of advanced age or malnutrition. In the pilot study, the safety and efficacy of concurrent erlotinib and radiotherapy was tested in 18 patients [34]. The median time of overall survival and progression-free survival was 21.1 and 12 months, respectively. Two-year overall survival, progression-free survival, and local-regional relapse-free survival were 44.4%, 38.9%, and 66.7%, respectively. Grade 3 esophagitis and skin rash were observed in five (27.8%) and two (11.1%) patients, respectively. Radiation pneumonitis of grades 2 and 5 was observed in one patient each. No grade 3/4 impaired liver function or hematological toxicity was observed. The results indicated that concurrent erlotinib and radiotherapy are tolerable and effective.

Concurrent chemoradiation is the standard of care for locally advanced esophageal cancer, and the question of whether or not the addition of anti-EGFR therapy can further improve the treatment outcome has also been explored. In a phase II study, 80 patients with locally advanced esophageal and EGJ cancers (the majority of the patients with adenocarcinomas) were treated with gefitinib added to perioperative concurrent chemoradiation [35]. Gefitinib did not increase toxicity, except for development of rash in 42 (53%) and diarrhea in 44 (55%). Compared with a historical series of 93 patients given concurrent chemoradiation without gefitinib, 3-year overall survival was marginally improved (42% versus 28%, $p = 0.06$). Patients who experienced gefitinib-related diarrhea appeared to have improved outcomes.

Erlotinib has similarly been evaluated in combination with chemoradiotherapy. In a phase II trial, 62 patients were treated with neoadjuvant chemoradiation plus erlotinib and bevacizumab [36]. The reported pathologic complete response rate of 29% and median overall survival of 30 months were comparable with historical controls treated with chemoradiation alone, suggesting little role for the combination, at least in an unselected patient population. However, in another phase II study in patients with locally advanced esophageal SCC, 24 patients received 60 Gy of RT in 30 fractions along with paclitaxel and cisplatin [37]. Erlotinib 150 mg/day was given concurrently on days 1 through 42. Acute grade 3 toxicities included leukopenia, esophagitis, and skin rash; four patients were unable to complete both cycles of chemotherapy. Twenty-two patients responded to therapy, including 11 patients with CRs. The 2-year overall survival and loco-regional control rates were 87.5 and 70.1%, respectively. These initial results suggest that this regimen has potential to enhance local control and improve survival in patients with esophageal cancer. Based on this promising result, a phase III trial of chemoradiotherapy plus erlotinib versus chemoradiotherapy in esophageal SCC is ongoing (NCT00686114). The study was a 2x2 factorial design, with erlotinib and elective nodal irradiation as factors. The preliminary result of this study was released in the 2014 European Society for Medical Oncology. At the interim analysis, 195 patients were recruited and 104 death events occurred. Although no significant difference in median overall survival was found between the two groups with or without erlotinib (20.0 months versus 17.8 months, $p = 0.37$), a highest 2-year survival rate of 61.6% and a median survival of 40.2 months was observed in patients treated with elective nodal irradiation plus erlotinib. The final analysis is expected.

Chemoradiation with cetuximab has been extensively studied in phase II trials, including in the neoadjuvant setting. In the Swiss Group for Clinical Cancer Research phase Ib/II trial (SAKK 75/06), 28 patients with locally advanced esophageal adenocarcinoma or SCC were treated with induction cisplatin, docetaxel, and cetuximab followed by radiation (45 Gy) along with concurrent cisplatin and cetuximab [38]. A promising result of 32% complete response rate was reported with this regimen, and 19 patients (68%) showed complete or near complete pathologic regression. Surgery (R0 resection) was performed in 25 patients; no deaths at 30 days and no treatment-related mortality after 12 months were observed. However, this result was in contrast to the findings of the Eastern Cooperative Oncology Group (ECOG) 2205 trial, which evaluated a neoadjuvant regimen of cetuximab in combination with 5-FU/oxaliplatin and radiotherapy in patients with operable esophageal adenocarcinoma [39]. The ECOG 2205 trial was prematurely closed after an excessive number of early deaths: four of 18 patients who underwent surgery died postoperatively as a result of acute respiratory distress syndrome (ARDS). The doses of cetuximab and radiation used were similar between the two trials. The difference in toxicity of ECOG 2205 and SAKK 75/06 might be explained by the radiosensitizing chemotherapy consisting of oxaliplatin and infusional fluorouracil in the ECOG 2205 trial. Because of the nature of the phase II trial of SAKK 75/06 and the small sample size, efficacy results should be interpreted with caution. Nevertheless, preliminary findings demonstrated with this EGFR-targeting preoperative regimen for esophageal cancer are promising, and have prompted the initiation of the phase III trial (SAKK 75/08) to test the efficacy of the described regimen against the same regimen without cetuximab.

The approach of cetuximab combined with definitive chemoradiotherapy has also been tested in numerous studies. The results of the SCOPE1 study, a 2-arm randomized phase II/III

study comparing cisplatin/capecitabine/radiation with or without cetuximab, have recently been reported [40]. After 258 patients (129 assigned to each treatment group) were recruited, the study was stopped without continuation to phase III because of inutility. Fewer patients were treatment failure-free at 24 weeks (the primary endpoint for the phase 2 trial) in the cetuximab group (66·4%) than in the chemoradiotherapy only group (76·9%). The cetuximab group also had shorter median overall survival (22.1 months vs 25.4 months; p=0.035). Patients who received cetuximab had more non-hematological grade 3 or 4 toxicities (79% vs 63%; p=0.004). Therefore, the addition of cetuximab to chemoradiotherapy resulted in more toxicity, less protocol treatment being delivered, and worse overall survival than with chemoradiotherapy alone. The negative outcome in the SCOPE1 study seems to be a result of tumor-specific interactions and biology that are not fully understood, or overlapping toxicities that preclude the delivery of effective standard treatment. However, the 2-year overall survival in all patients was 49%, and 56% in those receiving chemoradiotherapy only. The very encouraging outcomes seen with definitive chemoradiotherapy alone should provide an excellent platform to test more targeted therapeutic approaches, incorporating biomarker-driven systemic therapies and newer radiotherapy technologies to safely intensify treatment.

Radiation Therapy Oncology Group 0436 is a randomized phase III trial of cisplatin, paclitaxel, and radiation therapy to 50.4 Gy with or without cetuximab in inoperable esophageal cancer. In 2012, the investigators undertook a planned interim analysis to document superiority of the cetuximab arm as measured by clinical complete response rate. Disappointingly, the study failed to meet this end point and closed to further adenocarcinoma enrollment.

Nimotuzumab has been approved for the treatment of head and neck tumors and glioma in several countries and is under clinical trials for other solid tumors. For esophageal cancer, nimotuzumab has been found to enhance the radiosensitivity of esophageal SCC cells in in vitro assay [41]. Recent clinical studies have demonstrated that nimotuzumab in combination with irradiation is safe and tolerable and that it yields encouraging outcomes in ESCC patients. In a retrospective analysis of 66 patients with esophageal SCC treated with a combination of nimotuzumab and radiation or chemoradiation, 52 of the 66 patients received nimotuzumab combined with chemoradiation and 14 received nimotuzumab plus radiation [42]. Patients tolerated the treatment well. Grade 3-4 adverse events and toxicities occurred in 50% of the patients. The median overall survival and progression-free survival were 26.0 months and 16.7 months, respectively. More clinical data are needed to confirm this result.

FUTURE DIRECTIONS

It is well known that overexpression of EGFR is associated with poor prognosis in esophageal cancer. Accordingly, several EGFR inhibitors have been explored in esophageal cancer during recent decades, including locally and advanced setting. Although results less favorable than expectations for the entire population investigated were observed in these trials, there were subgroups of patients who might obtain benefits from EGFR inhibitors. In metastatic colorectal cancer, the efficacy of the anti-EGFR antibodies is limited to patients without activating mutations in KRAS, BRAF and PIK3CA. Similarly, esophageal cancer patients with certain biological characteristics may do better with EGFR inhibitors, and these

characteristics, especially molecular biomarkers, may be useful for stratification and outcome prediction.

Given the nature of anti-EGFR therapies, EGFR gene variation and protein overexpression might be a candidate for predictive biomarker in esophageal cancer. At present, no positive relationship has been found between protein overexpression (detected by immunohistochemistry) and response to anti-EGFR treatment. These results may be due to the heterogeneity of study populations or lack of a standardized assay for determining EGFR status. In a recently reported study evaluating EGFR protein expression using 6 representative scoring systems in esophageal SCC, a great disparity was found, and only 1 scoring system significantly correlated with overall survival [43]. EGFR gene copy number variation may be more reliable than protein expression in predicting prognosis. However, reports on the relationship between gene copy number and prognosis have been equivocal and the predictive of response to anti-EGFR therapy has not been clear. Although EGFR-activating mutations correlate with efficacy of EGFR TKIs in the treatment of patients with NSCLC, the prevalence of these mutations in esophageal cancer is not understood well.

As for p-Akt and p-Erk, two phosphorylated proteins respectively essential for the activation of two main EGFR downstream signaling pathways, a few clinical studies have tested their predictive value in regimens containing EGFR inhibitors. In a Phase II trial of erlotinib in gastroesophageal junction and gastric adenocarcinomas, no correlation was found between p-Akt and clinical outcome [44]. However, in an exploration study based on 32 tumor samples to identify the subgroup of ESCC patients who are sensitive to nimotuzumab therapy, significantly better overall survival was observed in patients with coexistence of high EGFR expression and low p-Akt expression ($p = 0.030$), indicating that high EGFR and low p-Akt expression may predict a clinical benefit of EGFR antagonists such as nimotuzumab combined with radiotherapy or chemoradiotherapy [45].

The predictive value of KRAS gene mutation to EGFR-targeted antibodies (cetuximab and panitumumab) was not consistent in solid tumors. Mutations in the KRAS gene have been shown in multiple phase III trials to predict lack of efficacy in patients with metastatic colorectal cancer. However, the addition of cetuximab to paclitaxel plus carboplatin in NSCLC did not improve overall survival when KRAS was not mutated. The exact prevalence of KRAS mutations in esophageal cancer is unclear, with reported rates of 3-30% [46]. In the REAL 3 study, the incidence of KRAS mutations and its potential effect on panitumumab efficacy in the first 200 patients was pre-planned to determine whether molecular selection for the ongoing study was indicated [47]. KRAS, BRAF and PIK3CA mutations and PTEN expression were assessed in pre-treatment biopsies. Results from 175 assessable biopsies showed: mutations in KRAS (5.7%), BRAF (0%), PIK3CA (2.5%), and loss of PTEN expression (15.0%). There was no significant association between any molecular change and response to panitumumab plus chemotherapy. This contrasts with the effect of KRAS mutations in metastatic colorectal cancer; possibly due to the small sample sizes of the trials and relative rarity of KRAS, BRAF, and PIK3CA mutations. Further biomarker analyses are ongoing in the full trial cohort.

Increasingly, biomarker identification is an integral part of the development of new anticancer drugs. The success of TKI in non-small-cell lung cancer and cetuximab in colorectal cancer are good examples for the development of anti-EGFR therapies in esophageal cancer. However, no encouraging positive result was found in completed phase III trials, including locally and advanced settings, which may be related to unselected population

and suboptimal combination with chemotherapy or chemoradiotherapy. Biomarkers to predict anti-EGFR efficacy are urgently required with the development of this strategy in esophageal cancer. The continued search for biomarkers in blood and tumor tissue, or via functional imaging, is essential to increase treatment efficacy in esophageal cancer. Techniques such as gene-expression profiling and next-generation sequencing might help to provide further information regarding the driver genetic events in this disease. Furthermore, the evaluation of genetic aberrations in pathways linked to EGFR signaling could still offer the prospect of identification of a low-frequency biomarker that identifies a subpopulation of patients benefiting from anti-EGFR targeted therapy in this setting.

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