Targeted Therapies for Non–Small Cell Lung Cancer: An Evolving Landscape

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Abstract

Over the past decade, a multitude of targeted agents have been explored in the treatment of advanced non–small cell lung cancer (NSCLC). Thus far, two broad classes of agents have been implemented in clinical practice: (a) vascular endothelial growth factor (VEGF)-directed therapies and (b) antagonists of the epidermal growth factor receptor (EGFR). In the former category, the agent bevacizumab (a monoclonal antibody) has shown landmark improvements in survival when added to cytotoxic therapy. Small molecule tyrosine kinase inhibitors (TKI) targeting the VEGF receptor (i.e., sunitinib, sorafenib, and vandetanib) show activity in phase II clinical studies. With respect to EGFR-directed therapies, the TKIs gefitinib and erlotinib have shown significant benefit, and have uncovered valuable information about the biology of lung cancer. Outside of therapies directed specifically at VEGF- and EGFR-mediated signaling, trials evaluating insulin-like growth factor-1 receptor (IGF-IR)-targeting agents, cyclooxygenase-2 (COX-2) inhibitors, c-met inhibitors, irreversible pan-HER inhibitors, mammalian target of rapamycin (mTOR) inhibitors, and histone deacetylase (HDAC) inhibitors are ongoing. Inhibitors of ALK show great promise in patients with the relevant gene translocation. Herein, the clinical development of novel therapies for NSCLC is described, including some discussion of relevant biomarkers and determination of synergy with both cytotoxic therapy and other targeted agents.

Introduction

A decade ago, oncologists struggled to determine the optimal platinum-containing doublet for the treatment of metastatic non–small cell lung cancer (NSCLC). Trials to assess the subject abounded, and the resulting data left the oncologist in a state of clinical equipoise (1, 2). Fortunately, with a greater understanding of tumor biology, numerous targeted agents have emerged to address the apparent plateau achieved with cytotoxic therapy. In the clinic, monoclonal antibodies and tyrosine kinase inhibitors (TKI) directed at vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) signaling have had the greatest tangible effect. Novel therapies targeted to ALK translocations in lung cancer have recently been developed. The agent PF-02341066, which targets the EML4-ALK fusion protein, has shown promising activity in NSCLC in a phase I clinical trial (3). Furthermore, on the horizon are a number of novel agents directed at unique molecular targets, including pan-HER inhibitors, insulin-like growth factor-1 receptor (IGF-IR)-targeting therapies, cyclooxygenase-2 (COX-2) inhibitors, c-met inhibitors, irreversible pan-HER inhibitors, mammalian target of rapamycin (mTOR) inhibitors, and histone deacetylase (HDAC) inhibitors (summarized in Fig. 1). Herein, the enlarging portfolio of clinical trials to facilitate development of these agents is described.

VEGF- and VEGFR-Directed Therapies

Monoclonal antibodies

Bevacizumab. Bevacizumab, a monoclonal antibody with specificity for VEGF, has improved clinical outcome in a wide spectrum of malignancies, including breast cancer, glioblastoma multiforme, colon cancer, and ovarian cancer (4–7). Likewise, several studies support the use of bevacizumab in NSCLC. A randomized, phase II trial showed improvement in response rate (RR; 31.5% versus 18%), and median overall survival (OS; 17.7 versus 14.9 months), with the addition of bevacizumab to carboplatin and paclitaxel chemotherapy (8). Subsequent to these results, the phase III Eastern Cooperative Oncology Group (ECOG) 4599 trial randomized in 878 patients to carboplatin-paclitaxel with or without bevacizumab, excluding patients with squamous cell histology because of increased risk of pulmonary hemorrhage (9). Patients with advanced or recurrent nonsquamous NSCLC
received six cycles of chemotherapy. In patients receiving bevacizumab, the treatment was administered as maintenance therapy following the completion of chemotherapy until evidence of disease progression or intolerable adverse effects. As in the phase II experience, OS was improved with the addition of bevacizumab (12.3 versus 10.3 months, \( P = 0.003 \)). Notably, the publication of ECOG 4599 marked the first report from a randomized, phase III trial of survival in excess of 1 year in the setting of metastatic NSCLC. Key exclusion criteria in this study included brain metastases, squamous histology, and the presence of hemoptysis. Though these criteria remain relevant to clinical practice, the prospective PASSPORT trial suggests the safety of bevacizumab in the setting of brain metastases (10). In this study, treatment-naïve patients with previously treated brain metastases received bevacizumab with platinum-based doublet therapy or erlotinib, at the physician’s discretion. Second-line patients received either bevacizumab with single agent chemotherapy or erlotinib, also at the physician’s discretion. With 106 safety-evaluable patients, there were no reported episodes of grade \( \geq 2 \) central nervous system hemorrhage. Furthermore, two grade 5 events were noted in bevacizumab-treated patients, both were pulmonary hemorrhage.

Several studies have aimed to determine the efficacy of distinct platinum doublets in combination with bevacizumab. The phase III AVAiL trial compared cisplatin and gemcitabine with either placebo, low-dose bevacizumab (7.5 mg/kg), or high-dose (15 mg/kg) bevacizumab (11). With 1,043 patients enrolled, the duration of follow-up thus far is insufficient to assess OS (the study’s primary endpoint; ref. 12). However, published results from this trial indicate an improvement in progression-free survival (PFS) with both high-dose bevacizumab (6.7 versus 6.1 months, \( P = 0.003 \)) and low-dose bevacizumab (6.5 versus 6.1 months, \( P = 0.03 \)), as compared with placebo. The use of two dose levels of bevacizumab with comparable efficacy results has elicited some degree of controversy about which level represents the optimal approach. Other platinum doublets have also shown promise in combination with bevacizumab. For instance, impressive phase II data for the combination of carboplatin, pemetrexed, and bevacizumab (reporting a RR of 55%) have spurred a phase III effort assessing the three-drug combination (13, 14).

Numerous efforts have focused on identifying subgroups of patients that may obtain particular benefit from the addition of bevacizumab to chemotherapy. Biomarker studies accompanying ECOG 4599 suggest that...
single nucleotide polymorphisms in VEGF, EGF, intercellular adhesion molecule-1 (ICAM-1), and WNK lysine deficient protein kinase 1 (WNK1) may predict response (15). As in other malignancies (i.e., breast cancer and pancreatic cancer), hypertension is additionally emerging as a biomarker of clinical benefit from bevacizumab (16, 17). Patients enrolled in ECOG 4599 who developed high blood pressure with bevacizumab therapy had a statistically significant improvement in OS as compared with patients who did not [hazard ratio (HR) 0.60, 95% confidence interval (CI) 0.43-0.81; P = 0.001; ref. 18].

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Other subset analyses paired with this trial include an extensive examination of age (19). In total, 224 patients enrolled in ECOG 4599 were over the age of 70 (26%). As compared with chemotherapy alone, there was a non-statistically significant improvement in RR (29% versus 20%, P = 0.063) with the addition of bevacizumab in this group. Grade 3 to 5 neutropenia, proteinuria, and bleeding did occur more frequently among older adults as compared with the remainder of the study population. A question remains about the true benefit of bevacizumab in a population of older adults with NSCLC.

**Aflibercept.** Aflibercept is a monoclonal antibody constructed from domains encompassed in VEGFR1 and VEGFR2, with a high affinity for VEGF (20). A phase I clinical trial of aflibercept showed dose-limiting toxicities of rectal ulceration and proteinuria at a 7 mg/kg dose intravenously every 2 weeks; therefore, 4 mg/kg has been established as the recommended phase II dose (21). In this study (including 47 patients with advanced solid tumors), three RECIST responses were noted. In a phase II clinical trial in patients previously treated with platinum-based chemotherapy and erlotinib, safety data are available for 33 patients thus far (22). The regimen (at a dose of 4 mg/kg intravenously every 2 weeks) seems to be safe and well tolerated, with no significant hemoptysis. Other ongoing efforts are exploring the role of aflibercept in lung cancer in combination with platinum-based doublets and single-agent docetaxel (20).

**Small molecule tyrosine kinase inhibitors**

**Sunitinib.** The small molecule inhibitor sunitinib targets a wide spectrum of membrane receptors, including VEGF receptor (VEGFR)-1, VEGFR-2, fetal liver tyrosine kinase receptor 3 (FLT3), stem cell factor receptor (SCF receptor, or KIT), platelet-derived growth factor receptor-alpha (PDGFR-alpha), and PDGFR-beta (23, 24). The drug has been approved for renal cell carcinoma (RCC) on the basis of phase III data (25, 26). In NSCLC, a phase II study in patients who failed platinum-based chemotherapy yielded an overall RR of 11.1% with sunitinib, comparable to other agents approved for second-line therapy (i.e., docetaxel, erlotinib, and pemetrexed; refs. 27-30). Trials exploring the combination of sunitinib with cytotoxic therapy are ongoing; as one example, the combination of cisplatin and gemcitabine with sunitinib (currently being explored in a phase I study) seems to be well tolerated (23). Unfortunately, data from phase III studies incorporating sunitinib have elicited concerns related to toxicity. A clinical trial of carboplatin, paclitaxel, and bevacizumab with or without sunitinib (SABRE-L) was closed prematurely after accrual of only 56 patients (31). SABRE-B and SABRE-R studies were also conducted in breast and RCC, employing combinations of bevacizumab and sunitinib; these trials were similarly subject to early termination because of safety concerns (32, 33). These data may be reflective of other experiences documenting significant challenges in administering the combination of sunitinib and bevacizumab because of vascular and hematologic toxicities (34). The combination of sunitinib with and without erlotinib has also been assessed (SUN 1058 and 1087; refs. 35, 36). Sunitinib is also being evaluated as maintenance therapy among patients who have completed platinum-based chemotherapy (CALGB 30607; ref. 37).

**Sorafenib.** Sorafenib has an affinity for a wide range of membrane receptors, including VEGFR-2, VEGFR-3, KIT, and FLT-3 (38). Now approved for use in both advanced hepatocellular carcinoma and metastatic RCC (mRCC) on the basis of phase III data, the role of sorafenib in advanced NSCLC is currently being explored (39, 40). In a “window of opportunity” study, patients who had not previously received therapy for metastatic NSCLC were treated with sorafenib, dosed at 400 mg twice daily (41). In this study, patients were examined weekly during the first two 4-week cycles, and those who progressed rapidly went on to receive standard chemotherapy. With 25 patients enrolled, median PFS was 2.9 months and overall RR was 12%. The trial was designed in two stages, but did not meet stage I efficacy criteria to proceed on to the second stage. In a subsequent double-blind, phase II randomized discontinuation study, patients with two or more prior therapies were treated with sorafenib (42). Of 342 patients enrolled, 97 patients were noted to have stable disease (SD) and thus randomized to either placebo or sorafenib. A prolonged PFS was observed with sorafenib therapy (3.8 versus 2.0 months, P = 0.01). These phase II data have spurned phase III efforts assessing sorafenib in combination with cytotoxic chemotherapy. As one example, the phase III ESCAPE trial has assessed the combination of carboplatin and paclitaxel with or without sorafenib (43). Unfortunately, an interim analysis suggested no improvement in OS (the primary endpoint of the study) and possibly increased mortality in patients with squamous histology, leading to the study’s early closure. The NEXUS trial is also examining the use of sorafenib therapy in this setting (44). This is a randomized phase III trial comparing cisplatin and gemcitabine with sorafenib versus placebo. Patients may receive up to six cycles of therapy, with maintenance of sorafenib versus placebo following therapy. The primary endpoint of the study is OS and enrollment is complete.

**Vandetanib.** Vandetanib is a dual TKI targeting VEGFR2 and EGFR, although its activity is likely mediated primarily through VEGFR-2 (45, 46). Single agent
therapy with vandetanib has been assessed in a randomized, double-blind dose-finding trial (47). This Japanese study identified that doses of vandetanib at 100, 200, and 300 mg elicited RRs of 17.6%, 5.6%, and 16.7%, respectively. More recently, vandetanib monotherapy has been compared with both carboplatin-paclitaxel and carboplatin-paclitaxel with vandetanib as first-line therapy (48). In a 2:1:1 randomization, patients either received vandetanib, vandetanib with chemotherapy, or chemotherapy alone. In an interim analysis, single-agent vandetanib did not result in improved PFS as compared with chemotherapy alone, and this arm was therefore discontinued. Ultimately, no significant improvement in PFS or OS was seen with the addition of vandetanib to carboplatin-paclitaxel.

Data for use of vandetanib as second-line therapy are accumulating. In a randomized, phase II study in patients who had failed on platinum-based chemotherapy, the addition of vandetanib to docetaxel significantly prolonged PFS from 12.0 weeks to 18.7 weeks (P = 0.037; ref. 49). Subsequent to this result, data from a phase III analysis employing the same randomization in 1,391 patients suggested an improvement in PFS (HR 0.79, 95% CI 0.70-0.90, P < 0.001) and a trend toward improvement in OS (HR 0.91, 98% CI 0.78-1.07; P = 0.196; ref. 50). However, the phase III ZEAL study, randomizing 534 patients to pemetrexed and vandetanib or pemetrexed with placebo as second-line treatment, failed to show an improvement in PFS with a median follow-up of 9 months. Nonetheless, the addition of vandetanib did improve the overall RR from 7.9 to 19.1% (P < 0.001; ref. 51). In a direct comparison to an approved second-line agent, the ZEST study randomized 1,240 patients who had progressed on one or two prior regimens to receive either erlotinib or vandetanib (52). In a preliminary analysis, both PFS and OS in each arm were similar.

**Pazopanib.** Similar to sunitinib, sorafenib, and vandetanib, pazopanib is a multitargeted tyrosine inhibitor that has shown preclinical activity in NSCLC, targeting VEGFR-1, -2, and -3, PDGFR-alpha and -beta, and c-kit (53). A neoadjuvant trial of pazopanib in stage I-II NSCLC used volumetric reduction via high-resolution computerized tomography to evaluate response. Using this approach, a decrease in tumor volume was seen among 20 of 26 patients (87%) enrolled (54). Three patients had standard partial responses by RECIST criteria. Patients received between 2 to 6 weeks of pazopanib therapy in total prior to surgical resection. Several ongoing studies of pazopanib are evaluating combinations of the agent with paclitaxel, pemetrexed, and erlotinib; refs. 55-57). The future success of VEGFR-TKIs will require a better understanding of patient selection and targeting of these agents.

**EGFR-Targeting Therapies**

EGFR is frequently overexpressed in NSCLC, and both EGFR and other members of the ErbB family of receptors (HER2, HER3, and HER4) may have a prognostic role in NSCLC (58, 59). Numerous agents have been developed to target this moiety. Like VEGF-targeted therapies, the majority of these agents can be classified as either monoclonal antibodies or small molecule inhibitors.

**Monoclonal antibodies: cetuximab**

Platinum-based chemotherapy in combination with the EGFR-targeting agent cetuximab has been assessed in several phase II efforts, with a suggestion of efficacy (60-65). Two subsequent phase III studies were developed on the basis of these data. In BMS-099, 676 patients who had not received prior therapy for metastatic disease were randomized to carboplatin and a taxane (either paclitaxel or docetaxel at the clinician’s discretion) with or without cetuximab (66). Molecular selection by EGFR expression was not required. No significant improvement in PFS or OS were observed, although there was a statistical improvement in overall RR (25.7% versus 17.2%, P = 0.007). The phase III FLEX trial assessed a distinct doublet regimen (cisplatin and vinorelbine) with or without cetuximab in patients with immunohistochemical evidence of EGFR expression in at least one positively stained tumor cell (67). Among 1,125 patients randomized, median OS was improved in those patients who received cetuximab (11.3 versus 10.1 months, P = 0.044). This benefit seemed to be consistent among patients with squamous histology; importantly, this stands in contrast to data for other agents (i.e., pemetrexed), in which a selective benefit is observed in those patients with adenocarcinoma. With respect to toxicity, both BMS-099 and FLEX identified higher rates of dermatologic toxicity with the addition of cetuximab. Interestingly, cetuximab also increased the rates of febrile neutropenia as compared with cisplatin and vinorelbine alone. The minimal benefit seen necessitates the ongoing search for a selection marker for cetuximab that might identify a sensitive population for improved efficacy.

Recent efforts have focused on KRAS and EGFR amplification or gene copy number to find a predictive marker for cetuximab. The role of EGFR amplification (assessed by fluorescence in situ hybridization, or FISH) has been explored in a phase II trial comparing concurrent or sequential carboplatin, paclitaxel, and cetuximab (68). Both PFS (6 versus 3 months, P = 0.0008) and OS (15 versus 7 months, P = 0.04) were prolonged in patients with FISH-positive tumors. Further evaluation of the importance of EGFR testing by FISH is ongoing in the Southwest Oncology Group study S08126. Patients with EGFR expression by immunohistochemistry (IHC) will receive chemotherapy or chemotherapy plus bevacizumab (on the basis of eligibility to receive bevaczumab) with or without concurrent cetuximab, and EGFR gene copy number will be analyzed and correlated with response to cetuximab. Given its established role in colorectal cancer, KRAS mutational status has been assessed in NSCLC patients receiving cetuximab (69). Interestingly, correlative analyses accompanying both BMS-099 and FLEX...
suggest no difference in clinical outcome on the basis of KRAS status (70, 71). In addition to laboratory biomarkers, much interest surrounds the use of rash as a predictor of cetuximab efficacy (72). A formal metric to assess rash in relation to cetuximab therapy has been established, termed the EGFR-inhibition related rash (EIRR) rating scale. The scale has been validated prospectively in a trial of cetuximab with pemetrexed in advanced NSCLC, and is being applied in larger efforts (73).

Outside the setting of advanced disease, cetuximab is being prospectively assessed in combination with cisplatin and gemcitabine as neoadjuvant therapy for stage IB-IIIA NSCLC (74). Early data from this study suggest appreciable RRs. Additionally, several clinical trials are assessing cetuximab in combination with radiation therapy (75–78).

Small molecule tyrosine kinase inhibitors

**Erlotinib.** The small molecule inhibitor erlotinib has directed activity toward EGFR, and has shown appreciable RRs in phase II trials of patients with previously treated advanced NSCLC (79). The subsequent National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) BR.21 trial randomized 731 advanced NSCLC patients who had received one to two prior chemotherapy regimens to receive either erlotinib or placebo (29). Both RR (8.9% versus <1%, P < 0.001) and median OS (6.7 versus 4.7 months, P < 0.01) were improved with erlotinib therapy. Furthermore, metrics assessing cough, dyspnea, and pain suggested an improvement in quality of life with erlotinib. Multivariate analyses assessing clinicopathologic features (done in a simultaneously reported companion study) suggested that adenocarcinoma histology, never-smoker status, and EGFR expression correlated with response (80).

In the BR.21 trial, molecular evaluation of EGFR expression by IHC, FISH, or mutation analysis did not show a significant survival advantage by multivariate analysis, but provided information for prospective studies (81). Recent data about the molecular selection of patients with EGFR-sensitizing mutations have improved our understanding of the most appropriate settings in which to use these agents. A prospective trial of EGFR mutation screening was conducted by the Spanish Lung Cancer Group (82). In this effort, a total of 2,105 patients with advanced NSCLC were assessed, with EGFR mutation shown in 350 patients (16.6%). Among 217 evaluable patients showing EGFR mutation and subsequently treated with erlotinib, PFS and OS were 14 and 27 months, respectively. EGFR mutations were more common among females (69.7%), never-smokers (66.6%), and patients with adenocarcinoma (80.9%). Exon 19 mutations were more common that L858R mutations (62.2% versus 37.8%). Notably, in multivariate analysis, an association was found between poor PFS and male gender (HR 2.94, 95% CI 1.72–5.03, P < 0.001) and the presence of L858R mutation (HR 1.92, 95% CI 1.19–3.10; P = 0.02).

The benefit of erlotinib therapy has been examined across several subsets of patients with advanced NSCLC. From an analysis of patients over the age of 70 enrolled in NCIC-CTG BR.21, it seems that older adults have similar PFS and OS benefit with erlotinib therapy (83). A prospective analysis of erlotinib monotherapy has been separately conducted in chemotherapy-naive patients age 70 or greater (84). In 88 patients, a median OS of 10.9 months was observed. Erlotinib therapy has also been examined in poor performance status (PS) patients. Although only ECOG PS 0-1 patients were enrolled in NCIC-CTG BR.21, a separate prospective effort assessed patients with a PS of 2. Using a randomized, phase II design, patients with no prior treatment for advanced disease received either erlotinib or platinum-based chemotherapy. A significant improvement in median OS was observed with chemotherapy (9.7 versus 6.5 months, P = 0.018; ref. 85).

To date, studies in unselected patient populations combining erlotinib with chemotherapy have been somewhat disappointing. In the phase III TALENT study, patients with advanced NSCLC were randomized to receive cisplatin and gemcitabine with either erlotinib or placebo (86). In this study, no difference in RR, time to progression, or median OS were observed. The TRIBUTE trial employed a similar design in 1,059 patients with advanced NSCLC, but evaluated a carboplatin-gemcitabine doublet (87). Again, no improvement in OS was observed in this study. A signal of activity was seen in patients characterized as never-smokers, in which a survival advantage was seen with erlotinib therapy (22.5 versus 10.1 months). Nonetheless, the clinical utility of chemotherapy with erlotinib remains questionable at this time.

As with other targeted therapies, erlotinib has been explored in a variety of other settings. For instance, erlotinib was recently assessed as maintenance therapy in the phase III SATURN study. In SATURN, patients who had completed four cycles of chemotherapy for advanced NSCLC received either erlotinib or placebo (88). A benefit in PFS with maintenance erlotinib was observed (HR 0.71, 95% CI 0.62–0.82, P < 0.0001). This benefit seemed to be present regardless of EGFR FISH or EGFR intron 1 CA repeat status (89). Although efficacy was seen in all patient subgroups, the largest benefit was seen in patients with EGFR mutation (HR 0.10, 95% CI 0.04–0.25, P < 0.0001). Several trials have also assessed the combination of erlotinib with or subsequent to radiation (90–92).

**Gefitinib.** Data from two separate randomized, phase II studies (IDEAL-1 and IDEAL-2) suggested limited toxicity and appreciable RRs with gefitinib in advanced NSCLC after failure of chemotherapy (93, 94). On the basis of these data, several phase III trials were launched. The Iressa Survival Evaluation in Lung Cancer (ISEL) study enrolled 1,692 patients with advanced NSCLC who had previously received chemotherapy (95). Patients were randomized to either gefitinib or placebo along with best supportive care. No difference in
median OS was observed in this study, although several clinical outcomes (objective RR, time to treatment failure, and OS) were significantly improved among Asian patients and higher RRs were noted in those patients with EGFR mutation (96).

Prospective validation of this finding in the EGFR-mutated subset is derived from several trials. Three phase II studies have assessed gefitinib in patients with EGFR mutation, with responses observed in 55 to 75% of patients (97–99). The INTEREST trial provides a direct comparison of gefitinib to chemotherapy in patients with advanced NSCLC who had progressed on prior platinum-based therapy (100). In this phase III study, 1,433 patients were randomized to receive either docetaxel or gefitinib. Correlative studies suggested no survival benefit among patients with EGFR amplification treated with gefitinib as opposed to docetaxel.

To further understand the role of clinicopathologic features versus molecular selection, the phase III iPASS study used several clinicopathologic criteria to identify a group of patients who may derive further benefit from gefitinib therapy (101). This multicenter study (conducted in East Asia) included chemotherapy-naïve, never-smokers with adenocarcinoma of the lung. A total of 1,217 patients were randomized to either gefitinib or carboplatin-paclitaxel. In this carefully selected population, PFS was superior with gefitinib therapy (HR 0.74, 95% CI 0.65-0.85, P < 0.001). In subset analyses, patients with EGFR mutation had superior PFS with gefitinib, whereas patients with wild-type EGFR had superior PFS with chemotherapy. This was the first study to definitively identify mutation status as an important predictive marker for EGFR-TKI therapy.

Subsequently, a phase III trial conducted in Japan (WIJTOG3405) enrolled only patients with chemotherapy-naïve advanced NSCLC harboring EGFR mutations (either an exon 19 mutation or a L858R point mutation; ref. 102). Patients were randomized to receive either gefitinib or cisplatin-docetaxel. The primary endpoint of the study was PFS, and with 177 patients randomized, the gefitinib group was noted to have significantly longer PFS (9.2 versus 6.3 months, P < 0.0001). The cisplatin-docetaxel group had an increased rate of myelosuppression, alopecia, and fatigue, whereas the gefitinib group had a higher rate of skin toxicity, liver dysfunction, and diarrhea. These results provide further support for use of gefitinib in a selected population.

Mirroring the experience with erlotinib and chemotherapy in combination, in unselected patients, cotreatment with gefitinib and chemotherapy has yielded disappointing results in phase III studies. In the INTACT-1 study, 1,093 patients with no prior therapy for advanced NSCLC were randomized to receive cisplatin and gemcitabine alone or with gefitinib (103). In contrast, INTACT-2 employed a similar randomization, substituting carboplatin-paclitaxel for cisplatin-gemcitabine (104). Neither study identified an improvement in OS with the addition of gefitinib.

Circumventing EGFR-TKI Resistance

Developing appropriate therapies for patients resistant to EGFR-TKIs requires a detailed understanding of mechanisms of resistance. It has been posited that whereas EGFR-mutated tumors are “addicted” to EGFR-mediated signaling and may be exquisitely sensitive to EGFR-TKIs, secondary mutations may arise that render these tumors resistant (105). These secondary mutations include mutations at T790M, which has been found in roughly half of tumors that are resistant to erlotinib and gefitinib (106). Alternatively, a “bypass mechanism” may render resistance; amplification of MET has been shown to activate PI3K (downstream of EGFR) in an ErbB3 (HER3)-dependent fashion (107).

Pan-HER inhibitors

Irreversible inhibitors of EGFR and related receptors have been suggested as a potential class of agents to overcome EGFR-TKI resistance. Several compounds with dual targeting of the ErbB family of receptors have shown clinical utility. The small molecule HKI-272 is a dual inhibitor of EGFR and HER2 tyrosine kinase domains (108). A phase I study enrolling 73 patients with advanced solid tumors included nine patients with NSCLC; no responses were noted in this subset (109). BIBW 2992 is a small molecule inhibitor with a similar spectrum of activity (110). Although a phase I trial of this agent showed no clinical responses in advanced solid tumors, the phase II LUX-Lung 2 trial (enrolling advanced NSCLC patients with EGFR mutation who failed first-line chemotherapy) yielded more impressive results (111, 112). Among 55 evaluable patients, 29 patients (53%) exhibited a PR. The pan-HER inhibitor PF-00299804 (with affinity for EGFR, HER2, and HER4) has also shown activity in NSCLC; in a phase II study enrolling advanced NSCLC patients who had progressed on both erlotinib and chemotherapy, two unconfirmed PRs (10%) have been reported among 20 evaluable patients (113). It is unclear about whether the irreversible inhibitors of ErbB family receptors will develop into a useful therapeutic strategy for evading EGFR-TKI resistance. For instance, it does seem that PF-00299804 has activity in preclinical models of gefitinib resistance; however, it may not overcome MET amplification (114). Furthermore, satisfactory inhibition of T790M mutated EGFR does not seem to occur at clinically relevant concentrations. The novel T790M-targeted agents discussed below may be a preferred approach in this setting.

Targeting T790M

A collaborative of leading investigators in thoracic oncology have established a series of criteria to define resistance to EGFR-TKIs (115). As noted in Table 1, mutations in EGFR that confer decreased sensitivity to gefitinib or erlotinib represent an important criterion; for instance, EGFR mutation at T790M has been implicated in 50 to 60% of patients with resistance to erlotinib (80, 116, 117).
Laboratory-based efforts have focused on developing agents to target this mutation. In a report from the Dana Farber Cancer Institute, a focused library of TKIs with common core scaffolds was tested in lung cancer cell lines harboring both T790M and KRAS mutations (109). From these efforts, three agents were derived (WZ4002, WZ3146, and WZ8040) that inhibited phosphorylation of EGFR in the aforementioned cell lines. In subsequent in vivo testing, WZ4002 induced tumor regression in murine models of T790M mutation.

**Targeting MET and HGF**

Independent of the T790M mutation, recent work suggests that resistance to EGFR-TKIs may be mediated through MET amplification (118). The MET proto-oncogene encodes a receptor tyrosine kinase, activated by hepatocyte growth factor (HGF). Amplification of MET has been shown to increase survival, invasiveness, and angiogenesis in cancer models (119). Several strategies to antagonize MET signaling are currently under investigation in lung cancer. For example, SCH-900105 is a humanized anti-MET antibody with specificity for free HGF, and is currently being combined with gefitinib in a phase I-II study in advanced NSCLC (120). The small molecule MET inhibitor ARQ-197 has been assessed in a phase II study, in which patients with advanced NSCLC who have received more than one prior chemotherapy are randomized to receive erlotinib with either ARQ-197 or placebo (36). In a recent press release, it was suggested that PFS was improved from 9.7 to 16.1 weeks with the addition of ARQ-197 (121).

**IGF-IR-Targeting Therapies**

IGF-IR activation has been conceptualized as a mechanism of bypassing ErbB-directed signaling in several malignancies (131). In the setting of NSCLC, increased expression of IGF-IR seems to occur in up to 70% of patients, and may correlate with other prognostic markers, including EGFR expression and amplification (132). IGF-IR itself may be a prognostic marker; an assessment of 77 patients treated with gefitinib monotherapy suggested
that IGF-IR expression by IHC correlated with OS (133). Agents targeting IGF-IR include CP-751,871, a monoclonal antibody that has been assessed in a randomized, phase II study in patients without previous therapy for advanced NSCLC (134). Patients received carboplatin and paclitaxel with or without CP-751,871. With 156 patients randomized at most recent report, there was a numerical increase in RR among patients receiving antibody. On the basis of promising results in patients with nonadenocarcinoma histology, a phase III trial was initiated, and recently closed at an interim analysis because of lack of efficacy (135). IMC-A12 is a distinct monoclonal antibody that has shown activity in advanced solid tumors in a phase I study; this agent and others like it (19D12, EM164, R1507, and AMG479) may ultimately play a role in therapy of NSCLC (136, 137). Several small molecule inhibitors of the IGF-IR tyrosine kinase domain (including AEW541, OSI906, and BMS536924) are currently in clinical development in combination with chemotherapy and EGFR-TKI therapy, as well (137).

mTOR Inhibitors

The serine-threonine kinase mTOR plays a critical role in cell growth and proliferation, sitting downstream of PI3K and Akt in signaling axes triggered by activation of receptor tyrosine kinases (138). The oral mTOR inhibitor everolimus has shown activity in VEGF-TKI refractory metastatic RCC (mRCC; ref. 139). The oral mTOR inhibitor temsirolimus (also active in mRCC) has been assessed in a randomized, placebo-controlled trial of 247 patients randomized at most recent report, there was a numerical increase in RR among patients randomized to carboplatin-paclitaxel with or without everolimus (140). With 94 patients randomized, overall RR with the addition of everolimus to chemotherapy was superior (34% versus 27%, P = 0.05), although no difference in PFS or OS was noted. IMC-A12 is a distinct monoclonal antibody that has been assessed in a randomized, phase II analysis of paclitaxel with celecoxib in 58 patients with platinum-refractory, advanced NSCLC (148). The study identified an objective response in 14 patients (24.1%), and SD in an additional 24 patients (41.3%). Phase III, placebo-controlled data for celecoxib are now available from the NVALT-4 study, in which patients advanced NSCLC were randomized to carboplatin-docetaxel with or without celecoxib (149). With 561 patients randomized, overall RR with the addition of celecoxib to chemotherapy was superior (32% versus 27%, P = 0.05), although no difference in PFS or OS were noted. Recent clinical investigations have also focused on more specific inhibitors of COX-2 with higher affinity, such as aprexisib (14). In parallel, correlative studies have biomarkers to predict response to COX-2 inhibitors. For example, in a randomized phase II trial comparing celecoxib with or without the 5-lipoxygenase inhibitor zileuton in advanced NSCLC, survival with celecoxib was inversely proportional to the level of COX-2 expression (150).

HDAC Inhibitors

Laboratory observations suggest synergy between HDAC inhibitors and platinum-based chemotherapy (151). These data are supported by a randomized, phase II study comparing carboplatin-paclitaxel with or without the HDAC inhibitor vorinostat (152). With 94 patients randomized, a significantly higher overall RR (34% versus 12.5%, P = 0.02) was observed with the addition of vorinostat. A trend toward improvement in PFS (6.0 versus 4.1 months; P = 0.48) and OS (13.0 versus 9.7 months, P = 0.17) was also noted.

Future Directions

With a growing list of targeted therapies at the oncologists’ disposal, a number of challenges arise. First, data are needed to determine rational combinations of these agents. As previously noted experiences combining cytotoxics and targeted therapies attest, synergy cannot always be reliably predicted from preclinical models and inevitably requires clinical validation. Multiple studies have assessed permutations of antiangiogenics, EGFR TKIs, and COX-2 inhibitors in a wide variety of settings within NSCLC, as delineated in Table 2. Outside of determining optimal combinations, further translational efforts are necessary to elicit biomarkers that may predict response to targeted therapies. The ongoing Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) study represents an effort...
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>No.</th>
<th>Key Results</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiangiogenic + EGFR TKI</td>
<td></td>
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<tr>
<td>Adjei et al. (160)</td>
<td>Phase I: sorafenib and gefitinib (refractory disease)</td>
<td>31</td>
<td>PR: 1 pt (3.2%) SD: 20 pts (64%)</td>
<td>No effect of gefitinib on sorafenib PK</td>
</tr>
<tr>
<td>Lind et al. (134)</td>
<td>Phase II: sorafenib and erlotinib (first line)</td>
<td>50</td>
<td>PR: 13 pts (26%) SD: 25 pts (50%)</td>
<td>18FDG-SUV uptake on PET more markedly decreased in responders compared with nonresponders</td>
</tr>
<tr>
<td>Gandara et al. (SWOG 0536; ref. 135)</td>
<td>Phase II: carboplatin and paclitaxel and bevacizumab (first line)</td>
<td>95</td>
<td>PR: 51 pts (54%) SD: 22 pts (23%)</td>
<td>No association of benefit with KRAS status (161)</td>
</tr>
<tr>
<td>Herbst et al. (31)</td>
<td>Randomized phase II: bevacizumab and chemotherapy OR bevacizumab and erlotinib OR chemotherapy (platinum failure)</td>
<td>120</td>
<td>1-year survival: Bevacizumab and chemotherapy: 53.8% Bevacizumab and erlotinib: 57.4% Chemotherapy alone: 33.1%</td>
<td>MALDI-TOF analyses suggest a proteomic profile for patients that may benefit from bevacizumab and erlotinib (44, 120)</td>
</tr>
<tr>
<td>Miller et al. (ATLAS; ref. 35)</td>
<td>Phase III: chemotherapy and bevacizumab → EITHER maintenance bevacizumab OR maintenance bevacizumab and erlotinib (first line)</td>
<td>768</td>
<td>PFS: Maintenance bevacizumab and erlotinib: 4.8 months Maintenance bevacizumab: 3.7 months</td>
<td>Three platinum doublets assessed (carboplatin and gemcitabine, docetaxel or paclitaxel); no effect upon clinical outcome</td>
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<td>Dual inhibition of EGFR</td>
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<tr>
<td>Ramalingam et al. (36)</td>
<td>Phase I: cetuximab and gefitinib (refractory)</td>
<td>13</td>
<td>PR: 0 pts (0%) SD: 4 pts (31.0%)</td>
<td>EGFR mutation was absent in all pts evaluated in this study</td>
</tr>
<tr>
<td>Felip et al. (121)</td>
<td>Phase I: pertuzumab and erlotinib (refractory)</td>
<td>15</td>
<td>Not reported</td>
<td>No DLTs with full dose pertuzumab and erlotinib</td>
</tr>
<tr>
<td>EGFR TKIs, and COX2 inhibitors</td>
<td></td>
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<tr>
<td>Reckamp et al. (55)</td>
<td>Phase I: erlotinib and celecoxib (refractory)</td>
<td>21</td>
<td>PR: 7 pts (33.3%) SD: 5 pts (23.8%)</td>
<td>MMP-9 level may predict response to this regimen (162)</td>
</tr>
<tr>
<td>Gadgeel et al. (56)</td>
<td>Phase II: gefitinib and celecoxib (refractory)</td>
<td>27</td>
<td>PR: 2 pts (7.4%) TTP: 2.2 months OS: 4.6 months</td>
<td>Most common AEs were skin rash and diarrhea</td>
</tr>
<tr>
<td>O’Byrne et al. (57)</td>
<td>Phase I-II: gefitinib and rofecoxib (refractory)</td>
<td>42</td>
<td>CR: 1 pt (2.3%) PR: 2 pts (4.8%) TTP: 2.0 months OS: 5.1 months</td>
<td>MALDI-TOF may distinguish responders and nonresponders</td>
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<tr>
<td>EGFR TKIs, and mTOR inhibitors</td>
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<td>Papadimitrakopolou et al. (143)</td>
<td>Phase I-II: erlotinib and everolimus (refractory)</td>
<td>61</td>
<td>CR: 1 pt (1.6%) PR: 3 pts (4.9%) SD: 17 pts (27.8%)</td>
<td>Most common DLTs were mucositis, rash, and diarrhea</td>
</tr>
<tr>
<td>Milton et al. (163)</td>
<td>Phase I-II: gefitinib and everolimus (refractory)</td>
<td>10</td>
<td>PR: 1 pt (16.7%)</td>
<td>PK data suggest limited interaction between agents</td>
</tr>
</tbody>
</table>

Abbreviations: AE, adverse effects; ATLAS, Assessment of Treatment with Lisinopril and Survival; CR, complete response; DLT, dose-limiting toxicity; MALDI-TOF, matrix-assisted laser desorption-ionization time-of-flight; MMP-9, matrix metalloproteinase 9; PK, pharmacokinetics; PR, partial response; pts, patients; TTP, time to progression; SWOG, Southwest Oncology Group.
that randomizes patients to a range of targeted agents (sorafenib, erlotinib, vandetanib, or erlotinib-bexarotene) on the basis of multiple molecular predictors (including KRAS mutation, EGFR mutation, BRAF mutation, etc.; ref. 153). With a total of 1255 patients randomized to date, the disease control rate at 1 year was 48% (154). Median OS was 9 months and 1-year survival was 39%. Better disease control has been observed with (1) EGFR mutation in the setting of erlotinib therapy (2), cyclin D1 positive and EGFR FISH amplification with bevacotene and erlotinib (3), VEGFR2 IHC-positive with vandetanib therapy, and (4) absence of EGFR mutation or high polysomy with sorafenib. The Lung Cancer Mutation Consortium (LCMC) is collaborative effort by 14 academic sites to screen patients with adenocarcinoma of the lung for known mutations and discover new mutations (155). The LCMC plans to genotype 1000 patients with advanced adenocarcinoma of the lung to determine important mutations.

Looking ahead, MET/Alk inhibitors and agents directed at the T790M EGFR mutation seem to be promising strategies to evade resistance. Along the axis of relevant signaling pathways in NSCLC (Fig. 1), there are various other therapeutic targets that warrant exploration. For example, drugs that inhibit PI3K and/or Akt signaling (i.e., BEZ235, perifosine, etc.) show promise in other malignancies and may ultimately play a role in the therapy of NSCLC (156–159). Given an emerging pipeline of targeted agents at the clinician’s disposal, the design of rational, scientifically driven trials is of the utmost importance. Although the landscape of NSCLC therapy has already been altered drastically by targeted agents, the pace of drug development will surely continue to modify this landscape in coming years.

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S.K. Pal: honoraria from Speakers Bureau, Novartis and Pfizer; consultant, Genentech. No other potential conflicts of interest were disclosed.

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