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Strategies for Overcoming Resistance to EGFR Family Tyrosine Kinase Inhibitors

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Abstract

The first-generation epidermal growth factor receptor tyrosine kinase inhibitors erlotinib and gefitinib have been incorporated into treatment paradigms for patients with advanced non-small cell lung cancer. These agents are particularly effective in a subset of patients whose tumors harbor activating *epidermal growth factor receptor* mutations. However, most patients do not respond to these tyrosine kinase inhibitors, and those who do will eventually acquire resistance that typically results from a secondary *epidermal growth factor receptor* mutation (eg, T790M), *mesenchymal-epithelial transition factor* amplification, or activation of other signaling pathways. For patients whose tumors have wild-type *epidermal growth factor receptor*, there are several known mechanisms of initial resistance (eg, *Kirsten rat sarcoma viral oncogene homolog* mutations) but these do not account for all cases, suggesting that unknown mechanisms also contribute. To potentially overcome the issue of resistance, next-generation tyrosine kinase inhibitors are being developed, which irreversibly block multiple epidermal growth factor receptor family members (eg, afatinib [BIBW 2992], PF-00299804) and/or vascular endothelial growth factor receptor pathways (eg, BMS-690514, XL647). In addition, drugs that block parallel signaling pathways or signaling molecules downstream of the epidermal growth factor receptor, such as the insulin-like growth factor-1 receptor and the mammalian target of rapamycin, are undergoing clinical evaluation. As drug resistance appears to be pleomorphic, combinations of drugs or drugs with multiple targets may be more effective in circumventing resistance.

Keywords

Epidermal growth factor receptor; tyrosine kinase inhibitor; non-small cell lung cancer; afatinib; BIBW 2992; PF-00299804; resistance

Introduction

The epidermal growth factor receptor (EGFR) family comprises 4 members— EGFR/(human epidermal growth factor receptor 1 [HER1]/ErbB1), HER2/ErbB2, HER3/ErbB3,

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and HER4/ ErbB4 —receptor tyrosine kinases (RTKs) that regulate downstream signaling pathways important to tumor cell proliferation, survival, migration, and metastasis.[1] The first-generation reversible EGFR tyrosine kinase (TK) inhibitors (TKIs) erlotinib (Genentech; South San Francisco, CA, US) and gefitinib (AstraZeneca; Wilmington, DE, US) have been incorporated into treatment paradigms for patients with relapsed or refractory advanced non-small cell lung cancer (NSCLC), but objective response rates (RRs) in unselected patient populations are modest: approximately 10% among patients in North America and 20% among patients in Asia.[2,3] Even when objective responses are achieved they are typically modest in duration, likely reflecting the presence of underlying or developing resistance mechanisms.[3-6]

Approximately 10% of patients harbor somatic gain-of-function *EGFR* mutations, such as in-frame deletions in exon 19 or point mutations in exon 21 (eg, L858R), that cluster around the adenosine-5'-triphosphate (ATP)-binding pocket of the EGFR TK domain and confer sensitivity to first-generation TKIs.[7,8] The presence of these activating mutations has been associated with higher RRs and improved outcomes with first-generation EGFR TKIs in numerous clinical trials and treatment settings.[9–11] In IPASS, first-line gefitinib provided significantly longer progression-free survival (PFS) and higher RRs than carboplatin/ paclitaxel in patients with activating *EGFR* mutations.[12] An analysis of 223 patients from 5 clinical trials evaluating gefitinib and erlotinib in chemotherapy-naive patients with NSCLC confirmed that the presence of *EGFR*-activating mutations correlated with improved outcome.[13]

Based on these observations, prospective clinical studies have been designed to select patients with *EGFR* mutations for TKI therapy. The Spanish Lung Cancer Group demonstrated the feasibility of large-scale screening for *EGFR* mutations among patients with advanced NSCLC and the use of screening results to guide treatment decisions with erlotinib.[14] In the selected patients, 24 patients had a complete response (CR), 115 had a partial response (PR), and 38 had stable disease (SD) with erlotinib; median PFS and overall survival (OS) were 14 and 27 months, respectively. Similarly, in a phase II trial, gefitinib produced a RR of 66% and a disease control rate (DCR) of 90% in the first-line treatment of patients with advanced NSCLC harboring *EGFR*-activating mutations.[15] Two phase III trials comparing chemotherapy to gefitinib as first-line treatment for advanced NSCLC patients with *EGFR*-activating mutations recently demonstrated gefitinib was associated with significantly improved PFS (hazard ratio [HR], 0.30; 95% confidence interval [CI], 0.22-0.41; $P < 0.001$[16] and HR, 0.49; 95% CI, 0.34-0.71; $P < 0.0001$)[17] although overall survival was not improved in any of these trials. Results from clinical trials assessing first-generation TKIs in patients with NSCLC who have activating *EGFR* mutations indicate that these patients eventually develop resistance to reversible EGFR TKIs, which may result from secondary acquired *EGFR* mutations or other resistance mechanisms unrelated to *EGFR* genotype[3] (Figure 1).

New strategies are needed for overcoming resistance. Genetic testing for specific *EGFR* mutations may help identify patients who may most likely benefit from EGFR TKIs early in the treatment process. This review discusses the mechanisms underlying resistance to the first-generation EGFR TKIs and ongoing clinical efforts aimed at identifying new treatment strategies for overcoming resistance mechanisms.

Factors Contributing to Resistance

EGFR Resistance Mutations

The T790M point mutation in exon 20 of *EGFR* is found in approximately 50% of the NSCLC tumors from patients who respond initially to reversible first-generation EGFR

TKIs and then develop resistance.[18,19] However, the T790M mutation may also be present prior to treatment with erlotinib or gefitinib and, therefore, may also contribute to primary resistance. Some patients who respond may have T790M mutations in a small percentage of tumor cells before treatment with erlotinib or gefitinib.[20,21] During treatment with a first-generation TKI, clonal selection may allow the T790M-expressing cells to assume an increasingly larger percentage of the tumor mass over time.[20,21] In addition, the T790M mutation may confer a growth advantage to tumor cells, particularly when it occurs in conjunction with a primary *EGFR*-activating mutation.[18]

Several other *EGFR* mutations have been associated with resistance to erlotinib and gefitinib. In 1 study, secondary *EGFR* kinase mutations were identified in the tumors of 8 of 16 patients who had progressive disease (PD) after initial responses to erlotinib or gefitinib.[22] Of these, 7 patients had a T790M mutation, which occurred in conjunction with a deletion in exon 19 (5 cases) or a L858R mutation (2 cases), and 1 patient had a secondary D761Y point mutation in exon 19 in conjunction with a primary L858R-activating mutation (not evident in the pretreatment specimen). Other investigators have reported secondary mutations in exon 21 (eg, T854A) that may contribute to resistance to first-generation TKIs.[23]

KRAS Mutations

Mutations in signaling molecules downstream of EGFR, such as the retrovirus-associated DNA sequences (RAS) family of proteins, may also contribute to resistance to EGFR TKIs.[24] Approximately 15% to 30% of NSCLC tumors contain activating mutations in *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*), which occur most frequently in codons 12 and 13 of exon 2.[25,26] Activation of *KRAS* has been proposed as a mechanism of primary resistance to gefitinib and erlotinib,[24] presumably by upregulation of the v-raf 1 murine leukemia viral oncogene homolog 1 (RAF1)/mitogen activated protein kinase (MAPK) pathway, which promotes survival and proliferation.[27] Interestingly, activating *KRAS* mutations are found almost exclusively in tumors with a wild-type *EGFR* genotype.[11,28,29]

Several studies have shown that the presence of *KRAS* mutations correlates with lower RRs and poorer clinical outcomes to first-generation EGFR TKIs in patients with advanced NSCLC.[11,28,30,31] In the TRIBUTE study, among patients with tumors carrying *KRAS* mutations, erlotinib plus paclitaxel/carboplatin was associated with a shorter median time to progression (TTP; $P = 0.03$) and shorter median OS ($P = 0.019$) than chemotherapy alone.[30] In a biomarker analysis from the BR.21 trial, which evaluated erlotinib after failure of standard chemotherapy, patients whose tumors had wild-type *KRAS* had a survival advantage with erlotinib versus placebo (HR, 0.69; 95% CI, 0.49-0.97; $P = 0.03$), but patients whose tumors had mutant *KRAS* did not (HR, 1.67; 95% CI, 0.62–4.50; $P = 0.31$).[11] Thus, the presence of mutant *KRAS* has been associated with resistance to first-generation TKIs, suggesting that an alternative therapeutic approach should be considered.

MET Amplification

The mesenchymal-epithelial transition factor (MET) RTK appears to stimulate HER3-dependent activation of phosphatidylinositol-3-kinase (PI3K)/Akt signaling, thereby circumventing the effects of EGFR TKIs.[32] *MET* amplification occurs in approximately 20% of NSCLC patients who develop resistance after an initial response to erlotinib or gefitinib and have tumors harboring *EGFR* mutations[32,33] and in approximately 7% of NSCLC patients who undergo surgical resection.[34] In 1 study, *MET* amplification was significantly more common in tumors of NSCLC patients who developed resistance to gefitinib or erlotinib versus untreated patients (21% vs 3%; $P = 0.007$).[33] In another study,

of 4 tumor samples with *MET* amplification from patients who were resistant to gefitinib, 1 concurrently expressed the *EGFR* T790M mutation.[32] Results of an analysis of tumor samples from 51 NSCLC patients who received prior gefitinib treatment demonstrated that prominent membrane expression of activated c-MET (c-MET phosphorylated at Y1003) was associated with PD ($P = 0.019$) and a shorter TTP ($P = 0.0416$).[35] As such, cMET [pY1003] may be a potential marker of primary gefitinib resistance in NSCLC.

Other Signaling Pathways

Preclinical studies suggest that parallel signaling pathways like the vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) pathways may contribute to resistance to first-generation EGFR TKIs. In 1 study, exposure to anti-EGFR monoclonal antibodies for 2 consecutive cycles resulted in resistant tumor xenografts of human A431 squamous cell carcinoma.[36] Five of the 6 resistant tumors expressed 2- to 4-times higher levels of VEGF than the parental tumors, which correlated with their increased angiogenic potential in vitro as well as the increased tumor angiogenesis observed in vivo.[36] Increased VEGF expression has also been reported in human GEO colon cancer tumors following chronic treatment with gefitinib.[37] Although gefitinib was effective initially, tumor growth occurred following 11 to 12 weeks of continuous therapy and reached a growth rate comparable to that of untreated control tumors after another 10 weeks.[37] The resistant GEO cells exhibited 5-fold to 10-fold increases in VEGF expression compared with the wild-type GEO cells; of note, the gefitinib-resistant tumors were susceptible to vandetanib (AstraZeneca; Wilmington, DE, US), a vascular endothelial growth factor receptor (VEGFR)/EGFR TKI.[37]

The insulin-like growth factor-1 receptor (IGF-1R) activates many of the same signaling pathways as EGFR, leading to proliferation, survival, angiogenesis, and metastasis.[27] Following treatment with an EGFR TKI, upregulation of IGF-1R expression was observed in a primary human glioblastoma multiforme cell line that was resistant to EGFR TKIs; IGF-1R upregulation caused sustained signaling through the PI3K/Akt pathway and led to antiapoptotic and proinvasive effects.[38] Similarly, increased expression and activation of IGF-1R has been reported in androgen-independent prostate cancer cells with acquired resistance to gefitinib.[39] These resistant cells produced high levels of IGF2 ligand and were dependent on IGF-1R for growth. Evidence for crosstalk between EGFR and IGF-1R has also been reported in NSCLC, where activation of IGF-1R by amphiregulin, a ligand for EGFR, initiated a positive-feedback loop by stimulating further release of amphiregulin.[40]

Finally, the process of epithelial-mesenchymal transformation (EMT) has been associated with resistance to EGFR TKIs. EMT is characterized by loss of epithelial cell junction proteins such as E-cadherin and gain of mesenchymal markers such as vimentin and fibronectin.[41] Notably, EMT increases the potential for cancer cells to migrate to distant sites and plays a critical role in disease progression.[42] The sensitivity of NSCLC cell lines to erlotinib varies widely across a 100-fold half-maximal inhibitory concentration (IC_{50}) range and can be predicted by whether or not they have undergone EMT.[41,43] In general, cell lines that still expressed E-cadherin were more sensitive to erlotinib whereas those that expressed vimentin, fibronectin, or both were resistant to erlotinib.[41]

The expression of E-cadherin is regulated by 4 zinc finger transcription factors, one of which —ZEB1 —has been significantly associated with resistance to gefitinib.[44] ZEB1 inhibits E-cadherin expression by recruiting histone deacetylase (HDAC), which can be blocked by the HDAC inhibitor MS-275.[44] Notably, treating gefitinib-resistant NSCLC cells with MS-275 increased E-cadherin and EGFR expression and restored sensitivity to EGFR TKIs.[44]

E-cadherin expression was determined in a small subset of patients (87 [8%] of 1,079) who participated in the TRIBUTE trial.[43] In patients whose tumors expressed E-cadherin, median TTP was longer with erlotinib plus carboplatin/paclitaxel than with carboplatin/paclitaxel alone (34.0 vs 19.3 weeks; HR, 0.37; $P = 0.003$).[43] Conversely, median TTP did not differ significantly between treatment regimens in the E-cadherin– negative subgroup.[43] Additional analyses in larger cohorts will be needed to validate E-cadherin as a marker of resistance to EGFR TKIs in patients with advanced NSCLC.

In summary, there are multiple strategies that may be used to develop new agents that may overcome or delay the emergence of acquired resistance to first-generation EGFR TKIs. Specifically, there is a need for agents that reduce signaling through pathways downstream of EGFR (eg, KRAS), pathways that overlap or signal in parallel with EGFR (eg, MET, VEGFR, and IGF-1R), and through those that promote EMT. It should be noted that although this review focuses on resistance to EGFR TKIs, treatment strategies with EGFR-targeted monoclonal antibodies may have to overcome similar mechanisms of resistance (eg, *KRAS* mutation).

Strategies for Overcoming Resistance to EGFR Inhibitors

Next-generation EGFR TKIs include irreversible inhibitors that simultaneously target multiple members of the EGFR family (Table 1). The first-generation agents, gefitinib and erlotinib, bind to the catalytic site of the EGFR TK domain through competitive binding with ATP.[18] The irreversible binding mechanism of next-generation TKIs and resulting reduced off-rate may increase TKI effectiveness by prolonging the inhibition of EGFR signaling and reducing the emergence of resistance. An irreversible EGFR TKI may overcome resistance to gefitinib or erlotinib through covalently binding to EGFR and, once bound, will no longer be in a competitive, reversible equilibrium with ATP.[45] In 1 study, 49 NCI-H1650 bronchioloalveolar cell clones showed decreased sensitivity to gefitinib, but clones resistant to an irreversible inhibitor could not be established.[46] In addition, irreversible inhibitors reduced proliferation in cells with an *EGFR*-activating mutation as well as in those with a secondary, resistance-associated *EGFR* mutation.[46]

Two irreversible inhibitors of multiple EGFR family members are currently being evaluated for the treatment of NSCLC in phase III clinical trials: afatinib (BIBW 2992) (Boehringer Ingelheim; Ingelheim, Germany), an EGFR/HER2 inhibitor, and PF-00299804 (Pfizer; New London, CT, US), an agent with activity against EGFR, HER2, and HER4.[47,48] Other irreversible and/or multitargeted TKIs, including lapatinib (GlaxoSmithKline; London, UK) and neratinib (Pfizer; New London, CT, US), have also been evaluated in NSCLC.

Afatinib

Results from preclinical studies indicate that afatinib inhibits the kinase activity of wild-type and mutant forms of EGFR and HER2.[47] In cell-free assays, afatinib has a potency similar to that of gefitinib for inhibiting L858R EGFR (IC_{50} of 0.4 nM vs 0.8 nM) and comparable to lapatinib for inhibiting HER2 (IC_{50} of 14 nM vs 15 nM). However, afatinib has shown 100-fold greater activity against L858R-T790M *EGFR* double mutants than gefitinib (IC_{50}, 10 nM vs 1,013 nM).[47] Moreover, afatinib was more effective than erlotinib, gefitinib, and lapatinib in inhibiting the survival of human NSCLC cell lines harboring wild-type *EGFR* or the L858R/T790M double mutant.[47] In a xenograft model of the epidermoid carcinoma cell line A431, which expresses high levels of EGFR and detectable HER2 levels, afatinib was more effective in suppressing tumor growth than maximally tolerated doses of gefitinib or lapatinib.[47] Afatinib also showed activity in tumor xenograft models resistant to first-generation EGFR TKIs, including tumors harboring the L858R/T790M double mutant, and in models dependent on HER2 overexpression.[47]

Afatinib 40 to 50 mg/day was evaluated in a single-arm phase II trial (LUX-Lung 2) in patients with advanced lung adenocarcinomas harboring activating *EGFR* mutations.[49] Target accrual was 120 patients, with a total of 129 patients treated with afatinib —68 in the second-line and 61 in the first-line setting; most patients were Asian (n = 112) and never smokers (n = 82).[49] In the overall population, DCR was 86%, confirmed objective RR was 60%, median PFS was 14 months, and median OS was 24 months.[50] DCR, confirmed objective RR, and PFS were 83%, 59%, and 16.1 months, respectively, in patients with L858R *EGFR* mutations (n = 54) and were 93%, 69%, and 13.7 months, respectively, in patients with a deletion in exon 19 of *EGFR* (n = 52). Diarrhea and rash/acne were the most common drug-related adverse events (AEs), occurring in 95% (19% at grade 3) and 91% (21% at grade 3) of patients, respectively.[50]

Afatinib was evaluated in a phase IIb/III trial (LUX-Lung 1) in patients with advanced lung adenocarcinoma who had failed 1 or 2 lines of chemotherapy and progressed after ≥12 weeks of therapy with erlotinib or gefitinib.[51] Between May 2008 to September 2009, 585 patients were randomized and received best supportive care plus either afatinib or placebo. Median OS (the primary endpoint) was 10.78 months with afatinib versus 11.96 months with placebo (HR 1.08; 95% CI, 0.86–1.35). However, afatinib significantly prolonged PFS (a secondary endpoint) to 3.3 months (vs 1.1 with placebo; HR 0.38, P <0.0001) in this population that was clinically enriched for the presence of *EGFR*-activating mutations. Afatinib was also associated with significant improvements in the secondary endpoints of confirmed DCR of at least 8 weeks (58% vs 19%; P <0.0001) and confirmed ORR (11% vs 0.5% by investigator analysis and 7.4% vs 0.5% by independent analysis; P <0.01). The 2 most common AEs observed with afatinib were diarrhea (87%; 17% at grade 3) and rash/acne (78%; 14% at grade 3).

Afatinib is being evaluated in an exploratory phase II study in patients with advanced NSCLC who were never smokers or light ex-smokers and who fall into 1 of 3 categories: (1) tumor harboring *EGFR/HER1* mutation and prior erlotinib or gefitinib failure, (2) tumor with *EGFR/HER1* FISH positivity and prior erlotinib or gefitinib failure, or (3) tumor harboring *HER2* mutation.[52] In a preliminary report of this study, all 3 evaluable patients were female, nonsmokers, had failed prior chemotherapy, and had tumors harboring mutations in the kinase domain of *HER2*. All 3 patients achieved PRs with afatinib 50 mg/day with concomitant improvements in symptoms and performance status.[52] A randomized, open-label, phase III study (LUX-Lung 3) is also evaluating afatinib versus pemetrexed/cisplatin as first-line therapy in patients with NSCLC tumors harboring *EGFR*-activating mutations (NCT00949650). Another randomized, open-label, phase III study (LUX-Lung 6) is evaluating afatinib versus cisplatin/gemcitabine chemotherapy as first-line therapy in patients with *EGFR* mutations in China, Korea, and India (NCT01121393). Afatinib is also being explored in combination with cetuximab for NSCLC. Preclinical analyses showed the combination was associated with CRs in mice with tumors harboring the T790M mutation or the L858R mutation.[53] A phase I trial to evaluate the combination of afatinib with cetuximab is currently recruiting NSCLC patients with progressive disease following treatment with erlotinib or gefitinib (NCT01090011).

PF-00299804

PF-00299804 is an irreversible pan-HER TKI that inhibits the kinase activity of wild-type EGFR (IC_{50}, 6 nM), HER2 (IC_{50}, 45.7 nM), and HER4 (IC_{50}, 73.7 nM).48 It is effective against NSCLC cell lines with the following double mutations: *EGFR* exon 19 deletion and L858R mutation and L858R/T790M mutations.[48] PF-00299804 has shown activity in NSCLC cell lines with *HER2* amplification and in those carrying the *HER2* Ins774YVMA insertion mutation, but not in those with *KRAS* mutations.[48] In an NSCLC cell line harboring the *EGFR* T790M mutation that maintained HER3/PI3K/Akt phosphorylation,

PF-00299804, but not gefitinib, completely inhibited the HER3 signaling pathway and caused substantial apoptosis.[48] Similarly, in tumor xenograft models harboring the *EGFR* T790M mutation, PF-00299804, but not gefitinib, was effective in inhibiting tumor growth.[48]

PF-00299804 was also evaluated in A431 human squamous cell carcinoma and H125 human NSCLC xenograft models.[54] In the A431 xenografts, PF-00299804 was administered once daily for 14 days, producing an average tumor growth delay of 45 days at a dose of 11 mg/kg. Several animals had a PR or a CR, defined as reductions in tumor mass of ≥50% and ≥75% from baseline, respectively, at doses of 11 to 100 mg/kg. In H125 xenografts, PF-00299804 at doses of 30 or 65 mg/kg once daily for 14 days produced tumor growth delays of 9.1 and 10.2 days, respectively, although none of the animals had a PR or a CR. In these models, mean body weight declined by approximately 20% in animals treated with PF-00299804 at doses of 30 mg/kg or more.[54]

In a 2-arm, phase II trial evaluating PF-00299804 in patients with advanced NSCLC who had failed 1 or 2 prior chemotherapy regimens as well as prior treatment with erlotinib, patients with adenocarcinomas were enrolled in 1 arm of the study and patients with other NSCLC histologies were enrolled in the other arm.[55] Preliminary results have been reported for the first 66 patients: 44 patients with adenocarcinomas and 22 patients with nonadenocarcinomas.[55] As of August 2009, of 36 evaluable patients with adenocarcinoma and 5 patients with nonadenocarcinoma, the DCR was 67% and 40%, respectively; SD >6 months occurred in 2 patients with adenocarcinoma and 1 patient with nonadenocarcinoma.[55] The most common AEs of any grade were diarrhea (82%), skin toxicity (77%), fatigue (59%), stomatitis (28%), and vomiting (23%).[55] This study suggests that PF-00299804 may have clinical activity in patients with advanced NSCLC after the failure of prior chemotherapy and erlotinib.

In the first-line setting, PF-00299804 is being tested in a phase II, open-label trial in patients with advanced lung carcinoma who were never smokers or former light smokers.[56] Among the first 29 evaluable patients, there was 1 CR, 6 PRs, and 16 patients with SD for ≥16 weeks. In a subanalysis of 14 evaluable patients with *EGFR* mutation-positive disease, tumor shrinkage was observed in all cases. The most common treatment-related AEs were diarrhea and dermatitis acneiform for all grade events (79% and 49%, respectively) and grade 3 events (9% for both). Another phase II trial evaluated PF-00299804 versus erlotinib as second-line or third-line therapy in 188 patients with advanced NSCLC. PF-00299804 was associated with improvements in median PFS (HR, 0.681; 95% CI, 0.490-0.945; $P = 0.019$) and objective RR (17.0% vs 4.3%; $P = 0.009$) and clinical benefit rate (response or SD ≥24 weeks; 27.7% vs 13.8%; $P = 0.03$).[57] However, there were imbalances between treatment arms of this study in the percentage of patients with performance status of 2 (PF-00299804, 19.1% vs erlotinib, 3.2%) and with tumors harboring *EGFR* mutations (PF-00299804, 20.2% vs erlotinib, 11.7%).[57]

PF-00299804 is being evaluated in patients with *KRAS* wild-type NSCLC refractory to at least 1 chemotherapy regimen and erlotinib in another phase II study.[58] Among 62 evaluable patients, 3 achieved a PR and 35 had SD. AEs included diarrhea (86%), fatigue (40%), rash (45%), and stomatitis/mucosal inflammation (23%). In Korea, an open-label, single-arm phase I/II trial is evaluating PF-00299804 in patients with advanced NSCLC and wild-type *KRAS* who have failed treatment with chemotherapy and an EGFR TKI.[59] For 42 patients in the phase II portion, preliminary results demonstrated an objective RR of 15%, clinical benefit rate (PR or SD ≥24 weeks) of 25%, and a 4-month and 6-month PFS rate of 48% and 32%, respectively. Treatment-related diarrhea (grade 3, 14.3%), paronychia (grade 3,

7.1%), and rash, stomatitis, pruritus and dermatitis acneiform (grade 3; all 2.4%) were the most common AEs observed.

Other EGFR Family TKIs

Lapatinib is a reversible dual EGFR/HER2 TKI that has been evaluated for the treatment of NSCLC. Two schedules of lapatinib (1,500 mg once daily and 500 mg twice daily) were evaluated as first-line or second-line treatment in a phase II multicenter trial in patients with advanced NSCLC.[60] Among 56 patients with bronchioloalveolar carcinoma histology or no smoking history, there were no objective responses and 14 patients (25%) had SD for ≥24 weeks. Of the remaining 75 patients, which included patients with other histologies or a smoking history, 1 (1.3%) had a PR and 16 (21%) had SD. Three patients with *EGFR* mutations failed to respond to lapatinib, although 1 of 2 patients with *HER2* amplification did achieve a 51% decrease in tumor size (albeit unconfirmed). There were no notable differences in the most common treatment-related AEs between the 2 dose schedules (1,500 mg vs 500 mg), which included diarrhea (60% vs 50%), rash (48% vs 41%), fatigue (37% vs 30%), nausea (38% vs 24%), and anorexia (26% vs 23%).[60] However, the trial was stopped due to lack of efficacy after 131 patients had been randomized to 1 of the 2 lapatinib schedules.[61] Results from this study suggest that lapatinib has limited single-agent activity in patients with advanced NSCLC.

Neratinib (HKI-272) is an irreversible EGFR/HER2 TKI.[62] In a 3-arm phase II trial, patients with advanced NSCLC were assigned to receive neratinib if they progressed after ≥12 weeks of erlotinib or gefitinib therapy and had tumors with an *EGFR* mutation (arm A) or wild-type *EGFR* (arm B), or if they had never received an EGFR TKI but had adenocarcinoma and a light (≤20 pack-year) smoking history (arm C).[62] Patients initially received neratinib 320 mg/day, but the dose was decreased to 240 mg/day because of dose delays and reductions associated with diarrhea. Overall, 3 (1.9%) of 158 patients had objective responses and 14 (9%) of 158 patients had SD for ≥6 cycles (24 ± 2 weeks), with an objective RR of 3.4% for arm A and 0% for arms B and C. Overall median PFS was 15.3 weeks (90% CI, 14.7-15.9) and was 15.3 (90% CI, 11.9-15.7), 16.1 (90% CI, 15.0-23.9), and 9.3 (90% CI, 6.4-18.9) weeks in arms A, B, and C, respectively. The most common neratinib-related AEs, regardless of grade, were diarrhea (91%), nausea (55%), fatigue (37%), vomiting (35%), anorexia (32%), and abdominal pain (32%); grade 3/4 AEs with an incidence ≥5% were limited to diarrhea (28%) and dyspnea (11%).[62] Thus, neratinib demonstrated limited efficacy in patients who were previously treated with first-generation EGFR TKIs and is no longer in development for the treatment of NSCLC.

Reasons underlying the modest clinical activity of lapatinib and neratinib in NSCLC are unknown, especially in light of robust responses observed in other cancers (eg, breast cancer).[63–66] One explanation may be that breast cancer is a largely HER2-driven disease, and *HER2*-resistance mutations have not yet been identified. In addition, the role of EGFR in breast cancer has not been fully established. Similarly, *EGFR*-activating mutations akin to those described in NSCLC have not yet been identified in breast cancer. The strong EGFR-driven component of NSCLC combined with the development of resistance likely precludes the prolonged use of reversible or weak irreversible inhibitors in NSCLC. For example, Godin-Heymann and colleagues showed that cells harboring an *EGFR* T790M mutation were resistant to neratinib and that this resistance could only be overcome with suprapharmacologic concentrations of neratinib (≥1 μM).[67] In the phase II trial by Besse and colleagues, 12 patients (7%) had T790M mutations, and none responded to neratinib.[62] These findings suggest that the treatment of advanced NSCLC patients with neratinib at maximally tolerated doses may not overcome potential development of the *EGFR* T790M mutation that is commonly associated with resistance to first-generation reversible TKIs.

Simultaneous Inhibition of EGFR and VEGF/VEGFR Pathways

An alternative approach for overcoming resistance to first-generation EGFR TKIs is to simultaneously target other pathways, such as the VEGF/VEGFR pathway. Two agents with this profile, vandetanib, an inhibitor of the EGFR, VEGFR, and rearranged during transfection (RET) TKs,[68] and BMS-690514 (Bristol-Myers Squibb; New York, NY, US), an EGFR, HER2, and VEGFR kinase inhibitor,[69] have been evaluated in NSCLC.

Results from 4 phase III clinical trials evaluating vandetanib in patients with advanced NSCLC have been reported. Results from the ZEAL trial (N = 534) indicated that the addition of vandetanib to pemetrexed significantly improved objective RR (P <0.001), but there was no significant improvement in PFS or OS compared with chemotherapy alone.[70] In the ZODIAC trial (N = 1,391), which evaluated vandetanib in combination with docetaxel, significant improvements with vandetanib were observed in the objective RR (17% vs 10%; P = 0.0001) and PFS (median 4.0 vs 3.2 months; HR, 0.79; 97.58% CI, 0.70–0.90; P <0.0001) versus chemotherapy alone, but there was no significant improvement in OS.[71] Results of the ZEST trial (N = 1,240), which evaluated vandetanib versus erlotinib in patients with advanced NSCLC, did not demonstrate significant differences in objective RR, PFS, or OS.[72] Results were also presented from another phase III trial, ZEPHYR, of vandetanib following chemotherapy and treatment with an EGFR TKI in patients with recurrent NSCLC. Vandetanib treatment resulted in an improvement in PFS (HR, 0.63; 95.2% CI, 0.54–0.74; P <0.0001) and objective RR (2.6% vs 0.7%: P = 0.028); however, the primary endpoint of prolonged OS was not met.[73] Based on these results, application for vandetanib approval in NSCLC has been withdrawn.[74]

In a phase II trial, BMS-690514 200 mg daily was administered to 60 patients with advanced NSCLC, and 11 (39%) of 28 erlotinib-naive patients and 7 (22%) of 32 erlotinib-resistant patients achieved disease control.[69] The DCR was higher in patients whose tumors harbored an *EGFR* mutation versus those with wild-type *EGFR* (75% vs 28%). BMS-690514 reduced tumor burden by 48% in an erlotinib-naive patient whose tumor had a codon 13 *KRAS* mutation and produced SD in 2 erlotinib-resistant patients with tumors harboring *EGFR* T790M mutations. The most common AEs included diarrhea (90%), skin rash (31%), asthenia (29%), anorexia (27%), hypertension (26%), and reversible acute renal insufficiency (11%).[69] BMS-690514 is currently being compared with erlotinib in a randomized phase II trial in patients with advanced NSCLC (NCT00743938).

XL647 (Exelixis; South San Francisco, CA, US and Symphony Evolution, Inc.; Rockville, MD, US) is an oral TKI with activity against EGFR, HER2, and VEGFR2.[75] In a phase I trial of patients (N = 31) with advanced solid malignancies who received XL647 350 mg/day, 2 of 4 patients developed clinically asymptomatic QT interval (QTc) prolongation.[75] XL647 was subsequently evaluated at a dose of 300 mg/day in a phase II trial in 23 patients with advanced NSCLC who had developed resistance after initial clinical benefit with erlotinib or gefitinib or whose tumors harbored an *EGFR* T790M mutation.[29] Preliminary results were reported for 8 evaluable patients: 1 had a PR and 7 had SD. However, 2 of the patients with SD discontinued therapy because of AEs, which included a grade 4 embolus and a grade 2 creatinine elevation in a patient with 1 functional kidney.[29] In another phase II study, XL647 350 mg/day was administered for 5 days every 2 weeks to 41 patients with untreated advanced NSCLC with adenocarcinoma histology.[76] Patients were eligible for inclusion if they also met at least 1 of the following criteria: Asian, female, or minimal (<15 pack-years) smoking history or no smoking history. Of 36 evaluable patients, 10 (28%) achieved a PR; of those, 7 had *EGFR*-activating mutations detected in their tumor tissue. Common AEs included grade 1 or 2 diarrhea, rash, fatigue, nausea, and clinically asymptomatic QTc prolongation.[76] No new trials evaluating XL647 in NSCLC patients are planned.

Inhibition of Parallel Signaling Pathways

Several inhibitors of signaling pathways that complement the EGFR pathway are also being evaluated in clinical trials in patients with advanced NSCLC. In vitro analyses conducted by Zucali and colleagues showed that DN-30, an anti-cMET monoclonal antibody, acted synergistically with hepatocyte growth factor to enhance the inhibition of growth by gefitinib in activated cMET [pY1003]-expressing cell lines.[35] In addition, blockade of cMET with a cMET TKI (PHA-665752) restored the sensitivity of NSCLC cells to gefitinib.[32]

Several MET inhibitors (ARQ 197 [ArQule, Inc.; Woburn, MA, US], XL184 [Exelixis; South San Francisco, CA, US], and MetMAb [Genentech; South San Francisco, CA, US]) are being tested in phase II trials in combination with erlotinib in patients with NSCLC (NCT00777309, NCT00596648, and NCT00854308, respectively). ARQ 197 is a non-ATP competitor of the MET protein, and has shown preliminary clinical activity as monotherapy[77,78] and in combination with erlotinib[79] in phase I clinical trials. In 1 trial, ARQ 197 was administered in 21-day cycles at escalating doses of 120 mg, 240 mg, and 360 mg twice daily in combination with erlotinib 150 mg/day.[79] Although no objective responses were observed in 25 treated patients with solid tumors, 3 of 3 evaluable patients with NSCLC achieved SD for durations of 14 to 32 weeks. Two patients experienced treatment-related serious AEs: neutropenia with the 360-mg dose and sinus bradycardia with the 240-mg dose.[79] Data from a global randomized phase II trial of erlotinib plus ARQ 197 or placebo (N = 167) indicated a nonsignificant improvement PFS in the ARQ 197 arm (16.1 vs 9.7 weeks in the placebo arm; HR, 0.81; 95% CI 0.57–1.15; $P = 0.23$).[80] However, a significant PFS benefit was demonstrated in a planned multivariable Cox regression model that adjusted for histology and genotype (for which imbalances were observed at baseline) and other prognostic factors (HR, 0.68; 95% CI, 0.47–0.98; $P < 0.05$); improvements in PFS were observed among patients with nonsquamous histology and with tumors harboring wild-type *EGFR* or *KRAS* mutations. In both arms, rash and diarrhea were the most common all-grade AEs, with similar incidences between the arms (64% and 52% for rash; 48% and 53% for diarrhea).

XL184, a small molecule MET TKI, has shown preclinical activity as monotherapy in EGFR TKI–resistant cell lines and in HCC827GR6 xenograft tumors when administered in combination with erlotinib.[81] Finally, MetMAb is a monovalent antagonist antibody to the MET receptor that has demonstrated preclinical activity in pancreatic[82] and glioblastoma models.[83–85] Data demonstrating the effects of XL184 or MetMAb in patients with NSCLC have not yet been presented.

PF-02341066 (Pfizer; New London, CT, US) is an inhibitor of the MET and anaplastic lymphoma kinase (ALK) TKs; it has been estimated that 1% to 6% of unselected patients with NSCLC have tumors with an echinoderm microtubule-associated protein like-4 (EML4)-ALK translocation.[86] Results were recently presented for a 2-part phase I trial of PF-02341066 in patients with *ALK* fusion-positive advanced NSCLC with varying extent of pretreatment (median of 3 prior regimens), for which the RR was 64% and DCR was 90% among the first 50 evaluable patients.[87] Monotherapy with PF-02341066 versus docetaxel or pemetrexed (investigator choice) is being evaluated in an ongoing phase III study in patients with NSCLC harboring an *ALK* aberration (NCT00932893). Patients progressing on chemotherapy in this phase III trial may be considered for inclusion in a single-arm phase II trial of PF-02341066 as monotherapy (NCT00932451). Also ongoing is a phase I/II trial of the safety, efficacy, and pharmacokinetics of erlotinib alone or combined with PF-02341066 in patients with advanced NSCLC of adenocarcinoma histology (NCT00965731). As ALK alterations are reciprocally exclusive of *EGFR* mutations,[88] these agents may be useful in patients with *EGFR* wild-type tumors that are less sensitive to first-generation EGFR TKIs.

A number of monoclonal antibodies and TKIs have been developed that target the IGF-1R.[89] Of these, the most advanced in clinical development for NSCLC is the anti–IGF-1R monoclonal antibody figitumumab (CP-751,871 [Pfizer; New London, CT, US]).[90,91] In a phase II trial, patients (N = 156) with previously untreated advanced NSCLC were randomly allocated in a 2:1 ratio to receive paclitaxel/carboplatin with or without figitumumab (10 to 20 mg/kg) every 3 weeks for up to 6 cycles.[90] Overall RR was 54% in the paclitaxel/carboplatin plus figitumumab arm compared with 42% in the chemotherapy alone arm (P <0.0001). Exploratory analyses by dose and histology revealed that among patients with squamous cell carcinomas and adenocarcinomas, overall RR was 62% in patients who received paclitaxel/carboplatin plus figitumumab 20 mg/kg versus 33% in patients who received chemotherapy alone (P = 0.0478).[90] The addition of figitumumab 20 mg/kg to chemotherapy also provided improved PFS compared with chemotherapy alone (HR, 0.46; 95% CI, 0.18 to 0.75; P = 0.0058). RRs and PFS did not differ for patients with unspecified histologies. Grade 3/4 hyperglycemia was noted in 15% and 8% of patients in the combination and chemotherapy alone arms, respectively.[90] Patient enrollment in a phase III clinical trial testing figitumumab in combination with paclitaxel/carboplatin was halted for futility.[92] Serious AEs in the combination arm included dehydration, hyperglycemia, and hemoptysis.

The heat shock protein (HSP) 90 chaperone mediates conformational changes for the EGFR family, MET, and various downstream kinases, including Akt.[93] HSP90 inhibitors may be a viable strategy for the treatment of NSCLC because *EGFR* mutations associated with resistance to first-generation EGFR TKIs do not compromise the ability of HSP90 to regulate EGFR family members.[93] HSP90 inhibitors have been shown to suppress EGFR-mediated signaling in erlotinib-sensitive and erlotinib-resistant cell lines, including those with L858R/T790M double mutation.[93] Moreover, in these resistant cells, HSP90 inhibitors prevented signaling by MET- and IGF-1R–dependent mechanisms. IPI-504 (Infinity Pharmaceuticals; Cambridge, MA, US), an HSP90 inhibitor, is being evaluated in phase I/II trial (NCT00431015) in patients with relapsed or refractory NSCLC.

The mammalian target of rapamycin (mTOR) inhibitor everolimus (Novartis; Cambridge, MA, US) was evaluated in a phase II trial of patients with advanced NSCLC who progressed after ≤ 2 prior chemotherapy regimens or chemotherapy plus a first-generation EGFR TKI.[94] Patients received everolimus 10 mg/day until PD or unacceptable toxicity. Everolimus produced objective responses in 7.1% of patients who had previously failed chemotherapy and in 2.3% of patients who had failed chemotherapy and an EGFR TKI. Overall, everolimus provided disease control in 47% of patients; median PFS was 2.7 and 2.6 months in the subgroups who had and had not received prior EGFR TKI therapy, respectively. Fatigue, dyspnea, stomatitis, anemia, and thrombocytopenia were the most frequently reported grade ≥ 3 AEs that were associated with everolimus.[94] A phase I trial is being conducted to explore the feasibility of adding everolimus to carboplatin/paclitaxel as first-line therapy in patients with NSCLC.[95] In a phase I/II trial evaluating everolimus plus erlotinib versus erlotinib alone in 133 patients with advanced NSCLC who progressed after ≥ 2 prior lines of chemotherapy, preliminary results demonstrate a 3-month DCR of 39.4% versus 28.4% and a median PFS of 2.9 months versus 2.0 months, respectively. In the combination group, the most common grade 3/4 AEs reported in ≥ 4 patients were stomatitis (32%), asthenia (11%), and diarrhea (8%).[96]

Conclusions

Only a small number of patients initially respond to first-generation EGFR inhibitors and among those who respond acquired resistance is common. A number of mechanisms of resistance have been identified, but they do not account for all cases of resistance to

treatment, suggesting that there are other unknown mechanisms of resistance. It appears that treatment resistance is pleomorphic and that many mechanisms can coexist in the same cell population. Therefore, combinations of therapies or therapies with multiple targets may be more effective. For next-generation EGFR TKIs, it will be important to determine whether acquired resistance still develops with the activation of compensatory signaling pathways. Many agents discussed herein are being evaluated in combination (eg, an EGFR inhibitor in combination with a MET or mTOR inhibitor) in the hope that resistance mechanisms will be overcome by simultaneously silencing EGFR signals and by blocking mechanisms of evasion. The strategy of targeting multiple tumorigenic pathways simultaneously (eg, EGFR and VEGFR) may also be an effective approach to overcome resistance to current therapy. As our understanding of intra- and inter-EGFR family signaling increases, strategies for the development of targeted agents for the treatment of NSCLC will likely evolve.

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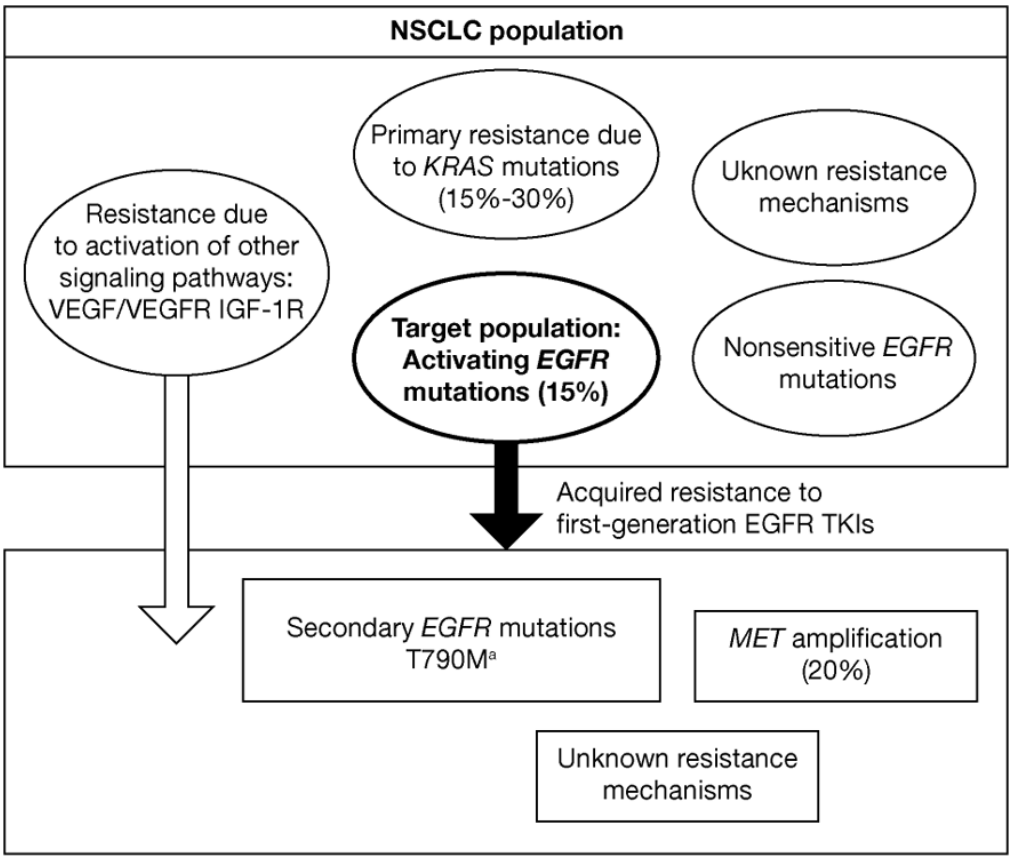

Figure 1.

Mechanisms of resistance to first-generation EGFR TKIs. The principal target population for first-generation EGFR TKIs is patients with activating *EGFR* mutations, primarily exon 19 deletions and exon 21 point mutations. Patients with *KRAS* mutations, activation of complementary signaling pathways, and nonsensitive *EGFR* mutations are typically resistant to these agents. Patients who initially respond may have the T790M mutation and may acquire resistance from *MET* amplification, or activation of alternative signaling pathways. Unknown mechanisms continue to play a part in both primary and acquired resistance. EGFR, epidermal growth factor receptor; IGF-1R, insulin-like growth factor-1 receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; MET, mesenchymal epithelial transition factor; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
[a]Indicates the T790M mutation may have been present prior to treatment.

Table 1

Targeted Agents in Clinical Development for Advanced NSCLC

Agent	Mechanism of Action	Status in NSCLC[a]
Afatinib	Irreversible EGFR/HER2 TKI	Phase III
PF-00299804	Irreversible pan-HER TKI	Phase III
PF-02341066	MET/ALK TKI	Phase III
ARQ 197	MET TKI	Phase II
XL184	MET/VEGFR/c-Kit/Flt3 TKI	Phase II
MetMAb	Anti-MET monoclonal antibody	Phase II
BMS-690514	EGFR/HER2/VEGFR TKI	Phase II
Everolimus	mTOR inhibitor	Phase II
IPI-504	HSP90 inhibitor	Phase I/II

[a]Based on ClinicalTrials.gov.

ALK, anaplastic lymphoma kinase; c-Kit, stem cell factor receptor; EGFR, epidermal growth factor receptor; Flt3, fms-like tyrosine kinase 3; HER, human epidermal growth factor receptor; HSP, heat shock protein; MET, mesenchymal-epithelial transition factor; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.