Intrinsic resistance to EGFR tyrosine kinase inhibitors in advanced non-small-cell lung cancer with activating EGFR mutations

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Abstract: Identifying activating EGFR mutations is a useful predictive strategy that helps select a population of advanced non-small-cell lung cancer (NSCLC) patients for treatment with EGFR tyrosine kinase inhibitors (TKIs). Patients with sensitizing EGFR mutations (predominantly an in-frame deletion in exon 19 and an L858R substitution) are highly responsive to first-generation EGFR TKIs, such as gefitinib and erlotinib, and show improved progression-free survival without serious side effects. However, all patients with activating EGFR mutations who are initially responsive to EGFR TKIs eventually develop acquired resistance after a median progression-free survival of 10–16 months, followed by disease progression. Moreover, ~20%–30% of NSCLC patients have no objective tumor regression on initial EGFR TKI treatment, although they harbor an activating EGFR mutation. These patients represent an NSCLC subgroup that is defined as having intrinsic or primary resistance to EGFR TKIs. Different mechanisms of acquired EGFR TKI resistance have been identified, and several novel compounds have been developed to reverse acquired resistance, but little is known about EGFR TKI intrinsic resistance. In this review, we summarize the latest findings involving mechanisms of intrinsic resistance to EGFR TKIs in advanced NSCLC with activating EGFR mutations and present possible therapeutic strategies to overcome this resistance.

Keywords: NSCLC, EGFR mutation, EGFR TKIs, intrinsic resistance, T790M

Introduction

Primary lung cancer is one of the most common malignancies and a major cause of cancer-related mortality worldwide, accounting for ~1.6 million deaths per year.1 Approximately 85% of all primary lung cancers are non-small-cell lung cancers (NSCLCs), and adenocarcinoma is the most common histologic subtype of NSCLC. A majority of NSCLC patients present with locally advanced or metastatic disease and cannot undergo surgical resection when they are initially diagnosed. The overall therapeutic outcome of NSCLC is far from satisfactory. The 5-year survival rate of metastatic NSCLC is <5%, with a median overall survival (OS) of <12 months. The benefits and efficacy of cytotoxic chemotherapy and radiation therapy are limited, and they cause relatively serious side effects, affecting the patients’ quality of life.2

In the past decade, significant improvements have been made due to the development of targeted therapies, such as EGFR tyrosine kinase inhibitors (TKIs), for advanced NSCLC. Several large Phase III clinical trials have demonstrated that patients with a sensitizing exon 19 deletion or an exon 21 substitution mutation are highly responsive to first-generation EGFR TKIs, such as gefitinib and erlotinib, compared to traditional platinum-based doublet chemotherapy, with a prolonged time...
### Table 1 Clinical response rate and survival results of EGFR-mutant or EGFR wild-type NSCLC patients treated with EGFR TKIs as first-line therapy

| Study            | Study name       | Year | Treatments | Mutated EGFR | Wild-type EGFR |
|------------------|------------------|------|------------|---------------|----------------|
| Mok et al\(^2\)  | IPASS            | 2009 | G          | 132 71.2  9.5 | 91 1.1 3.1 11.2 |
| Mitsudomi et al\(^3\) | WJT0G3405        | 2010 | G          | 86 62.1  9.2 | 0 0 0 0 |
| Maemondo et al\(^4\) | NEJ002          | 2010 | G          | 114 73.7 10.8 | 0 0 0 0 |
| Zhou et al\(^5\)  | OPTIMAL          | 2011 | E          | 83 82.0 13.1 | 0 0 0 0 |
| Han et al\(^6\)   | First-SIGNAL     | 2012 | G          | 26 84.8  8.0 | 27 25.9 2.1 18.4 |
| Rosell et al\(^7\) | EURTAC           | 2012 | E          | 86 64.0  9.7 | 0 0 0 0 |
| Lee et al\(^8\)   | TOPICAL          | 2012 | E          | 28 4.8 10.4 | 0 0 0 0 |
| Sequist et al\(^9\) | LUX Lung-3       | 2013 | E          | 230 56.0 11.1 | 0 0 0 0 |
| Wu et al\(^10\)   | LUX Lung-6       | 2014 | E          | 224 66.9 11.0 | 0 0 0 0 |
| Wu et al\(^11\)   | ENSURE           | 2015 | E          | 217 62.7 11.0 | 0 0 0 0 |

**Abbreviations:** NSCLC, non-small-cell lung cancer; G, gefitinib; E, erlotinib; A, afatinib; TKIs, tyrosine kinase inhibitors; ORR, objective response rate; mPFS, median progression-free survival; mOS, median overall survival.

EGFR and activating EGFR mutations in NSCLC

EGFR is a member of the ErbB family, which also includes HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). EGFR is auto-phosphorylated at tyrosine residues when it binds to its ligands, including EGF and transforming growth factor-α. As a potent oncogenic driver, EGFR activation further activates downstream signaling pathways, such as PI3K/Akt/mTOR and RAS/RAF/MAPK, which promote cell proliferation and survival and inhibit apoptosis.\(^5\) In 2004, somatic mutations in the tyrosine kinase domain of EGFR were characterized in NSCLC. The mutated EGFR is constitutively active in ~20% of NSCLC patients, with significantly increased proportions in adenocarcinoma, females, those of Asian ethnicity, and nonsmokers.\(^6,7\) EGFR expression and high EGFR gene copy number are also found in 10%–30% of NSCLC patients. Earlier studies showed that constitutive activation of the EGFR signaling pathway was initiated by EGFR gene amplification. Subsequent studies found that EGFR activation was triggered by EGFR mutations but not by EGFR amplification, although both activating EGFR mutations and amplification could occur in cancer tissues.\(^8\) Patients with EGFR gene amplifications or mutations have a different prognosis compared to those without these genetic alterations. Common activating mutations include an in-frame deletion in exon 19 and an L858R substitution in exon 21, which account for >90% of all EGFR mutations in NSCLC. Generally, activating mutations in the EGFR gene are mutually exclusive from other gene mutations and result in constitutive activation of EGFR and downstream signaling pathways independent of ligand binding and EGFR expression, eventually promoting the growth and spread of the cancer.\(^9\)

### Treatment of NSCLC with EGFR TKIs

Several small-molecule TKIs targeting EGFR, such as reversible competitive inhibitors of ATP, including erlotinib and gefitinib, and irreversible inhibitors, including afatinib, dacomitinib, and neratinib, have been developed to block EGFR-mediated downstream signaling activation. In lung cancer cells with EGFR mutations, gefitinib and erlotinib...
selectively bind to the tyrosine kinase region of the intracellular domain of EGFR and significantly attenuate the autophosphorylation of EGFR, reduce the subsequent activation of the PI3K/Akt/mTOR and RAS/RAF/MAPK pathways, and inhibit cell proliferation and promote apoptosis. Irreversible inhibitors also show potent activity against proliferation in cells with activating EGFR mutations and gatekeeper T790M mutations by binding covalently to EGFR.

Several EGFR TKIs have been explored and evaluated as potent agents for the treatment of NSCLC with activating EGFR mutations in large randomized Phase III clinical trials (Table 1). Clinical use of EGFR TKIs has promoted a change in the approach to patients with advanced diseases, particularly lung adenocarcinomas. The IRESSA Pan-Asia Study (IPASS) was the first randomized clinical trial comparing gefitinib (n=609) to chemotherapy (paclitaxel plus carboplatin, n=608) as a first-line therapy for patients with advanced NSCLC, although only East Asian patients who were light or nonsmokers were enrolled. The objective response rate (ORR) in the overall population was significantly higher in patients treated with gefitinib monotherapy than those treated with chemotherapy (43.0% versus 32.2%, respectively). In the gefitinib-treated arm, the ORR was 71.2% in patients with EGFR mutations versus 1.1% in patients without EGFR mutations. Subgroup analysis in 261 patients with EGFR mutations showed that gefitinib resulted in a longer PFS than chemotherapy (9.5 versus 6.3 months, respectively). Several large Phase III trials further confirmed the superiority and efficacy of EGFR TKIs over standard chemotherapy, including the WJTOG3405, NEJ002, OPTIMAL, and ENSURE studies in Asian patients and the EURTAC study in European patients. These studies consistently found that NSCLC patients with EGFR mutations had a high ORR ranging from 58% to 83%, with a median PFS of 10–16 months, when they received reversible EGFR TKIs. However, EGFR TKIs were not more effective than chemotherapy as a second-line therapy for previously treated NSCLC patients who had wild-type EGFR. Although there was no benefit in terms of OS due to the influence of subsequent treatments in trials using first-generation EGFR TKIs, an irreversible EGFR inhibitor, afatinib, significantly prolonged OS of the subgroup of patients with exon 19 deletion mutations in the whole population from the LUX-Lung 3 and/or LUX-Lung 6 studies. However, no significant difference was found in the OS of patients with exon 21 mutations. Thus, the researchers concluded that the tumors with EGFR exon 19 deletions and L858R mutations should be considered two distinct populations that should be studied separately and will require different treatment strategies in the future. The clinical difference in OS observed with afatinib compared to traditional chemotherapy in patients with the exon 19 deletion could be attributed to a difference in mechanism between afatinib and the first-generation EGFR TKIs. Moreover, the rechallenge with EGFR TKIs could also affect survival.

Overall, patients receiving TKIs tolerated them well, and only displayed mild adverse events, including skin rash and diarrhea. TKIs improved the quality of life and delayed the deterioration of symptoms. There are more patients of Asian ethnicity who benefited from TKI treatment due to a high EGFR (nearly 50%) mutation frequency compared to those of Caucasian ethnicity (<20%). In the clinical management of advanced NSCLC, EGFR TKIs have been approved around the world as the standard first-line therapy for a subset of patients whose tumors harbor EGFR mutations. Because EGFR TKIs are costly and may have deleterious effects on individuals without activating mutations, EGFR mutation testing is recommended prior to the initiation of TKI treatment, which allows for selection of optimal advanced NSCLC.

Unfortunately, similar to standard platinum-based chemotherapy, all patients with mutant EGFR will ultimately develop resistance to EGFR TKI therapy after a period of promising clinical response. The efficacy of EGFR TKIs is largely limited by either acquired (secondary) or intrinsic (primary) resistance. The elucidation of the molecular mechanisms underlying acquired resistance to EGFR TKIs is an active area of clinical and translational research since Kobayashi et al first identified a secondary mutation associated with acquired resistance to gefitinib. These established mechanisms include development of a secondary EGFR T790M gatekeeper mutation, MET amplification, HER2 amplification, epithelial–mesenchymal transition (EMT), and transformation of NSCLC to small-cell histology. Moreover, ~20%–30% of patients with mutated EGFR have intrinsic resistance to EGFR TKI therapy, and some patients do not show an acceptable response to treatment. The mechanisms of intrinsic resistance to EGFR TKIs are currently not understood well.

**Mechanisms of intrinsic resistance to EGFR TKIs in EGFR-mutated NSCLC**

Similar to drug resistance associated with chemotherapy, resistance to EGFR TKIs includes intrinsic (primary) and acquired (secondary) resistance. Intrinsic resistance is generally defined as a de novo inactivation of EGFR TKIs, whereas
acquired resistance is generally defined as relapse of the disease following a period of clinical response. The intrinsic resistance to EGFR TKIs in NSCLC patients can be divided into resistance in patients with mutant EGFR and resistance in patients with wild-type EGFR. Specifically, Jackman et al recently proposed detailed criteria for acquired resistance in patients with mutant EGFR. These include 1) patients harbor an activating EGFR mutation that is associated with clinical response to first-generation EGFR TKIs, 2) patients achieve a partial or complete response, or develop a stable disease in response to EGFR TKI monotherapy (>6 months), 3) disease progression occurs despite uninterrupted exposure to EGFR TKIs in accord with response evaluation criteria in solid tumors, and 4) patients receive additional systematic treatment after discontinuation of EGFR TKIs. However, clinical criteria for intrinsic resistance to EGFR TKIs have not been established. In all Phase III trials evaluating the efficacy of EGFR TKIs in previously untreated EGFR-mutant NSCLC, intrinsic resistance refers to development of progressive or stable disease as the best response to EGFR TKIs in ≤2 months. Other investigators defined intrinsic resistance as progressive or stable disease in <3 or 4 months without any evidence of an objective response when using EGFR TKIs. In contrast to acquired resistance to EGFR TKIs, intrinsic resistance is more complicated. Although the molecular basis of intrinsic resistance to EGFR TKIs remains to be clarified, genetic mutations and molecular alterations from several preclinical and retrospective studies present clues to direct further investigations.

Smoking status

It is well known that activating EGFR mutations are more frequently found in nonsmokers than smokers. Additionally, current smokers experience a lower clinical benefit from EGFR TKI therapy compared to never smokers as well as former smokers. A more recent meta-analysis showed that the PFS benefit for never smokers was better than that for ever smokers, although all patients had an activating EGFR mutation and received EGFR TKI treatment. Thus, smoking-related lung cancer is viewed as an independent disease that is different from nonsmoking-related lung cancer with respect to genetic profile, prognosis, and response to targeted therapy, as well as other treatment strategies. Cigarette smoke has been found to activate Src kinase and lead to aberrant activation of EGFR or cytochrome P450 1A1, which is involved in the metabolism of TKIs, or promote degradation of EGFR by inducing the release of reactive oxidative species. Nicotine exposure has been found to induce resistance to EGFR TKIs in EGFR-mutated NSCLC PC-9 cells via an EGFR signal. However, Lee et al identified eleven patients who showed primary resistance to EGFR TKIs (disease progression <3 months) among 197 consecutive EGFR-positive patients who received EGFR TKI therapy, in which EGFR genotype and smoking did not predict PFS for targeted treatments.

Genetic alterations of EGFR

Some NSCLC patients exhibit intrinsic resistance to EGFR TKIs, although they have an activating EGFR mutation (Figure 1). It has been shown that insertion mutations in exon 20 of the EGFR gene are associated with oncogenic transformation of cells and confer primary resistance to EGFR TKIs, with the exception of the insertion A763_Y764insFQEA in the C-terminal helix of EGFR. This mutation represents ~5%–10% of all known EGFR gene mutations. In in vitro experiments, regular treatment with gefitinib or erlotinib was unable to inhibit EGFR signaling and induce cell apoptosis because this mutation decreased the affinity for EGFR TKIs, but the irreversible inhibitor CL-387,785 was efficacious. Further clinical investigations showed that the majority of patients with insertions in exon 20 of the EGFR gene did not demonstrate a clinical response when treated with TKIs, including reversible and irreversible inhibitors, although these patients had an OS similar to that of patients with EGFR exon 19 or 21 mutations.

T790M is a frequently reported secondary mutation of the EGFR gene and is detected in >60% of patients with acquired resistance to EGFR TKI therapy. This point mutation causes a substitution of methionine for threonine at position 790 in exon 20 of the EGFR gene. The methionine side chain leads to steric hindrance that affects the ability of EGFR TKIs to bind to the ATP-kinase pocket. Interestingly, a recent report showed that the T790M resistance mutation may be associated with genetic susceptibility to lung cancer. The T790M mutation has been described as a minor clone in a small group of treatment-naïve NSCLCs harboring activating EGFR mutations, suggesting its association with intrinsic EGFR TKI resistance (Table 2).

Generally speaking, the T790M mutation leads to poor response to EGFR TKIs. Patients who harbor both resistant T790M and an activating EGFR mutation have a reduced ORR and a shorter PFS compared with those with an activating EGFR mutation but without T790M. However, there are no differences between the two groups regarding OS. Furthermore, patients with wild-type EGFR still have a significantly longer PFS. Thus, the EGFR
TKI benefit for patients with a baseline EGFR T790M detected by standard molecular analysis is limited, and initial treatment with EGFR TKIs may not be a first-line option for these patients. However, it is currently unclear whether T790M-positive patients should be excluded from EGFR TKI therapy.

The frequency of the T790M mutation in pretreated tumor samples with activating EGFR mutations has been reported and ranges from 1% to 65%, which is dependent on the techniques used to analyze this mutation.\textsuperscript{37,42–45,47–52} For example, the T790M mutation was found in 25.2% of pretreated NSCLC tumors using a highly sensitive method, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and only 2.8% by direct sequencing.\textsuperscript{43} A study reported that 79.9% of TKI-naïve tumor specimens with EGFR mutations were T790M-positive by digital polymerase chain reaction (PCR).\textsuperscript{48} The T790M mutation was detected by nanofluidic digital PCR in all EGFR-mutated NSCLC samples regardless of EGFR TKI treatment, and the ratio of the number of T790M alleles to that of activating mutation alleles (T/A) is associated with resistance to EGFR TKIs, indicating that highly sensitive and quantitative detection of T790M is also important for evaluation of the contribution of T790M to EGFR TKI resistance.\textsuperscript{47}

**Genetic alterations in bypass signaling**

Genetic alterations in genes other than EGFR and its associated ErbB family members can occur with activating EGFR mutations and may be responsible for the decreased sensitivity of NSCLC to EGFR TKI treatment (Figure 1). These alterations include EGFR signal-related and non-EGFR signal-related mutations. Several studies have shown that genetic alterations are associated with acquired EGFR TKI
resistance in TKI-treated tumors or intrinsic resistance in TKI-naïve tumors.

For example, somatic exon 20 insertions have been found in the HER2 gene in NSCLC specimens. These mutations lead to constitutive phosphorylation and activation of HER2 as well as EGFR and confer resistance to gefitinib or erlotinib. 53 Somatic PIK3CA mutations in the catalytic domain of PIK3CA have been identified in NSCLC tumors after TKI treatment, which is one mechanism related to acquired resistance to EGFR TKI. 54 These mutations have also been detected in 1%–3% of tumor samples harboring activating EGFR mutations prior to TKI therapy. In in vitro experiments using EGFR-mutant cell lines, PIK3CA mutations contributed to consistent activation of PI3K and attenuated TKI-induced apoptosis. 55 Interestingly, PIK3CA mutations have been found in NSCLC with EGFR/KRAS mutations, and patients with single PIK3CA mutations had shorter OS than those with PIK3CA-EGFR/KRAS co-mutations or wild-type PIK3CA or wild-type EGFR/KRAS. 56

The PTEN controls the PI3K/Akt signal pathway and acts as a tumor suppressor gene. Mathematical models showed that several downstream factors, including pAkt, pSTAT3, and pERK, were largely inhibited in the presence of erlotinib, but pAkt levels did not decrease with the loss of PTEN, suggesting that cells that lost PTEN were resistant to erlotinib. 57 Sos et al found that loss of PTEN expression contributed to primary resistance to EGFR TKIs. In this study, there was one patient with PTEN loss among 24 patients with mutated EGFR. Further in vitro study indicated that H1650, a lung adenocarcinoma cell line with an exon 20 deletion in the EGFR gene, also harbored a PTEN loss of function and exhibited innate resistance to erlotinib. The effect of PTEN loss on TKI efficacy was related to activation of Akt and EGFR in EGFR-mutant cells. 58 In another study by Cetin et al, reduced PTEN protein expression levels were observed in 17 (34%) of the cases. The loss of PTEN expression could be an intrinsic mechanism of EGFR TKI resistance in advanced NSCLC. 59 In addition to the described genetic mutations,
Microenvironment
The tumor microenvironment and stroma are critical for cancer progression or metastasis. Growing evidence has indicated the involvement of alteration of the tumor microenvironment in the development of resistance to EGFR TKIs. For example, cancer-associated fibroblasts (CAFs) in the tumor microenvironment contribute to intrinsic resistance to EGFR TKIs in NSCLC with mutated EGFR. In a mouse model, an EMT-derived subpopulation of CAFs expressed a marker of gefitinib resistance, epithelial membrane protein-1. Furthermore, EGFR TKI-resistant CAFs secreted paracrine factors that mitigated the EGFR TKI-mediated blockade of pEGFR and pMAPK.60 Lung adenocarcinoma cells with activating EGFR mutations (PC9 and HCC827) showed intrinsic resistance to gefitinib when they were cocultured with podoplanin-expressing CAFs by direct contact, whereas separate coculturing of lung cancer cells and CAFs did not induce resistance to gefitinib.61,62 The podoplanin-mediated pERK expression induced EMT and activation of the Hedgehog signaling pathway, which were associated with primary EGFR TKI resistance. Further clinicopathological investigations revealed that a significantly lower response was observed in EGFR-mutated patients with podoplanin-positive CAFs compared with those with podoplanin-negative CAFs following gefitinib therapy (53% versus 83%, respectively).63

Integrin and cadherin are responsible for cellular adhesion to the extracellular matrix, and play important roles in mediating cell–cell adhesion. Recently, EGFR-mutant NSCLC cells expressing increased levels of integrin β-1 were found to promote resistance to EGFR TKIs by activating the Akt signaling pathway. The expression levels of integrin β-1 in patients who were resistant to EGFR TKIs were significantly higher than those in patients who were sensitive to EGFR TKIs.61 Yamauchi et al found that N-cadherin expression was markedly upregulated in gefitinib-resistant PC9 cells with mutated EGFR as well as NSCLC cells with wild-type EGFR. Inhibition of N-cadherin expression induced caspase-dependent apoptosis in association with inactivation of the PI3K/Akt signaling pathway.64 Other cytokines and noncoding RNAs might play an important role in mediating primary resistance to EGFR TKIs. For example, microRNA (miRNA) expression signatures demonstrated that three miRNAs, miR-21, miR-27a, and miR-218, were related to primary resistance to EGFR TKIs in NSCLC patients who had EGFR exon 19 deletions.65 These results indicate that the clinical difference in outcome for patients with resistance to EGFR TKIs could be attributed to different miRNA expression patterns, which may distinguish between sensitive and resistant patients with activating EGFR mutations. However, these results are from a small sample of individuals.

Molecular alterations of related molecules
Molecular alterations of specific molecules might also play a role in primary resistance to EGFR TKIs in NSCLC patients with activating EGFR mutations, independent of the T790M mutation. MET protein expression and phosphorylation are commonly found in NSCLC tumors before EGFR TKI treatment. Furthermore, MET activation was significantly associated with poor response to subsequent EGFR TKI treatment, regardless of the EGFR status.66 As a ligand of the MET receptor, HGF can confer resistance on NSCLC cells harboring activating EGFR mutations by phosphorylation of MET and activation of the PI3K/Akt pathway.67 In a report involving 97 lung patients with mutant EGFR, high levels of HGF expression were found in 29% of tumors with intrinsic resistance compared to none with the EGFR T790M mutation and 4% with MET amplification. High HGF expression might be more common than other mutations in tumors with primary resistance and may promote intrinsic resistance to EGFR TKIs by activating the MET signaling pathway.68 Moreover, HGF was responsible for reducing susceptibility to irreversible EGFR TKIs in NSCLC with EGFR T790M mutations.69 Recent investigations by Park et al revealed that CRIPTO1 expression in NSCLC with mutated EGFR is likely a major mechanism that leads to intrinsic resistance to EGFR TKIs. All EGFR-mutated NSCLC tumors that were resistant to erlotinib expressed higher levels of CRIPTO1, whereas only 30% of EGFR-mutated NSCLC tumors that were sensitive to erlotinib showed CRIPTO1 expression. Further in vitro studies showed that CRIPTO1-induced erlotinib resistance
was linked to activation of the SRC signaling pathway via downregulation of miR-205 expression.29

As a proapoptotic molecule of the Bcl-2 family, BIM is responsible for apoptosis triggered by a variety of molecules, including EGFR TKIs.70 In vitro experiments, EGFR TKI-induced apoptosis in sensitive EGFR-mutated cells was associated with BIM expression, whereas cisplatin-induced apoptosis was not triggered by overexpression of BIM in different NSCLC cells regardless of the EGFR status.71 BIM deletions are more frequently found in East Asian than Caucasian patients, with a prevalence of 12.3%.72 Marker analyses from the EURTAC study showed that patients with erlotinib whose tumors expressed low or intermediate levels of BIM mRNA had a lower ORR (34.6% versus 87.5%, respectively) and unfavorable PFS (7.2 versus 12.9 months, respectively) and OS (22.1 versus 28.6 months, respectively) compared to those whose tumors expressed high levels of BIM mRNA.73 The molecular mechanisms underlying BIM regulation are still not fully understood. One possibility is that only specific active BIM isoforms affect the sensitivity of cancer cells to EGFR TKIs. A polymorphism may result in a BIM isoform that lacks the proapoptotic BH3 domain, promoting resistance to EGFR TKIs. Ng et al found that patients with this polymorphism showed a reduced median PFS compared to those without this polymorphism (6.6 versus 11.9 months, respectively).72 In a Chinese study involving 166 patients who received EGFR TKI therapy and had an activating mutation, the median PFS (4.7 versus 11.0 months; 25% versus 66%, respectively) and the ORR were lower in patients with BIM deletions than those without this variation.73 However, the effect of BIM deletion on the sensitivity to EGFR TKIs and prognosis of lung cancer patients remains controversial. Some investigators believe that this BIM deletion cannot account for the intrinsic resistance in EGFR-mutant NSCLC patients to erlotinib or gefitinib.23 A meta-analysis of six studies was conducted to evaluate the association between the BIM deletion and resistance to EGFR TKIs in NSCLC patients. The BIM deletion may represent a negative predictive biomarker for tumor response in NSCLC patients treated with EGFR TKIs, with an ORR of 0.39 (95% confidence interval [CI]: 0.23–0.67). This polymorphism was significantly correlated with poor PFS (hazard ratio [HR]: 1.37; 95% CI: 1.09–1.7) and OS (HR: 1.25; 95% CI: 1.08–1.45).74 Recently, Wu et al provided evidence suggesting that paxillin expression may confer primary resistance to EGFR TKIs in lung cancer cells with mutated EGFR in vitro and nude mouse models. Paxillin induced ERK activation and modulated the stability of BIM by phosphorylation at serine 69. Importantly, tumors expressing high levels of paxillin exhibited an unfavorable response to EGFR TKIs compared to those with low levels of paxillin (65% versus 26%). Moreover, the OS and PFS were significantly shorter in patients with high levels of paxillin compared to those with low levels of paxillin.75 Thus, BIM deletion causes partial, but not absolute, intrinsic resistance to EGFR TKI treatment. Several molecular mechanisms could synergistically contribute to intrinsic resistance to EGFR TKIs, such as genetic alterations and posttranscriptional regulation of the BIM gene.

Heterogeneity of mechanisms of resistance

Lung cancer is a disease with heterogeneous features, and a higher homogeneity has been found in EGFR mutation status. EGFR mutation heterogeneity in tumors could explain why a subset of patients had mixed response to EGFR TKIs. Thus, intratumor heterogeneity could be responsible for intrinsic resistance. Firstly, multiple intrinsic resistance mechanisms could co-occur in a single tumor sample. Bean et al found that MET amplification occurred with T790M mutations in EGFR-mutated tumors with acquired resistance to EGFR TKIs.76 Both EGFR T790M and MET amplification have been found in a minor population of NSCLC cells before exposure to EGFR TKI treatment.43,77 Secondly, mutation ambulance and category is different in different sites within same metastatic organ. As a sample, a large study of EGFR mutation heterogeneity between primary lung tumors and their metastases revealed that the overall discordance rate was 13.9% in 180 patients, but patients with multiple pulmonary nodules had the highest discordance rate of 24.4%.78 Although the T790M mutation is usually detected in tumors after exposure to EGFR TKIs, it also occurs prior to EGFR TKI treatment. In patients with acquired resistance, T790M mutation could be detected in one primary site, but it could disappear in another site and/or other resistance mutations could be found in another metastatic site including HER2 amplification and MET amplification.40 Thirdly, EGFR mutation status or mechanism of resistance could be different in different metastatic sites, despite the use of highly sensitive methods. Multiple biopsies might elucidate the relevance and degree of heterogeneity distribution of driver gene mutations regarding the metastatic locations and different parts of the same tumor at the time of initial diagnosis. However, single biopsy is sometimes not accessible. Liquid biopsy that measures the serum level of circulating tumor DNA, microdissection of small tumor foci, deep sequencing of
small amounts of tumor tissues, and more sensitive detection techniques such as digital PCR and MALDI-TOF-MS would help answer the critical question of high intratumor heterogeneity in EGFR mutation and heterogeneity of clinical response to EGFR TKIs.

Overcoming intrinsic resistance to EGFR TKIs

Acquired resistance to EGFR TKIs has been widely investigated, and many treatment strategies have been explored. However, few studies have been specifically designed to delay or overcome intrinsic TKI resistance. In clinical practice, no standard approach exists to overcome this resistance. However, there are several overlapping mechanisms between intrinsic and acquired resistance, and strategies to overcome acquired resistance could also be applied to intrinsic resistance.

Second-generation EGFR TKIs

The irreversible EGFR TKIs, such as afatinib, neratinib, and dacomitinib, bind covalently to the Cys-797 residue of EGFR. These inhibitors suppress the activation of EGFR and PI3K/Akt and inhibit proliferation in cells with mutant EGFR, even those harboring a resistant T790M mutation. Nevertheless, the LUX-Lung 1 trial comparing afatinib and a placebo as a second-line or third-line therapy for patients who progressed following gefitinib or erlotinib therapy failed to demonstrate an improvement in OS for afatinib (10.8 versus 12 months). Patients treated with afatinib had a low ORR of 7% and a prolonged median PFS of only 2.2 months compared to those with the placebo. Clinical use of afatinib is presently limited because of dose-limiting toxicity related to concurrent inhibition of wild-type EGFR, such as diarrhea and rash. Currently, treatment with afatinib is not recommended for NSCLC patients with mutated EGFR when they are refractory to erlotinib or gefitinib. Therefore, second-generation EGFR TKI monotherapy does not appear to be an alternative strategy to overcome primary resistance.

Third-generation EGFR TKIs

The T790M mutation in exon 20 is the most common mechanism associated with acquired resistance to EGFR TKIs and accounts for ~50% of all acquired resistance in NSCLC patients who have lung cancer with an EGFR mutation. In addition, this mutation is most often present in pretreated patients with activating EGFR mutations. The third-generation EGFR TKIs, such as AZD9291, CO1686, and HM61713, have been developed to specifically target the T790M EGFR mutation (Table 3). These compounds were shown to be active in cell experiments and murine models of lung cancer driven by EGFR T790M. AZD9291 irreversibly and selectively inhibits T790M mutation-positive EGFR as well as sensitizing EGFR. Recently, Jänne et al reported the final results in a Phase I study, including a dose-escalation cohort of 31 patients and a dose-expansion cohort of 222 patients. The ORR among all patients was 51% (95% CI: 45%–58%). A total of 127 patients with centrally confirmed EGFR T790M mutations significantly responded to AZD9291, with an ORR of 61% (95% CI: 52%–70%) compared to 21% (95% CI: 12%–34%) in patients without the EGFR T790M mutation. The most frequently reported side effects were rash, pruritus, and diarrhea, without dose-limiting toxicity. In November 2015, a tablet formulation of AZD9291 (osimertinib) was granted accelerated approval by the US Food and Drug Administration as a second-line therapy for metastatic EGFR T790M mutation-positive NSCLC that progressed after erlotinib or gefitinib treatment. Several large prospective clinical trials evaluating the efficacy of AZD9291 in EGFR-mutated NSCLC are ongoing, including a Phase II, single-arm, open-label study (NCT02094261) in second-line T790M-positive patients and a Phase III study comparing AZD9291 to platinum/pemetrexed chemotherapy in second-line T790M-positive patients (NCT02151981). The results from the AURA2 Phase I/II study (NCT02094261) showed that pretreated patients with activating EGFR mutations and T790M mutations benefited from an 80 mg once-daily dose of AZD9291, with an ORR of 71% and an

Table 3 Clinical response rate and survival results of EGFR-mutant or EGFR wild-type NSCLC patients treated with the third-generation EGFR TKIs

| Study | Mutated EGFR | Wild-type EGFR | NSCLC |
|-------|--------------|----------------|-------|
|       | N ORR (95% CI, %) | mPFS (95% CI, months) | N ORR (95% CI, %) | mPFS (95% CI, months) | N ORR (95% CI, %) | mPFS (months) |
| AZD9291 | 138 | 61 (52–70) | 9.6 (8.3–NR) | 62 | 21 (12–34) | 2.8 (2.1–4.3) | 239 | 51 (45–58) | 8.2 |
| CO1686 | 46 | 59 (45–73) | 13.1 (5.4–13.1) | 17 | 29 (8–51) | 5.6 (1.3–NR) | 83 | 21.7 | |
| HM61713 | 48 | 29.2 | 18.8 (11.5–20.5) | 34 | 11.8 | 10.0 (5.7–11.5) | 36 | 50 |
| ASP8273 | 15 | 80 | 21.8 | |

Abbreviations: TKIs, tyrosine kinase inhibitor; NSCLC, non-small-cell lung cancer; ORR, objective response rate; mPFS, median progression-free survival; NR, not reached.
immature PFS of 8.6 months.83 An international Phase III study (NCT02296125) has been designed to assess the safety and efficacy of AZD9291 versus EGFR TKIs as a first-line treatment for patients with locally advanced or metastatic NSCLC with activating EGFR mutations.

CO1686 (rociletinib) is another promising third-generation irreversible TKI that selectively inhibits mutant EGFR, in particular the T790M drug-resistant mutation, in NSCLC models.84 In an initial expansion of a Phase II/II study, 130 patients received the free-base form of rociletinib (150 mg once daily to 900 mg twice daily) or the hydrog bromide salt form (500 mg twice daily to 1,000 mg twice daily). The ORR among the 46 patients with T790M mutations was 59% (95% CI: 45%–73%), and the rate among the 17 patients without T790M mutations was 29% (95% CI: 8%–51%).85 Subsequent interim Phase II data reported that the ORR among the 81 patients with T790M mutations was 51%. The T790M-positive subset with mature data showed an ORR of 60%. The frequent side effects included nausea, diarrhea, and grade 3 hyperglycemia. At the recommended Phase II dose (625 mg twice daily), the ORR was 60% with the duration of response ranging from 85 to 184+ days. In the T790M-negative group, the overall response rate was 28%. Two clinical trials, including a Phase II trial of rociletinib as a second-line therapy (NCT02147990) and a Phase III trial comparing rociletinib to erlotinib in a first-line setting (NCT02186301), are ongoing. Other compounds, including HM61713 and ASP8273, have also been shown to target T790M and common exon 19 deletions and exon 21 EGFR substitution mutations, with an ORR ranging from 29.2% to 77.8%. In particular, treatment with HM61713 delivered a comparable ORR of 29.6% in patients who progressed following treatment with first-generation TKIs within 4 weeks, which indicated that HM61713 was active in patients with intrinsic resistance to EGFR TKIs. In conclusion, the third-generation EGFR TKIs appear to be promising in patients who progressed on treatment with EGFR TKIs. Greater efficacy and benefits will be achieved in T790M mutation-positive patients than T790M mutation-negative patients. Ongoing studies may determine whether the third-generation EGFR TKIs are also active against advanced NSCLC with intrinsic resistance due to the presence of de novo T790M mutations as an initial treatment strategy.86,87

**Chemotherapy plus EGFR TKIs**

Platinum-based doublet chemotherapy is the current standard treatment for patients with advanced NSCLC who failed after receiving first-line first-generation EGFR TKIs if the patients are not eligible for clinical trial participation. Currently, standard chemotherapy should also be considered in patients who are primarily resistant to EGFR TKIs.

Several clinical studies have shown that concurrent administration of gefitinib or erlotinib with cytotoxic chemotherapy did not confer a survival advantage over chemotherapy alone in NSCLC patients with previously untreated advanced NSCLC.95–99 These early studies with a concurrent combination of chemotherapy and EGFR TKIs were designed before the discovery of the EGFR mutations and included unselected NSCLC patients. In contrast, sequential administration of EGFR TKIs after chemotherapy or intercalated chemotherapy and the EGFR TKI erlotinib might be more effective than chemotherapy plus placebo.91–93 A Phase III IMPRESS study showed no significant improvement in PFS with continued use of gefitinib plus pemetrexed/cisplatin doublet chemotherapy compared to chemotherapy alone in 265 EGFR mutation-positive patients who progressed on first-line treatment with EGFR TKIs.94 However, a recent Phase II study showed that 33 EGFR-mutant patients were treated with gefitinib (days 1–56 and restarted on day 134) and three cycles of cisplatin plus docetaxel, in which 12 patients achieved a 2-year PFS, with a rate of 40.2%.95 The results from this study suggested that the addition of standard chemotherapy might prevent the development of acquired resistance to EGFR TKIs only in specific patients with activating EGFR mutations. In conclusion, the validation of specific patients needs clarification when considering the application of chemotherapy plus EGFR TKIs for patients with acquired or intrinsic resistance in large clinical and translational studies.

In addition, a combination of irreversible afatinib and the EGFR-specific antibody cetuximab overcame T790M-mediated resistance in preclinical models.86 In a Phase II study, 126 mutant EGFR-positive patients who progressed on erlotinib or gefitinib treatment were enrolled.97 The ORR was 29% in all patients, but the ORR was comparable in tumors with T790M mutations and tumors without T790M mutations (32% versus 25%). The median PFS and median duration of response to the combination of afatinib and cetuximab was 4.7 (95% CI: 4.3–6.4) and 5.7 months (range: 1.8–24.4 months), respectively. However, there is a lack of data regarding the efficacy of chemotherapy plus EGFR TKIs in advanced NSCLC with intrinsic resistance due to the presence of de novo T790M mutations.
Blockade of bypass signaling
MET amplification represents 5% of the mechanisms associated with acquired resistance to EGFR TKI treatment. Moreover, intrinsic resistance to EGFR TKIs can develop through upregulation of the MET ligand FGF or MET amplification in lung tumors previously untreated with gefitinib or erlotinib. In a single-arm Phase II trial investigating the safety and efficacy of the MET inhibitor INC280 plus gefitinib in EGFR-mutated and MET-positive NSCLC patients who progressed after prior EGFR TKI treatment, six unconfirmed patients (15%) had a partial response to the combined treatment out of 41 patients.98 A Phase III study compared the MET inhibitor tivantinib (ARQ197) plus erlotinib to erlotinib alone in previously treated patients with advanced NSCLC. Addition of the MET inhibitor to erlotinib did not increase the OS regardless of EGFR status.99 The dual MET-VEGF inhibitor cabozantinib plus erlotinib produced an unconfirmed partial response of 11% in EGFR-mutated NSCLC patients following progression on EGFR TKI therapy.100 In an in vivo model of intrinsic resistance to EGFR TKIs, a combination treatment targeting EGFR and MET simultaneously was highly active against tumors codriven by mutated EGFR and MET amplification.101

PIK3CA mutation has been reported as a mechanism associated with intrinsic and acquired resistance to EGFR TKIs. Targeting the PI3K pathway could be a novel strategy to overcome TKI resistance. A dual inhibitor of PI3K/mTOR, NVP-BEZ235, was found to effectively inhibit the growth of gefitinib-resistant NSCLC cells in vivo as well as in vitro.102 A Phase II study of the Akt inhibitor MK-2206 plus erlotinib showed a 9% ORR in advanced mutant EGFR-positive NSCLC previously treated with erlotinib, with a PFS of 4.4 months.103 Therefore, specific inhibitors targeting MET, PI3K, or other pathways may be promising treatments for NSCLC patients with mutations associated with intrinsic resistance to EGFR TKIs.

Other strategies against EGFR TKI resistance
Other approaches to overcome resistance include the use of antiangiogenic agents, immunotherapy, and other strategies. A recently published study of sorafenib in NSCLC patients who relapsed after EGFR TKI therapy showed that sorafenib failed to prolong survival with a disease control rate of 32.8%. The median PFS and OS were 3.7 (95% CI: 3.5–3.9 months) and 7.4 months (95% CI: 5.7–9.2 months), respectively.104 The disease control rate was not associated with BIM deletion and EGFR mutation status, and identification of biomarkers to determine the population who can benefit from sorafenib treatment is needed. Bevacizumab in combination with platinum has been approved for treating metastatic non-squamous NSCLC.105,106 The results from BeTa trial showed that combination of bevacizumab and erlotinib did not provide survival benefit to 636 previously treated unselected patients, regardless of the EGFR status.107 However, a small Phase II study in Japan investigated the efficacy of gefitinib plus bevacizumab as a first-line treatment in metastatic NSCLC with activating EGFR mutations. Although this clinical study did not meet the primary end point, the median PFS for overall patients and patients with exon 19 deletions were 14.4 and 18.0 months, respectively.108 Another trial (JO5567) in Japan was the first prospective randomized trial to compare the efficacy and safety of erlotinib plus bevacizumab to erlotinib alone in advanced EGFR-mutant non-squamous NSCLC in a first-line setting. The addition of bevacizumab to erlotinib improved PFS by ~6 months compared to erlotinib alone (16.0 versus 9.7 months).109 No new safety issues were identified, and the combination treatment did not cause a significant decrease of the quality of life. The BELIEF study recently presented at the 2015 European Cancer Congress demonstrated that addition of bevacizumab to erlotinib significantly improved the ORR (76.1%) and PFS (13.8 months; 95% CI: 10.3–21.3) independent of T790M status.110 In contrast, another retrospective study indicated that the T790M mutation might have an adverse effect on the combination of EGR TKIs and bevacizumab in patients with activating EGFR mutations. Both the ORR (0% versus 18%) and the PFS (3.3 versus 4.0 months) were significantly lower in T790M-positive patients compared with T790M-negative patients.111 Thus, the addition of bevacizumab to EGFR TKIs appears to be a favorable and well-tolerated strategy for EGFR mutation-positive individuals, even for those with intrinsic resistance to EGFR TKIs. Bevacizumab addition may enhance the antitumor activity of anti-EGFR therapy and/or partially reverse intrinsic resistance by increasing intratumoral concentration of EGFR TKIs.

The PD-1/PD-L1 checkpoint inhibitors, including nivolumab and pembrolizumab, showed an unprecedented survival benefit compared with chemotherapy and have been already approved for clinical use in advanced NSCLC.112–114 In squamous NSCLC, nivolumab was shown to be highly efficacious regardless of PD-L1 expression by immunohistochemistry. In the case of non-squamous NSCLC, nivolumab tended to be more effective in patients whose tumors expressed high levels of PD-L1 than those whose tumors expressed low levels of PD-L1.113 A small retrospective
study demonstrated that the high initial expression of PD-L1 was correlated with the presence of activating EGFR mutations.\textsuperscript{114} Interestingly, the PD-L1 expression was significantly upregulated in cultured EGFR-mutant tumor cells and markedly increased in tumor cells in a subset of EGFR-mutant NSCLC patients after EGFR TKI treatment.\textsuperscript{115,116} A small study (NCT01454102) investigated the efficacy of the anti-PD1 antibody nivolumab plus erlotinib in EGFR mutation-positive NSCLC patients who progressed on TKI therapy. Of the 20 patients who progressed after receiving erlotinib, 15\% achieved partial response.\textsuperscript{117} This subtype of EGFR mutation-positive NSCLC was highly eligible for PD-1/PD-L1 immunotherapy because the patients had high expression of PD-L1. Thus, PD-1/PD-L1 inhibitors appear to be an alternative strategy for EGFR-mutant patients who are intrinsically resistant to EGFR TKIs.\textsuperscript{118} Other targeted drugs also showed modest activity against NSCLC with mutant EGFR. In particular, the PPAR-gamma agonist rosiglitazone potentiated the antiproliferative effects of gefitinib by increasing PTEN expression.\textsuperscript{119} Treatment with vorinostat, a HDAC inhibitor, induced apoptosis by restoring the expression of BIM and overcoming gefitinib resistance in EGFR-mutated lung adenocarcinoma cells in vitro and vivo due to the BIM polymorphism.\textsuperscript{120}

**Conclusion**

In the past decade, a better understanding of the biological mechanisms of NSCLC had helped to identify critical biomarkers and oncogenic drivers. Additionally, individualized care has been explored to ensure that patients receive the correct clinical treatment. A growing number of therapeutic choices are available with excellent clinical responses. In particular, NSCLC patients benefit from first-generation EGFR TKIs if they have tumors with activating somatic EGFR mutations. Unfortunately, developing acquired resistance to EGFR TKIs ultimately limits the long-term effectiveness of such treatments, and a variety of mechanisms, including EGFR T790M secondary mutations, have been shown to contribute to this process. Many investigators have attempted to explore specific therapeutic strategies to overcome acquired resistance to EGFR TKIs. Additionally, intrinsic resistance remains another major challenge, and molecular mechanisms of intrinsic resistance have not been investigated widely. Although de novo EGFR T790M mutations, rare exon 20 insertions, or proapoptotic BIM gene deletions have been described in a small group of EGFR-mutant NSCLC patients, the majority of resistant cases cannot be explained by these mutations, and the mechanistic basis of intrinsic EGFR TKI resistance in patients supposed to be responsive remains largely unknown. Furthermore, lack of specific predictive makers associated with intrinsic resistance does not allow for effective selection of patients with initial resistance to EGFR TKIs prior to using erlotinib or gefitinib. Given that primary resistance is present in ~20\%–30\% of NSCLC patients with EGFR mutations, it is urgent to elucidate the mechanisms associated with innate resistance to EGFR TKIs and explore novel treatments to overcome this resistance. Future directions combating intrinsic resistance might focus on the following: 1) clinical standards, which should be established to identify patients who are initially resistant to EGFR TKIs, although they have sensitizing EGFR mutations; 2) cytotoxic chemotherapy, which remains the only standard therapy option for patients with intrinsic TKI resistance; 3) third-generation EGFR TKIs as they are a promising choice for a small group of untreated patients with T790M mutations; 4) coexistent genetic alteration, which might be responsible for de novo resistance to EGFR TKIs in a small subset of patients, and stratification analysis of global genetic mutations, which is needed before treatment choice; 5) novel specific and effective inhibitors of MET or other pathways or combination therapy with new targeted drugs to demonstrate improved efficacy in patients with intrinsic resistance due to activation of bypass signaling;\textsuperscript{128} and 6) further investigations of mechanisms associated with intrinsic resistance, including the microenvironment, epigenetic and genetic alterations, as well as expression and alterations of critical mediators considering concurrent resistance mechanisms and potential tumor heterogeneity in individual patients. New approaches, including initial global genetic investigations at the time of diagnosis, novel therapies targeting specific subgroups of lung cancers, and potential combined treatment strategies, are able to maximize the duration of clinical response to current targeted therapies and further improve long-term survival.

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The authors report no conflicts of interest in this work.

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