Lung Cancer: Targets and Therapy

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REVIEW

Development of personalized treatments in lung cancer: focusing on the *EGFR* mutations and beyond

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Abstract: Lung cancers with *epidermal growth factor receptor* (*EGFR)* gene mutation account for ~40% of adenocarcinoma in East Asians and ~15% of that in Caucasians, which makes them one of the most common molecularly defined lung cancer subsets. The role of *EGFR* mutation as a strong predictive biomarker of response to EGFR-tyrosine kinase inhibitors (TKIs) was finally confirmed by the biomarker analysis of Iressa Pan-Asian Study (IPASS). Since the 2004 discovery of *EGFR* mutation in lung cancer, the *EGFR* mutation and EGFR-TKI treatment have been widely studied. These include characteristics of lung cancers with *EGFR* mutations; clinical efficacies and adverse effects of EGFR-TKIs in patients with *EGFR*-mutated lung cancers; development of novel EGFR-TKIs that may prolong progression-free survival of these patients or overcome resistance to first-generation EGFR-TKIs (gefitinib and erlotinib); optimal treatment schedules for EGFR-TKIs to delay emergence of resistance; molecular mechanisms of acquired resistance to EGFR-TKIs; treatment strategies after patients acquire resistance to EGFR-TKIs; and predictive biomarkers for EGFR-TKIs among patients with *EGFR*-mutated lung cancers. Some of these results are widely accepted, while others are apparent only in cell line models, preclinical animal models, or retrospective analyses (and sometimes conflict with each other). In this review, we summarize accumulated reports from the past decade, especially focusing on unanswered but important clinical questions in treating patients with *EGFR*-mutated lung cancers.

Keywords: epidermal growth factor receptor mutation, predictive biomarkers, personalized therapy, molecular target, adjuvant therapy, acquired resistance

Introduction

Next year, 2014, will mark the tenth anniversary of the discovery of somatic mutations of the *epidermal growth factor receptor* (*EGFR*) gene in non-small-cell lung cancers. The *EGFR* mutation is of both clinical and investigational interest because its presence strongly predicts efficacy of EGFR-tyrosine kinase inhibitors (TKIs) that have been applied in clinic a few years before.

In the decade after this discovery, clinicians and researchers have found clinical, pathological, and prognostic characteristics of *EGFR*-mutated lung cancers, and began several clinical trials, enrolling "selected" patients. These trials[1–4] showed the superiority of EGFR-TKIs in progression-free survival (PFS) compared with platinum-doublet chemotherapy, which had been the gold-standard regimen, in patients with *EGFR*-mutated lung cancers.

However, this clinical success has invited other questions for researchers: (1) why does EGFR-TKI efficacy vary among patients with *EGFR*-mutated lung cancers?; (2) why do patients who show dramatic initial responses acquire resistance, and

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how can this be avoided or overcome?; (3) what is the best EGFR-TKI for a particular patient?; and (4) what is the most appropriate EGFR-TKI treatment schedule?

In this review, we summarize accumulated studies on *EGFR*-mutated lung cancers and EGFR-TKIs from the last 10 years. In addition, we discuss the further issues that must be addressed to optimize outcomes of patients with *EGFR*-mutated lung cancers.

Basic EGFR information

EGFR is a transmembrane receptor tyrosine kinase (Figure 1). Upon binding to its ligands (epidermal growth factor, transforming growth factor alpha, amphiregulin, etc), EGFR forms homodimers or heterodimers with the other family members (ERBB2, ERBB3, or ERBB4),[5] which stimulates intrinsic receptor tyrosine kinase activity and triggers autophosphorylation of specific tyrosine residues within their cytoplasmic regulatory domains. These phosphorylated tyrosine residues activate several signaling pathways, including mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositol 3-kinase (PI3K)/AKT pathway, and the signal transducer and activator of transcription pathways. These pathways promote cell proliferation, migration and metastasis, evasion from apoptosis, or angiogenesis, all of which are associated with cancer phenotypes.

Because various cancers, including lung cancers, often overexpress EGFR, which is reportedly associated with poor prognosis,[6] EGFR is regarded as a promising molecular target in cancer therapy. However, all four Phase III trials that enrolled unselected patients and compared chemotherapy plus EGFR-TKI to chemotherapy alone gave negative results for addition of the EGFR-TKIs.[7–10] In addition, one EGFR-TKI, gefitinib, even failed to show survival advantage over placebo in unselected previously treated

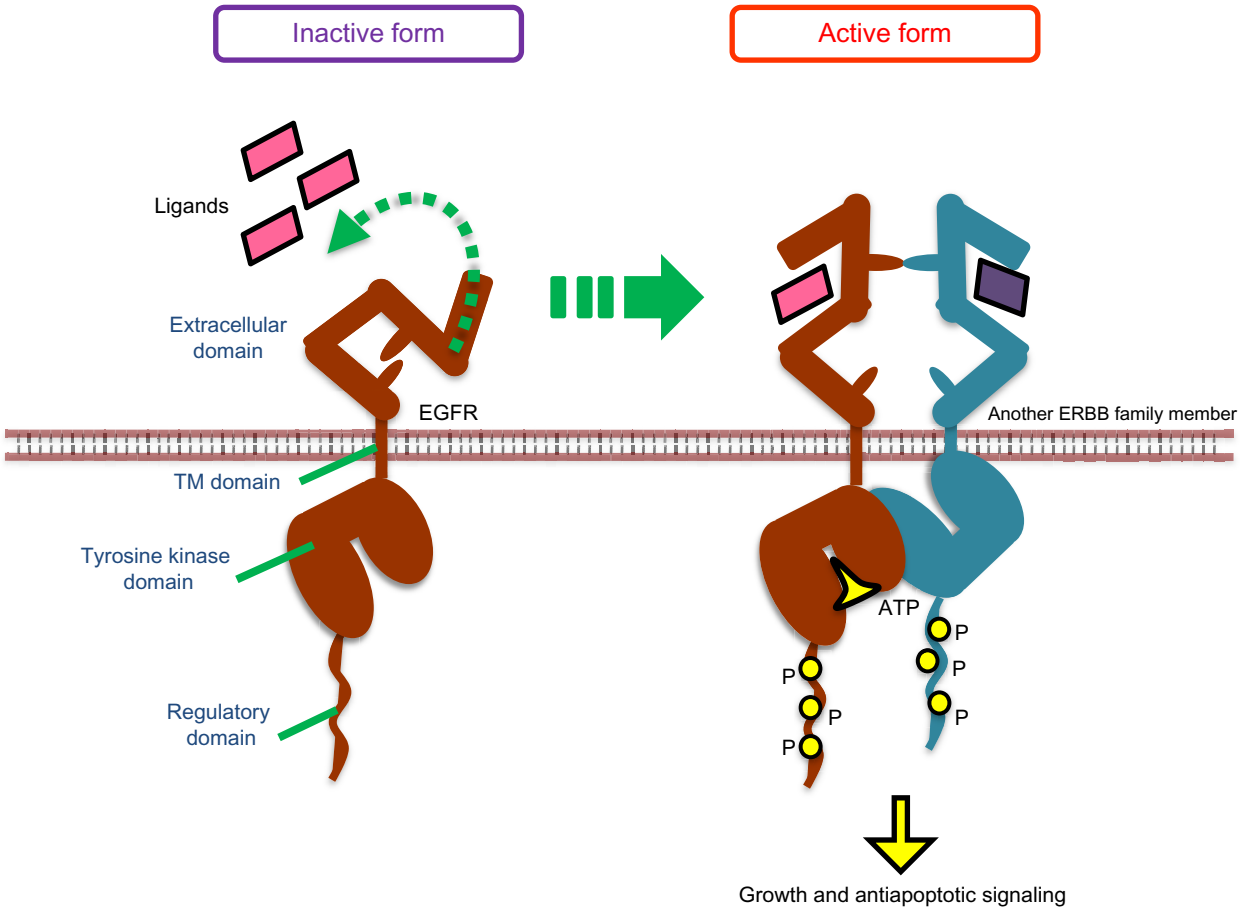

Figure 1 Structure and activation mechanism of epidermal growth factor receptor (EGFR).
Notes: The EGFR protein consists of extracellular, transmembrane, tyrosine kinase, and regulatory domains. EGFR undergoes conformation changes when a specific ligand binds to its extracellular domain, and EGFR forms homodimers or heterodimers with the other ERBB family members (ERBB2, ERBB3, or ERBB4). In doing so, their respective kinase domains dimerize asymmetrically, in a tail-to-head orientation, which stimulates intrinsic tyrosine kinase activity of the receptors and triggers autophosphorylation of specific tyrosine residues within the cytoplasmic regulatory domains. These phosphorylated tyrosine residues serve as specific binding sites for several adaptor proteins, inducing proliferative or antiapoptotic signaling pathways, such as mitogen-activated protein kinase, phosphatidylinositol 3-kinase/AKT, and the signal transducer and activator of transcription pathways.
Abbreviations: TM, transmembrane; P, indicates phosphorylation of tyrosine residues; ATP, adenosine triphosphate.

patients.[11] These failures in early-phase EGFR-TKI therapy development ironically support the importance of biomarker-based treatment in lung cancers.

Established evidence of *EGFR*-mutated lung cancers and EGFR-TKI treatment

EGFR somatic activating mutations, which occur in the EGFR-tyrosine kinase domain, were discovered in 2004 by two independent groups in Boston, MA, USA.[12,13] *EGFR* mutations usually occur in the first four exons of tyrosine kinase domain (exons 18–21) and reportedly induce ligand-independent activation of EGFR, followed by activation of downstream proliferative and antiapoptotic signaling.

Lung cancers with *EGFR* mutation account for ~40% of adenocarcinoma in East Asians and ~15% of those in Caucasians,[14] which makes them one of the most common molecularly defined lung cancers subset. The role of *EGFR* mutation as a strong predictive biomarker of response to EGFR-TKI treatment has been reported in several retrospective analyses,[15] and finally confirmed by the biomarker analyses of Iressa Pan-Asian Study (IPASS).[16]

For chemotherapy-naïve patients with *EGFR*-mutated lung cancers, six Phase III trials have proved PFS of EGFR-TKI treatment (gefitinib, erlotinib, or afatinib) is superior to that of platinum-doublet chemotherapy (Table 1).[1–4,17,18] Although these prospective trials could not prove the power of EGFR-TKIs to prolong overall survival (OS) because of a high crossover rate between the two arms, two retrospective analyses observed that EGFR-TKI treatment was an independent favorable predictor for OS among patients with *EGFR*-mutated lung cancers but not in those without *EGFR* mutation.[19,20] Because median survival time in the 2002 Eastern Cooperative Oncology Group (ECOG) 1594 study, which compared four different platinum-doublet chemotherapies, ranged from 7.4 to 8.1 months,[21] median survival time >24 months, as shown in Table 1, is extremely noteworthy.

On the other hand, clinicians experienced that not all patients with *EGFR*-mutated lung cancers responded equally to EGFR-TKIs. Accumulated data show that patients harboring the two most common mutations, exon 19 deletion mutation and L858R point mutation (exon 21), respond very well, followed by G719X point mutation (exon 18) and L861Q point mutation (exon 21), whereas tumors with exon 20 insertion mutation show intrinsic resistance to first-generation EGFR-TKIs, gefitinib and erlotinib.[15] Coexistence of pretreatment T790M gatekeeper mutation (in the section: How does acquired gefitnib-erlotinib resistance occur after promising initial responses?), found by direct sequencing in ~0.5% of lung cancers with activating *EGFR* mutation,[22] reportedly also causes inherent resistance. The role of less common *EGFR* mutations as predictors for EGFR-TKI response is unclear because of their scarcity.[23,24]

The use of *EGFR* mutation status as a predictive biomarker requires knowing whether all cancer cells in one lung cancer patient harbor the same *EGFR* mutational status, ie, if the *EGFR* mutational status is homogenous or not. Because *EGFR* mutations are also identified in precursor lesions of lung adenocarcinoma or lung adenocarcinoma

Table 1 Summary of PFS and OS in prospective studies that compared EGFR-TKIs with platinum-doublet chemotherapies

Study	Patient group	EGFR-TKI	N	PFS (months)		HR for PFS (95% CI)	OS (months)	
				TKI	Chemotherapy		TKI	Chemotherapy
1. Subset analyses of patients selected by clinical background								
IPASS[86]	Asian	Gefitinib	261	9.5	6.3	0.48 (0.36–0.64)	21.6	21.9
First-SIGNAL[87]	Korean	Gefitinib	42	8.4	6.7	0.61 (0.31–1.22)	30.6	26.5
2. Phase III trials for patients selected by *EGFR* mutation								
NEJ002[2]	Japanese	Gefitinib	228	10.8	5.4	0.32 (0.24–0.44)	27.7	26.6
WJTOG3405[1]	Japanese	Gefitinib	172	9.6	6.6	0.52 (0.38–0.72)	35.5	38.8
OPTIMAL[4]	Chinese	Erlotinib	154	13.7	4.6	0.16 (0.10–0.26)	22.7	28.9
EURTAC[3]	Caucasian	Erlotinib	173	9.7	5.2	0.37 (0.25–0.54)	19.3	19.5
LUX-Lung 3[17]	Caucasian 26% Asian 72%	Afatinib	345	11.1	6.9	0.58 (0.43–0.78)	N/A	N/A
LUX-Lung 6[18]	Asian	Afatinib	364	13.7	5.6	0.28 (0.20–0.39)	N/A	N/A

Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; N/A, not applicable; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; IPASS, IRESSA Pan-Asian Study; First-SIGNAL, First-line Single Agent Iressa versus Gemcitabine and cisplatin Trial in Never-smokers with Adenocarcinoma of the Lung; NEJ, North East Japan; WJTOG, West Japan Thoracic Oncology Group; OPTIMAL, A Randomized, Open-label, Multi-center Phase III Study of Erlotinib Versus Gemcitabine/Carboplatin in Chemo-naive Stage III/IV Non-Small Cell Lung Cancer Patients With EGFR Exon 19 or 21 Mutation; EURTAC, European Randomised Trial of Tarceva vs. Chemotherapy; LUX-Lung 3, LUX-Lung 6, A Randomised, Open-label, Phase III Study of BIBW 2992 Versus Chemotherapy as First-line Treatment for Patients With Stage IIIB or IV Adenocarcinoma of the Lung Harbouring and EGFR Activating Mutation.

in situ,[25] this mutation is assumed to occur in early phases of lung carcinogenesis, indicating that all lung cancer cells retain the same *EGFR* mutation. However, early reports observed discordant *EGFR* mutational status between primary tumors and lymph node metastases, and others observed intratumoral heterogeneity of *EGFR* mutations.[26] As the cause of such heterogeneity of *EGFR* mutation, Yatabe, one of our principal coinvestigators, considered contamination of normal cells (eg, fibroblasts) and differences in gene copy number or expression level of mutated *EGFR*, if low sensitivity methods were used. To confirm this supposition, Yatabe et al performed a detailed analysis using high-sensitivity methods and identified no discordant mutation patterns among 77 paired primary and metastatic site samples or among 54 primary and recurrent tumor pairs.[26] In addition, to examine intratumor heterogeneity, three samples each from 50 lung cancers and 100 samples each from an additional five tumors were examined; the same mutations throughout each individual tumor were identified.[26]

These widely accepted investigations have changed treatment strategies for patients with *EGFR*-mutated lung cancers and improved their outcomes. However, these successes have also raised several clinical questions and problems.

Why does the efficacy of EGFR-TKIs vary among patients with lung cancers with sensitive *EGFR* mutations?

Disease control rates of gefitinib or erlotinib for patients with lung cancers with sensitive *EGFR* mutations in first-line Phase III studies were reported to be 93%~97%,[1,3,4] which implies that 3%~7% of *EGFR*-mutated lung cancers are inherently resistant to EGFR-TKIs even though they harbor "sensitive" *EGFR* mutations. Some researchers have focused on the molecular mechanisms of inherent resistance. As downregulation of the PI3K-AKT pathway is required for EGFR-TKI-induced apoptosis in *EGFR*-mutated lung cancers,[27] and inactivation of PTEN is a common cause of PI3K pathway activation in human cancers,[28] PTEN has been included as a candidate molecule in inherent resistance to EGFR-TKIs. An example of in vitro inherent resistance is seen in the H1650 lung cancer cell line, which harbors an *EGFR* mutation and homozygous deletion of *PTEN*. PTEN reconstitution by stable retroviral expression increased susceptibility to TKI-induced apoptosis in this cell line.[29] In addition, in a recent analysis using inherent resistance clinical specimens, Yano et al observed that high-level expression of hepatocyte growth factor, a ligand of MET proto-oncogene product, was detected in 29% of tumors and *MET* gene amplification in 4%, suggesting the participation of these molecules in intrinsic EGFR-TKI resistance in patients with *EGFR*-mutated lung cancers.[30] Clinical relevance of these molecules on inherent resistance to gefitinib or erlotinib, and treatment strategies to overcome these resistance mechanisms, should be determined in the future.

Among patients who respond to gefitinib or erlotinib, some show shorter PFS. Why do some *EGFR*-mutated lung cancers easily acquire resistance to EGFR-TKIs? To explain this phenomenon, Maheswaran et al analyzed pretreatment tumor specimens for a preexisting small population of cancer cells with T790M gatekeeper mutation (a resistant mutation described in the section: How does acquired gefitinib/erlotinib resistance occur after promising initial responses?) using a high-sensitivity method. Interestingly, pretreatment minor clones with T790M mutation were detected in 38% of *EGFR*-mutated lung cancers, and correlated with reduced PFS after EGFR-TKI treatment.[31] Rosell et al also identified pretreatment minor clones with T790M mutation in 35% of *EGFR*-mutated lung cancers, and observed shorter PFS in patients with pretreatment T790M mutation.[32] Most recently, Su et al analyzed pretreatment T790M mutation using mass spectrometry and next-generation sequencing, and found pretreatment T790M mutation to again be an independent predictor of decreased PFS after EGFR-TKI treatment.[33]

Meanwhile, as a predictive biomarker of EGFR-TKI response among patients with lung cancers with sensitive *EGFR* mutation, Faber et al[34] and Ng et al[35] studied the BCL2-interacting mediator of cell death (BIM), a proapoptotic BCL-2 family protein, upregulation of which is required for TKI-induced apoptosis. These investigations found low BIM-extra long (EL) isoform expression and an intronic deletion polymorphism of *BIM* that provided decreased expression of BIM-EL as predictors of diminished response to EGFR-TKIs in *EGFR*-mutated lung cancers.[34,35] Consistent with these results, Nakagawa et al observed that two *EGFR*-mutated lung cancer cell lines with *BIM* deletion polymorphism (PC3 and HCC2279) showed low susceptibility to gefitinib-induced apoptosis.[36] Interestingly, the intronic deletion polymorphism of *BIM* also conferred low sensitivity to imatinib in ABL1 kinase-driven chronic myeloid leukemia.[35]

On the other hand, Bivona et al identified FAS and NF-kB signaling as a suppressor of EGFR-TKI-induced cell death.[37] Following this observation, they analyzed IκB expression in *EGFR*-mutated lung cancers and found that low IκB expression (high NF-kB activation state) was predictive of worse

PFS, while IκB expression did not predict PFS among patients treated with chemotherapy.[37] Other studies have implied that low expression of LIM-domain-only 4 (LMO4)[38] or of PTEN[39] predicts a poor response to EGFR-TKI treatment.

These results are, however, controversial. For example, Fujita et al found pretreatment minor clones with T790M mutation in 79% of *EGFR*-mutated lung cancers, but this did not predict the response to gefitinib treatment.[40] Rosell et al also saw no difference in PFS between patients with and without pretreatment minor clones with T790M mutation in the erlotinib arm of the EURTAC trial.[41] Meanwhile, Lee et al analyzed 193 patients with *EGFR*-mutated lung cancers for *BIM* intronic deletion polymorphism and found that the *BIM* polymorphism did not predict PFS after EGFR-TKI treatment.[42] Such discrepancies might be caused by overlapping and interacting of molecular biomarkers. Rosell et al found that pretreatment minor clones with T790M mutation and increased *BRCA1* mRNA levels both significantly predicted a poor response to EGFR-TKI treatment, whereas low *BRCA1* levels neutralized the negative effect of pretreatment T790M mutation.[32] Table 2 summarizes these studies. Comprehensive analyses for these molecular biomarker candidates are needed to identify the most reliable predictive marker(s) for EGFR-TKI treatment.

How does acquired gefitinib/erlotinib resistance occur after promising initial responses?

Despite initial dramatic response, almost all patients with *EGFR*-mutated lung cancers eventually develop acquired resistance to gefitinib or erlotinib. A clinical definition of acquired resistance was proposed by Jackman et al in 2010,[43] and mechanisms underlying acquired resistance have been extensively analyzed. Acquisition of T790M gatekeeper mutation of the *EGFR*, which substitutes methionine for threonine at amino acid position 790,[44,45] is the most common mechanism of acquired resistance, reportedly up to 68%–83.3% if high-sensitivity method is used.[33,46] Initially, the larger methionine residue was thought to sterically block binding of gefitinib or erlotinib; however, a later study found increased ATP affinity of EGFR with T790M mutation as the mechanism of resistance.[47] Several EGFR-TKIs that can bind to EGFR with T790M are now under development (as described in the section: What is the most appropriate EGFR-TKI?).

The second candidate of acquired resistance mechanism is MET activation, either by gene amplification[48,49] or by high expression of the ligand (HGF).[50] Acquired resistance in in vitro models by MET activation is highly responsive to a combination of EGFR-TKI and MET-TKI,[48,50,51] but these results have not been confirmed in clinical settings. MET reportedly mediates several miRNAs, (miR-30b, miR-30c, miR-221, miR-222, miR-103, and miR-203) that affect gefitinib-induced apoptosis and epithelial-to-mesenchymal transition (EMT) of lung cancer cells.[52]

Other candidates of acquired resistance molecular mechanisms against EGFR-TKIs in *EGFR*-mutated lung cancers are not yet evaluated well in clinical samples, identified only in acquired resistance in vitro models or in observation of small numbers of clinical specimens obtained after EGFR-TKI treatment failure. Those resistant mechanisms include PTEN downregulation; *CRKL* amplification; NFkB signaling activation; AXL activation; *HER2* amplification; reactivation of ERK signaling by either an amplification of MAPK1 or

Table 2 Predictive biomarker candidates for poor response to gefitinib/erlotinib in patients with *EGFR*-mutated lung cancers

Candidate biomarkers	Molecular mechanism	Predictive biomarker for poor response	
		Pros	Cons
Preexistence of small population of cancer cells with T790M drug-resistant mutation	TKI treatment select preexistence drug-resistant cells	Maheswaran et al[31] Rosell et al[32,a] Su et al[33]	Fujita et al[40] Rosell et al[41]
Low BIM-EL expression	Upregulation of BIM (BIM-EL isoform) is required for TKI-induced apoptosis	Faber et al[34]	
BIM deletion polymorphism	The deletion polymorphism provides decreased expression of BIM-EL	Ng et al[35] Isobe et al[88]	Lee et al[42]
Low IκB expression	IκB suppresses NFκB activation that suppresses TKI-induced cell death	Bivona et al[37]	
Intermediate/high BRCA1 expression	DNA repair enzyme that may also repair TKI-induced DNA breakage	Rosell et al[32,a]	
Low LMO4 expression	Negative regulator of BRCA1 function	Karachaliou et al[38,a]	

Note: [a]These investigations analyzed the same cohort of patients.

Abbreviations: BIM, BCL2-interacting mediator of cell death; EGFR, epidermal growth factor receptor; EL, extra long; LMO4, LIM-domain-only 4; TKI, tyrosine kinase inhibitor; BRCA1, breast cancer 1 early onset.

by downregulation of negative regulators of ERK signaling; *BRAF* mutation; loss of EGFR mutant allele; EMT including stem cell-like features; or conversion to small-cell lung cancer.[53–60] Several reports that analyzed clinical specimens suggest that main molecular mechanisms of acquired resistance basically occur in a mutually exclusive fashion (as might be represented in a pie chart),[51,53,61] indicating the importance of molecular analyses after a lung cancer patient acquires resistance to first-line treatment with EGFR-TKI.

What is the most appropriate EGFR-TKI?

Because T790M secondary mutation is the most common acquired resistance mechanism to gefitinib or erlotinib, and *EGFR*-mutated lung cancers often harbor this mutation as a minor clone prior to treatment, several EGFR-TKIs that can suppress T790M mutation are now under development. To overcome T790M mutation, irreversible EGFR-TKIs, such as afatinib or dacomitinib, which covalently bond to cysteine 797 of EGFR, were initially developed. As a first-line therapy, afatinib showed superior PFS[17,18] compared with cisplatin/pemetrexed or compared with cisplatin/gemcitabine in patients with *EGFR*-mutated lung cancers (Table 1). However, it remains unclear which EGFR-TKI (gefitinib, erlotinib, or afatinib) is the most appropriate for first-line EGFR-TKI therapy. These EGFR-TKIs differ in efficacy and adverse effects; results of major prospective EGFR-TKI trials in a first-line setting are summarized in Tables 1 and 3 for efficacy and adverse effects, respectively. Many clinical trials analyzing the differences between these EGFR-TKIs are currently underway (eg, WJOG 5108 L, LUX-Lung 8, and ARCHER studies).

Irreversible EGFR-TKIs, so-called second-generation EGFR-TKIs, were also expected to overcome acquired resistance after treatment failure of gefitinib or erlotinib, because they showed in vitro activity in cancer cells with T790M mutation,[62,63] or exon 20 insertion mutations that confer de novo resistance to gefitinib or erlotinib,[64] at clinically achievable concentrations. However, these compounds are also active for wild-type EGFR;[62,63] therefore, dose limitation due to inhibition of wild-type EGFR is predicted to result in inadequate clinical activity against cancer cells harboring *EGFR* T790M mutation. Indeed, the LUX-Lung 1 study, which enrolled patients who received at least 12 weeks of gefitinib or erlotinib and experienced treatment failure, found no OS advantage in the afatinib arm compared with placebo.[65]

To overcome this drawback, chemical libraries were screened to find compounds that selectively inhibit mutant

Table 3 Summary of adverse effects in prospective studies of EGFR-TKIs

Study	Drug	Rash (%)		Paronychia (%)		Diarrhea (%)		Liver damage (%)		ILD (%)	
		All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3
WJTOG3405[1]	Gefitinib	85	2	32	1	54	1	70[a]	28[a]	2	1
NEJ002[2]	Gefitinib	71	5	–	–	34	1	55	26	5	3
EURTAC[3]	Erlotinib	80	13	–	–	57	5	6	2	1	1
OPTIMAL[4]	Erlotinib	43	2	4	0	25	1	37[a]	4[a]	–	–
Japan PII[89]	Erlotinib	83	14	66	1	81	1	33[a]	8[a]	5	2
LUX-Lung 3[17,b]	Afatinib	98	20	87	24	100	20	–	–	4	2

Notes: [a]Elevation of ALT; [b]Japanese subset.
Abbreviations: ALT, alanine transaminase; EGFR, epidermal growth factor receptor; ILD, interstitial lung disease; TKI, tyrosine kinase inhibitor.

EGFR, including T790M mutation, while sparing wild-type EGFR. These EGFR-TKIs are called "T790M-specific" or "third-generation" EGFR-TKIs, and several compounds are now under development.[66–68] Although no results from large clinical trials for these compounds have been reported, these T790M-specific EGFR-TKIs have shown promising results in preclinical settings, and one, CO-1686, recently showed tumor shrinkage in patients with *EGFR*-mutated lung cancer after acquisition of T790M mutation.[69]

What is the most appropriate treatment schedule for EGFR-TKI?

For patients with *EGFR*-mutated lung cancers, EGFR-TKIs are usually administered as continuous treatment. However, the superiority of this treatment schedule is not confirmed. To address this question, Chmielecki et al analyzed isogenic EGFR-TKI-sensitive and EGFR-TKI-resistant (due to T790M mutation) pairs of cell lines that mimic the behavior of human tumors. In their analyses, T790M-mediated EGFR-TKI-resistant cells showed slower growth compared with sensitive cells in a drug-free condition. Therefore, they evaluated an intermittent high-dose pulse of erlotinib (or afatinib) in conjunction with a continuous low-dose administration of erlotinib, and proposed that this treatment schedule may delay the acquisition of EGFR-TKI resistance.[70] An intermittent treatment schedule has also been suggested in preclinical models of *BRAF*-mutated melanoma;[71] however, it is not clear if these treatment schedules can be applicable to all patients with *EGFR*-mutated lung cancers.

Treatment strategies after acquisition of resistance to gefitinib/erlotinib

Although, theoretically, resistance mechanism-based treatments are desired, in clinical practice, EGFR-TKI is usually converted to cytotoxic chemotherapy after lung cancer patients acquire resistance to gefitinib or erlotinib. In the NEJ002 trial that compared gefitinib with carboplatin/paclitaxel in first-line settings, as much as 65% of the patients received chemotherapy, and 62% of this was platinum-based, as the second-line treatment in patients who were originally allocated to the gefitinib group.[72]

After acquisition of resistance to EGFR-TKIs, clinicians must pay attention to a phenomenon called "disease flare."[73] This phenomenon is reported to occur in 14 of 61 patients (23%), and is defined as hospitalization or death attributable to disease progression.[74] Disease flare has also been experimentally mimicked.[70] Results suggest that patients may benefit from continued treatment with an EGFR-TKI, even after developing T790M-mediated resistance (beyond progressive disease [PD]). This concept is now being tested in several clinical trials, including IMPRESS, in which patients with acquired resistance to gefitinib are randomized between cisplatin/pemetrexed and cisplatin/pemetrexed plus gefitinib treatment (ClinicalTrials.gov, number NCT01544179).[75]

Several auspicious candidate treatments other than conversions to chemotherapy with or without EGFR-TKIs have been suggested to overcome acquired resistance to gefitinib or erlotinib. One of these candidates is combination of an anti-EGFR antibody and an irreversible EGFR-TKI, afatinib. Anti-EGFR antibodies bind to EGFR and induce endocytosis and depletion of total EGFR from cell surface. Combination of an irreversible EGFR-TKI, afatinib, with cetuximab was assessed in in vivo models and achieved marked shrinkage of erlotinib-resistant tumors with T790M mutation.[76] Following this result, a Phase I/II study of afatinib combined with cetuximab was conducted, enrolling100 patients with clinically defined acquired resistance to EGFR-TKIs. Amazingly, disease control rate and response rate were 94% and 40%, respectively, and, interestingly, the response was similar in both T790M+ (38%) and T790M– (47%) tumors.[77] These results may suggest that most lung cancers depend on the EGFR signaling pathway even after acquisition of resistance to EGFR-TKIs, regardless of the presence of T790M mutation.

As a completely different approach, heat shock protein 90 (Hsp90) has been reported as a candidate molecule that may overcome acquired resistance to EGFR-TKIs. Hsp90 is a 90 kDa molecular chaperone that is required for folding, stabilization, and function of proteins, including several oncogene products such as EGFR, MET, and EML4-ALK. Some studies suggest that Hsp90 inhibition may be beneficial for patients with acquired resistance to EGFR-TKIs.[78,79]

EGFR-TKIs in adjuvant setting after surgical resection

Platinum-doublet adjuvant chemotherapy, the current standard of care for pathological stage II–III non-small-cell lung cancer patients after "curative" resection, improves the 5-year survival rate by only 5.4% compared with surgery alone.[80] Because pulmonary resection provides abundant tumor tissues for analyses, several biomarker-based clinical trials in adjuvant settings have been performed or are ongoing.[81] Some of these trials include EGFR status for selection of adjuvant chemotherapy. Although the prematurely terminated BR.19 trial could not show the efficacy of adjuvant gefitinib

therapy compared with placebo, even in the subset of patients with *EGFR* mutation,[82] a retrospective study found adjuvant EGFR-TKI to be associated with a lower risk of recurrence.[83] To confirm the role of adjuvant EGFR-TKI prospectively in patients with *EGFR*-mutated lung cancers, the ongoing, Phase III IMPACT trial is comparing gefitinib with cisplatin plus vinorelbine.[84] Also, to confirm the efficacy of biomarker-tailored adjuvant therapy, several ongoing Phase III trials[82] are comparing customized and standard treatment. In the TASTE study,[85] patients are assigned to three groups the a customized arm, with erlotinib for those with *EGFR* mutations, cisplatin plus pemetrexed for those without *EGFR* mutation and low ERCC1, and no treatment for those without *EGFR* mutation and high ERCC1, while all patients in the standard arm receive cisplatin plus pemetrexed.

Conclusion

Although the prognosis of patients with *EGFR*-mutated lung cancers has dramatically improved over the past decade, many questions remain. Research must continue to seek the best treatments for patients with *EGFR*-mutated lung cancers. We hope this review aids many clinicians and researchers in understanding advances in this area and leads to better clinical trials and translational research in the next decade.

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