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Review

Targeting epidermal growth factor receptor: Central signaling kinase in lung cancer

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ABSTRACT

Non-small cell lung cancer (NSCLC) is the leading cause of cancer mortality worldwide. Platinum-based doublets remain the current standard therapy for advanced NSCLC. However, overall survival (OS) has reached a plateau, even with the improvement in these regimens. Advances in the knowledge of molecular mechanisms of carcinogenesis have prompted the development of many novel molecular-targeted agents including the epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). Results of the recent phase III IPASS trial showed that the EGFR-TKI gefitinib has a superior progression-free survival (PFS) to the most commonly used platinum-based doublet carboplatin-paclitaxel as the first-line chemotherapy for pulmonary lung adenocarcinoma among nonsmokers in East Asia. This trial also demonstrated that the presence of *EGFR* mutation is the best predictor of gefitinib treatment compared with the other biomarkers including *EGFR* gene copy number. Despite the therapeutic benefit of EGFR-TKIs in NSCLC, most patients eventually develop resistance to these drugs. A secondary mutation of *EGFR* (T790M) and amplification of *MET* account for 70% of all cases of acquired resistance to EGFR-TKIs. This review summarizes the significance of *EGFR* mutations and the mechanisms of resistance to EGFR-TKIs in NSCLC, both of which are critical for patient selection to extend survival as well as to overcome resistance in NSCLC patients treated with EGFR-TKIs.

Contents

1. Introduction . . . 613
2. Strategy to target EGFR which is important for cell survival or proliferation in NSCLC . . . 615
3. The significance of *EGFR* mutations in NSCLC patients who receive EGFR-TKIs . . . 615
4. Application of proteomic approach to EGFR signaling network . . . 616
5. T790M secondary *EGFR* mutation as the mechanism of EGFR-TKIs resistance . . . 617
6. The other mechanisms of EGFR-TKIs resistance including *MET* gene amplification . . . 618
7. The strategy based on the monoclonal antibodies to EGFR in NSCLC . . . 619
8. Conclusion and clinical view of the molecular-targeted therapies in NSCLC . . . 619
Acknowledgements . . . 619
References . . . 619

1. Introduction

Non-small cell lung cancer (NSCLC) is characterized by high incidence and mortality rates worldwide [1]. Based on the associated moderate improvement in survival and quality of life, platinum-based chemotherapy constitutes standard first-line treatment for advanced NSCLC patients with good performance status [2–5]. However, NSCLC patients show rapid emergence of resistance against platinum-based chemotherapeutics resulting in limited overall survival (OS), even with the improvement in these regimens or the development of the second line chemotherapy in NSCLC [3,6,7]. Insight into the molecular events underlying oncogenesis and drug resistance is needed for the development of new treatment approaches such as molecularly targeted therapies [8]. The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), including gefitinib and erlotinib both of which compete with Adenosine TriPhosphate (ATP) for binding to the tyrosine kinase pocket of the receptor, have been

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Table 1
The brief overview of key phase III trials of EGFR-TKIs for NSCLC treatment.

(a) Single agent EGFR-TKI in the first-line chemotherapy for NSCLC

Trial [Ref.]	Patient selection	Treatment	PFS as primary end point	Significance	Results for EGFR-TKIs
IPASS [17,18]	Adenocarcinoma, Asian, Nonsmoker	Gefitinib ($N=609$) vs Carboplatin/Paclitaxel ($N=608$)	9.5 months ($N=132$) (PFS in *EGFR*-MT subset[a]) 6.3 months ($N=129$)	HR = 0.48 95% CI = 0.36–0.64 $P=0.001$	Positive
WJTOG3405 [15]	Japanese, *EGFR*-MT positive	Gefitinib ($N=86$) vs Cisplatin/Docetaxel ($N=86$)	9.2 months 6.3 months	HR = 0.489 95% CI = 0.336–0.710 $P=0.0001$	Positive
NEJ002 [16]	Japanese, *EGFR*-MT positive	Gefitinib ($N=98$) vs Carboplatin/Paclitaxel ($N=96$)	10.4 months 5.5 months	HR = 0.357 95% CI = 0.252–0.507 $P=0.001$	Positive

(b)EGFR-TKI combined with platinum-based doublets in the first-line chemotherapy for NSCLC

Trial [Ref.]	Patient selection	Treatment	OS as primary end point	Significance	Results for EGFR-TKIs
INTACT I [189]	None	Gefitinib with Cisplatin/Gemcitabine ($N=365$ for Gefitinib 500 mg/day; $N=365$ for 250 mg/day) vs Cisplatin/Gemcitabine ($N=363$)	9.9 months for each dose 10.9 months	$P=0.4560$	Negative
INTACT II [190]	None	Gefitinib with Carboplatin/Paclitaxel ($N=347$ for Gefitinib 500 mg/day; $N=345$ for 250 mg/day) vs Carboplatin/Paclitaxel ($N=345$)	8.7 months for 500 mg/day 9.8 months for 250 mg/day 9.9 months	$P=0.6385$	Negative
TRIBUTE [191]	None	Erlotinib with Carboplatin/Paclitaxel ($N=539$) vs Carboplatin/Paclitaxel ($N=540$)	10.6 months 10.5 months	HR = 0.995 95% CI = 0.86–1.16 $P=0.95$	Negative
TALENT [192]	None	Erlotinib with Cisplatin/Gemcitabine ($N=580$) vs Cisplatin/Gemcitabine ($N=579$)	43 weeks 44.1 weeks	HR = 1.06 95% CI = 0.90–1.23 $P=0.49$	Negative

(c) EGFR-TKI in the maintenance chemotherapy for NSCLC

Trial [Ref.]	Patient selection	Treatment	OS or PFS as primary end point	Significance	Results for EGFR-TKIs
SWOG0023 [186]	None	Gefitinib ($N=118$) vs (after Cisplatin/Etoposide/RT followed by Docetaxel) Placebo ($N=125$)	23 months (OS) 35 months	$P=0.013$	Negative
WJTOG0203 [179]	Japanese	Gefitinib ($N=298$) vs (after Platinum-based doublets) Placebo ($N=297$)	15.4 months ($N=235$) (OS in adenocarcinoma subset[b]) 14.3 months ($N=232$)	HR = 0.79 95% CI = 0.65–0.98 $P=0.03$	Positive in adeno
SATURN [180]	None	Erlotinib ($N=438$) vs (after Platinum-based doublets) Placebo ($N=451$)	12.3 weeks (PFS) 11.1 weeks	HR = 0.71 95% CI = 0.62–0.82 $P=0.0001$	Positive
ATLAS [181]	None	Erlotinib/Bevacizumab ($N=370$) vs (after Platinum-based doublets/Bevacizumab) Bevacizumab ($N=373$)	4.76 months (PFS) ↓ 3.75 months	HR = 0.722 95% CI = 0.592–0.881 $P=0.0012$	Positive

(d)EGFR-TKI after the second line chemotherapy for NSCLC

Trial [Ref.]	Patient selection	Treatment	OS as primary end point	Significance	Results for EGFR-TKIs
BR.21 [11,12]	None	Erlotinib (N = 488) vs Placebo (N = 243)	6.7 months 4.7 months	HR = 0.70 95% CI = 0.58–0.85 P = 0.001	Positive
ISEL [184]	None	Gefitinib (N = 1129) vs Placebo (N = 563)	5.6 months 5.1 months	HR = 0.89 95% CI = 0.77–1.02 P = 0.087	Negative
V15-32 [185]	Japanese	Gefitinib (N = 245) vs Docetaxel (N = 244)	11.5 months 14.0 months	HR = 1.12 95.24% CI = 0.89–1.40 P = 0.330	Negative
INTEREST [183]	None	Gefitinib (N = 723) vs Docetaxel (N = 710)	7.6 months 8.0 months	HR = 1.020 96% CI = 0.905–1.15[c]	Positive[c]

[a] IPASS trial also showed the superiority of gefitinib for PFS in the overall patient population of this study (HR = 0.74, 95% CI = 0.65–0.85, $P < 0.001$).
[b] In the overall patient population of WJTOG0203 trial, there was no significant difference for OS between gefitinib and placebo arms (HR = 0.86, 95% CI = 0.72–1.03, P = 0.11), although PFS was significantly improved in gefitinib arm (HR = 0.68, 95% CI = 0.57–0.80, $P < 0.001$).
[c] INTEREST trial established non-inferior survival of gefitinib compared with docetaxel because HR (96% CI) met the predefined non-inferiority criterion.

extensively studied as well as applied to the treatment of NSCLC [9–12], based on the biological significance of EGFR in this cancer [13,14]. Several recent phase III trials, WJTOG3405 [15], NEJ002 [16], or subset analysis of IPASS [17,18] demonstrated that single agent gefitinib brings superior progression-free survival (PFS) to platinum-based doublets if we select the NSCLC patients harboring *EGFR* mutation (Table 1a). Despite the therapeutic benefit of EGFR-TKIs, the efficacy of these agents in NSCLC is often limited by the emergence of drug resistance conferred either by a secondary T790M mutation of *EGFR* [19–22] or by acquired amplification of the *MET* gene [23], which are account for 70% of all cases of acquired resistance to EGFR-TKIs. Given these results, the selection of patients by *EGFR* mutation status or *MET* gene copy number is critical for longer survival as well as overcoming resistance in NSCLC patients treated with EGFR-TKIs. In this review, we summarize the significance of *EGFR* mutations and the mechanisms of resistance to EGFR-TKIs so that we can select NSCLC patients by appropriate biomarkers.

2. Strategy to target EGFR which is important for cell survival or proliferation in NSCLC

EGFR, a member of the ErbB family of receptor tyrosine kinases, is frequently overexpressed and negatively correlated with prognosis in many types of human malignancy including NSCLC [13,14,24,25]. EGFR is a 170-kDa plasma membrane glycoprotein composed of an extracellular ligand-binding domain, a transmembrane region and an intracellular tyrosine kinase domain with a regulatory COOH-terminal segment [26]. Binding of ligand to EGFR induces not only homo-dimerization with EGFR but also hetero-dimerization with the other members of the ErbB family of receptor tyrosine kinases such as Her2 (ErbB2), Her3 (ErbB3), and Her4 (ErbB4) [27]. The receptor dimerization results in consequent conformational changes, following activation of the receptor kinase and autophosphorylation of specific tyrosine residues within the COOH-terminal region of the protein (Fig. 1) [26,28,29]. These events trigger intracellular signaling pathways such as those mediated by the protein kinases Akt or extracellular-signal regulated kinase (Erk), both of which play fundamental roles in the control of numerous cellular processes [30–33]. Akt signaling, mainly associated with cell survival [34,35], is triggered by binding of the Src homology 2 (SH2) domain of phosphoinositide 3-kinase (PI3K) to phosphorylated tyrosine of Her3 which is hetero-dimerized with EGFR [36]. Although the autophosphorylation sites on EGFR do not include canonical PI3K binding sites, EGFR can activate PI3K via the docking protein Gab1 [37,38]. On the other hand, binding of Grb2 SH2 domain to EGFR results in RAS activation triggering Raf/MEK/Erk signaling which are mainly involved in cell proliferation (Fig. 1) [39,40]. In response to ligand-binding, the ligand-EGFR complex is rapidly internalized allowing EGFR to interact more with various signaling protein such as Grb2 or PI3K and to reach full and sustained activation of Akt or Erk signaling (Fig. 1) [41–43]. EGFR is either recycled back to the cell surface or proteolytically degraded. Recognition of the role of EGFR in oncogenesis has led to the development of EGFR-targeted therapies including both small-molecule TKIs that compete with ATP at the intracellular tyrosine kinase domain [44,45] and monoclonal antibodies (mAbs) that mainly compete with ligands at the extracellular domain (Fig. 2) [46–48].

3. The significance of *EGFR* mutations in NSCLC patients who receive EGFR-TKIs

Early clinical studies of EGFR-TKI gefitinib showed that higher response rates (RR) are apparent in the patients who are females, Japanese, nonsmokers with adenocarcinoma [9,10,49,50], raising

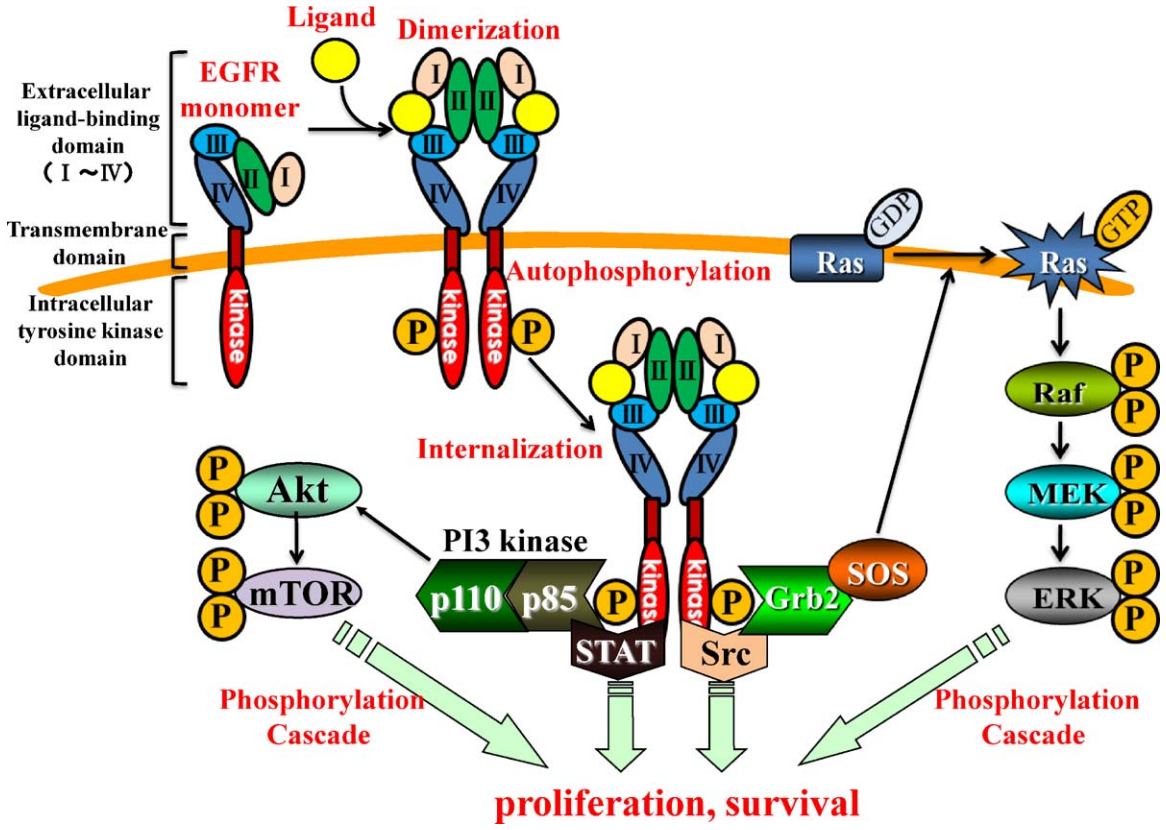

Fig. 1. Schema for EGFR signaling pathways in NSCLC. Ligand-binding induces conformational changes and dimerization of EGFR, following activation of the receptor kinase and autophosphorylation of specific tyrosine residues within the intracellular domain. These events as well as receptor internalization allow EGFR to interact with the SH2 domain of various signaling proteins such as Grb2 and PI3K. SOS-binding to the SH3 domain of Grb2 promotes exchanging GDP with GTP to activate RAS and Raf/MEK/Erk signaling which are mainly involved in cell proliferation, while PI3K triggers Akt signaling which is mainly associated with cell survival.

the rationale to identify predictive biomarker at the molecular level. In 2004, 3 groups reported that NSCLC patients with *EGFR* mutation experience a dramatic response to gefitinib or erlotinib [51–53]. Both deletions in exon 19 and a point mutation that substitutes an arginine for a leucine at codon 858 (L858R) in exon 21 are known to be the most common *EGFR* mutations (Fig. 3) [54–56]. The presence of *EGFR* mutation activates the receptor tyrosine kinase by disrupting autoinhibitory interactions [44], and induces higher phosphorylation of EGFR compared with wild-type *EGFR* [51,57]. Furthermore, NSCLC cells with *EGFR* mutation constitutively activate both EGFR and downstream signaling because of ligand-independent dimerization of the receptor [58–60]. On the other hand, *EGFR* mutation allows gefitinib to bind more tightly to EGFR compared with the wild-type *EGFR* [44,57]. These results suggests that the cells with *EGFR* mutation not only depend more on EGFR signaling but also have a better affinity for gefitinib-binding compared with those harboring wild-type *EGFR*, likely explaining their sensitivity to gefitinib. Interestingly, the patients with exon 19 deletion shows longer survival than those with L858R point mutation [61,62], in line with the results demonstrating that each mutation has a different phosphorylation pattern of EGFR as well as downstream signaling [58]. Recent phase III clinic trials, WJTOG3405 [15], NEJ002 [16], or subset analysis of IPASS [17,18] (Table 1a) as well as the combined analysis of 7 Japanese phase II trials of gefitinib monotherapy (ICHAMP) [63] demonstrated that single agent gefitinib in the patients with *EGFR* mutation shows significantly longer PFS than platinum-based doublets in the first-line chemotherapy. Furthermore, subset analysis of IPASS trial firmly confirmed *EGFR* mutation is the best predictor of gefitinib treatment compared with the other biomarkers including *EGFR* gene copy number [17,18].

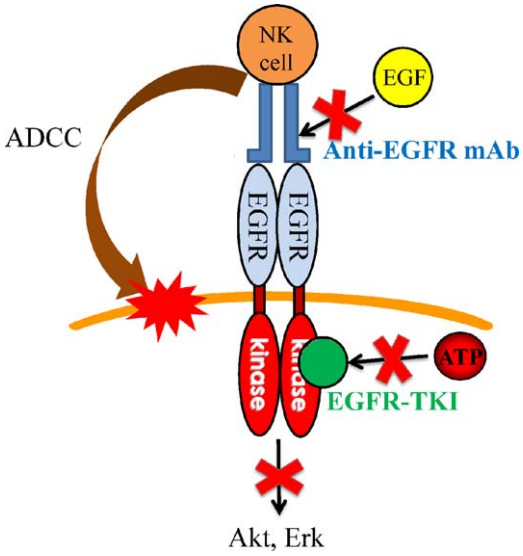

Fig. 2. Schema for how EGFR-targeted agents work. Anti-EGFR mAbs compete with ligands such as EGF at the extracellular domain of the receptor as well as exert ADCC. EGFR-TKIs compete with ATP at the intracellular tyrosine kinase domain of the receptor.

4. Application of proteomic approach to EGFR signaling network

Advances in quantitative proteomics and phosphoproteomics were broadly applied to understand the complexity of EGFR

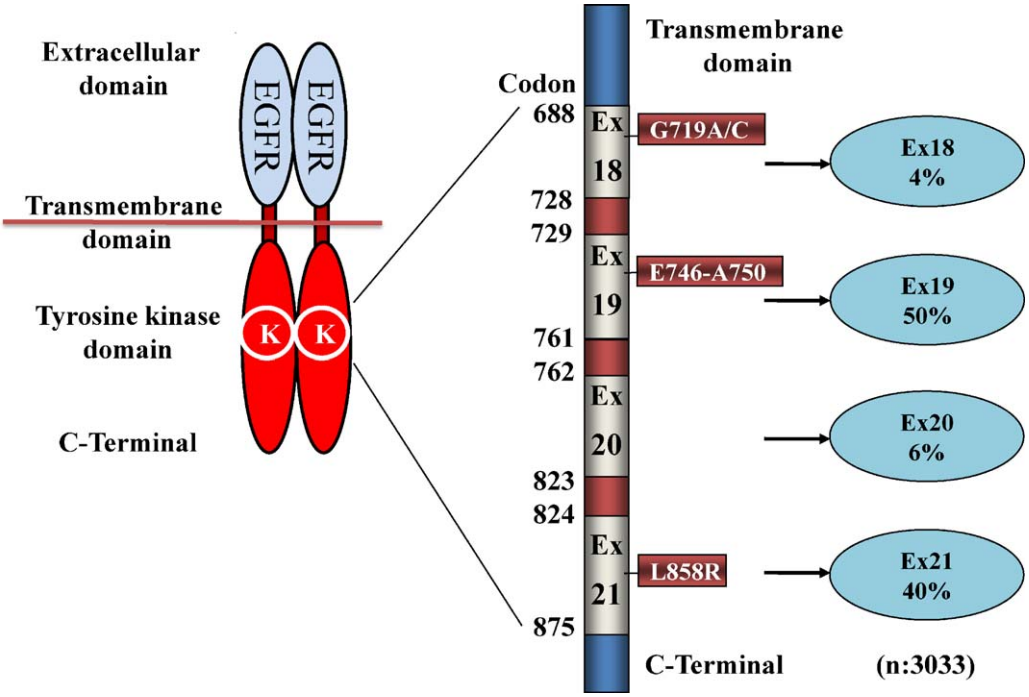

Fig. 3. Frequency of *EGFR* mutations in NSCLC ($n = 3033$) [54].

signaling network by detecting and quantifying both dynamics and regulation of EGFR phosphorylation sites, or mapping the interaction of the activated EGFR [64]. Using an unbiased and global quantitative proteomic approach, 53 activated tyrosine kinases including 25 receptor tyrosine kinases and 28 non-receptor tyrosine kinases were profiled and quantified from 41 NSCLC cell lines and over 150 NSCLC tumors [65]. Further ranking analysis of quantitative phosphorylation revealed the particular combinations of activated tyrosine kinases in given tumor. Integration of the rank of activated tyrosine kinases and the information of *EGFR* mutation status could classify each selected human lung tumor. Reverse phase protein array quantitation, one of the antibody-based quantitative proteomic approaches, firstly revealed that multiple and site-specific phosphoproteins in EGFR signaling are associated with *EGFR* mutation status in Laser Capture Microdissected NSCLC cells [66]. Their results demonstrated that synergistic changes of 6 phosphorylation sites of EGFR signaling network proteins are correlated with the presence of *EGFR* mutation.

Tumor-associated multiple tyrosine kinases identified by both mass spectrometry (MS) and antibody-based proteomic approaches provide the information to guide personal therapy of cancer by blockade of EGFR pathway. Quantitative proteomics has also proven the ability of predicting target selectivity of TKIs. Global proteomic analysis of phospho-tyrosine signaling tried to identify the signaling network of gefitinib-sensitive NSCLC cells with *EGFR* mutation and gene amplification [67]. In this study, semi-quantitative spectral counting approach showed a set of differentially tyrosine phosphorylated proteins by comparing the gefitinib-sensitive with gefitinib-resistant NSCLC cell line panels. Combination of immunoaffinity method and SILAC (stable isotope labeling with amino acids in cell culture), a quantitative MS method [68], has identified signaling network including receptor tyrosine kinases and their downstream molecules affected by gefitinib. Finally, core network of about 50 proteins involved in the pathways mediating drug response was identified by comparing the NSCLC cells harboring *EGFR* mutation with the *MET*-amplified gastric cancer cells. In clinic, a classification algorithm based on spectra from matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) was developed to classify patients by analysis of pretreatment serum and plasma, which specially fit to identify subgroups of NSCLC patients for clinical outcome after treatment with EGFR-TKIs such as gefitinib and erlotinib [69].

5. T790M secondary *EGFR* mutation as the mechanism of EGFR-TKIs resistance

Even though NSCLC patients with *EGFR* mutation show an initial dramatic response to EGFR-TKIs such as gefitinib or erlotinib, almost all of them acquire resistance to these drugs within 1 year as a serious clinical problem. A secondary point mutation in exon 20 of *EGFR* that substitutes methionine for threonine at amino acid position 790 (T790M) was identified in the NSCLC patients who developed acquired resistance to gefitinib or erlotinib [19,20]. The other reports indicated that 50% of NSCLC patients with acquired resistance to EGFR-TKIs have T790M secondary mutation [21,22]. These results suggested that *EGFR* mutation is associated with not only sensitivity but also resistance to EGFR-TKIs. T790M was also detected before treatment in a few cases of NSCLC patients who did not responded to gefitinib [70]. In addition, the NSCLC patients with low levels of T790M in circulating cells before EGFR-TKIs treatment showed significantly shorter progression-free survival than those without T790M [71]. These reports suggested that, similar to *KRAS* mutations [72–79], T790M is also involved in the cause of primary resistance to EGFR-TKIs and may become possible biomarker to identify the patients who do not respond well to EGFR-TKIs. Since T790M emerges as a minor population in tumor samples [80] or in the cells even after enforced exposure to gefitinib [81], the sensitive methods compared with direct sequence, such as polymerase chain reaction (PCR) invader [82,83], PCR clamp [84–87], Scorpion Amplification Refractory Mutation System (SARMS) [71,88–91], and Cycleave PCR assays [22,92,93], are now developing in clinic to examine *EGFR* mutations including T790M.

T790M is known to be located in the ATP-binding cleft of the EGFR structure and is thought to block EGFR-TKI binding due to alteration of the topology [19,20,45,94,95], similar to the

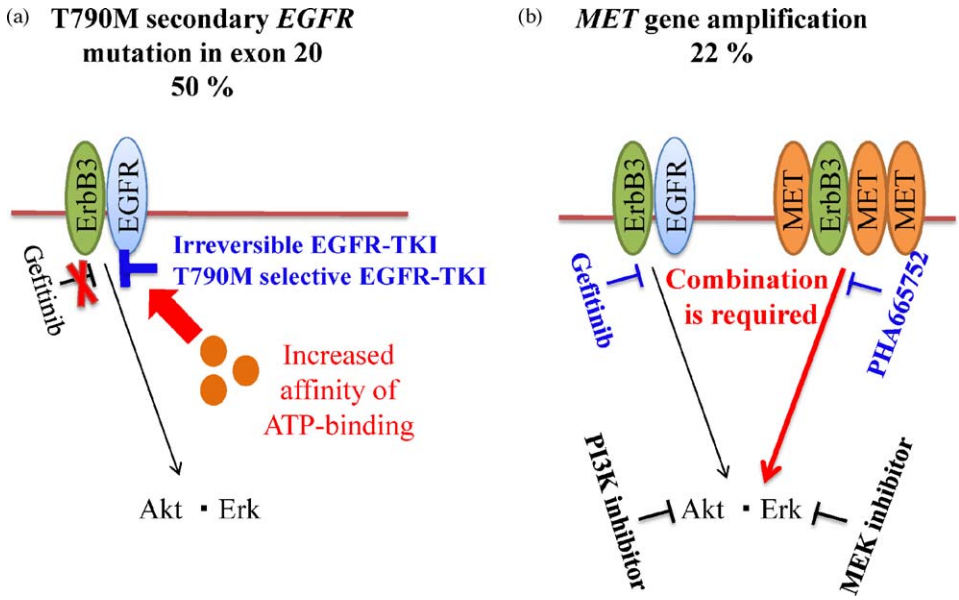

Fig. 4. Strategy to overcome acquired EGFR-TKIs resistance in NSCLC. (a) T790M secondary *EGFR* mutation in exon 20 is found in 50% of the NSCLC patients who acquired resistance to EGFR-TKIs such as gefitinib or erlotinib. In this situation, gefitinib cannot compete enough with ATP because of alteration of the topology or increased affinity of ATP-binding in the ATP-binding cleft. Irreversible EGFR-TKIs or T790M selective EGFR-TKIs are thought to be a potent strategy to overcome the resistance induced by T790M. (b) *MET* gene amplification is found in 22% of the NSCLC patients who acquired EGFR-TKIs resistance. In this situation, MET signaling is activated through Her3 (ErbB3) in addition to EGFR signaling. Both gefitinib and MET inhibitor PHA665752 are necessary to overcome the resistance induced by *MET* amplification. The combination of inhibitors which block the downstream molecules of both EGFR and MET, such as the PI3K inhibitor combined with the MEK inhibitor, could be one of the alternative strategies to overcome the resistance induced by *MET* amplification.

resistance mutations to imatinib in BCR-Abl (threonine-315 to isoleucine; T315I) [96] or kit (threonine-670 to isoleucine; T670I) [97]. Furthermore, the presence of T790M provides a growth advantage to the cells both *in vitro* and *in vivo* [98], suggesting that T790M itself has a oncogenic effects by enhanced kinase activity [57]. Recent study has also shown that the affinity of ATP-binding to the cleft is increased by the presence of T790M [99], explaining that irreversible EGFR-TKIs such as CL387,785 [100,101], PF00299804 [102,103], BIBW2992 [104], or HKI-272 [105] are thought to be one of the strategies to overcome the resistance induced by T790M (Fig. 4a). However, IC50 for irreversible EGFR-TKIs (CL387,785, PF00299804) in H3255 GR cells which acquired T790M by enforced gefitinib exposure are still 17 to 65 times higher than those in parental H3255 cells with exon 19 deletion [101,102]. In addition, recent chemogenomic profiling in NSCLC cell line panels demonstrated the limited activity of irreversible EGFR-TKIs in the cells with T790M [106]. Some investigators also pointed out the possibility that the treatment with irreversible EGFR-TKIs in the cells with T790M could induce the resistance to themselves [107–111]. These results suggested that single agent irreversible EGFR-TKI is not enough to reverse the resistance completely. Several preclinical reports showed that the other agents combined with irreversible EGFR-TKIs, such as BIBW2992 combined with either anti-EGFR mAb cetuximab [112] or PI3K/mammalian target of rapamycin (mTOR) inhibitor PI-103 [106], and HKI-272 combined with mTOR inhibitor rapamycin [113], are promising to overcome T790M. The dual inhibitors of vascular endothelial growth factor receptor (VEGFR) and EGFR such as vandetanib [114,115], EXEL-7647 [116], or BMS-690514 [117] as well as the anti-VEGF mAb bevacizumab [114] were effective in the cells with T790M as single agent therapy. The heat shock protein 90 (Hsp90) inhibitors such as geldanamycin [118], 17-DMAG [109,118], or CUDC-305 [119] are also thought to be the potent strategy against T790M. Most recently, EGFR-TKI which selectively inhibits EGFR carrying T790M, but not wild-type *EGFR* or sensitive *EGFR* mutation, is identified and expected to be developed in clinic [120].

6. The other mechanisms of EGFR-TKIs resistance including *MET* gene amplification

Some other rare secondary *EGFR* mutations, such as E709A, L747S, D761Y, Q787R, G796A, T854A, or H870R, could induce primary or acquired resistance to EGFR-TKIs in NSCLC [21,60,121–125]. However, the mechanisms of many cases of acquired resistance other than T790M had remained unclear. *MET* gene amplification has recently been identified as a novel mechanism of gefitinib resistance, being detected in 22% of tumor samples from NSCLC patients with *EGFR* mutations who acquired gefitinib resistance [23]. MET activation (phosphorylation) was also shown to be associated with progressive disease (PD) and shorter time to progression (TTP) in NSCLC patients treated with gefitinib or erlotinib [126], while we need to discuss more about the role of *MET* amplification for primary resistance to EGFR-TKIs in NSCLC patients [127–130]. Both MET and EGFR signaling activate PI3K via ErbB3 (Her3) in the gefitinib-resistant HCC827GR cells with *MET* amplification which were generated by enforced exposure of HCC827 cells harboring exon 19 deletion to gefitinib. In this situation, the combination of gefitinib and MET inhibitor PHA665752 is critical to shut down survival signaling in these cells (Fig. 4b) [23]. Although there are few studies describing the alternative therapy to overcome EGFR-TKIs resistance induced by *MET* amplification, the combination of inhibitors which block the downstream molecules of both EGFR and MET, such as the PI3K inhibitor combined with the MEK inhibitor, could be one of the strategies (Fig. 4b) [131]. In line with this possibility, Src inhibitor dasatinib alone effectively inhibits cell growth in the gefitinib-resistant HCC827GR cells with *MET* amplification based on the results showing that Src acts downstream of both EGFR and MET in these cells [132]. Another preclinical study has also shown that gefitinib combined with the oral fluoropyrimidine derivative S-1 could be an alternative therapy to overcome EGFR-TKIs resistance induced by acquired *MET* amplification [133]. Recent reports showed that *MET* amplification or MET activation without gene amplification could exist together with T790M secondary muta-

tion, and dual inhibition of EGFR and MET pathways is potent as well in this situation [134,135]. However, MET inhibitors did not affect cell viability or sensitivity to EGFR-TKIs in PC9GR, PC9ER, or PC9/VanR cells which acquired both T790M and MET activation without gene amplification by enforced exposure of PC9 cells harboring exon 19 deletion to gefitinib, erlotinib, or vandetanib [115,135]. These results suggested that MET activation without gene amplification in the cells carrying T790M is dependent on EGFR signaling to some extent unlike *MET* amplification. Even though several possible mechanisms of acquired EGFR-TKIs resistance, such as the involvement of insulin-like growth factor 1 receptor (IGF1R) signaling [136–138], the loss of PTEN [139,140], PI3K-dependent recruitment of Gab1/Shp2 [141], or hepatocyte growth factor (HGF) overexpression [142], were also reported, the cause of around 30% of the patients with acquired resistance have remained to be elucidated. It is critical to investigate the other mechanisms of EGFR-TKIs resistance as well as establish the strategy to overcome known resistant mechanisms.

7. The strategy based on the monoclonal antibodies to EGFR in NSCLC

The mAbs are thought to be another strategy to target EGFR by binding to the extracellular domain of the receptor (Fig. 2). There are several anti-EGFR mAbs well studied in basic and clinical researches in NSCLC, including cetuximab which is a chimeric mouse-human antibody of the immunoglobulin (Ig) G1 subclass [143–158], matuzumab [144,159–161] or nimotuzumab [162] which are humanized IgG1 mAbs, and a fully human IgG2 mAb panitumumab [163]. Structural studies have demonstrated how these mAbs can block EGFR signaling. Nimotuzumab directly blocks access of ligand to the domain III ligand-binding site of EGFR [48], while matuzumab prevents conformation change of EGFR which is required for receptor dimerization [164]. On the other hand, cetuximab blocks both ligand-binding and conformation change of the receptor at the domain III of EGFR [46]. Independent of preventing ligand-binding or receptor dimerization, anti-EGFR mAb exerts an antibody-dependent cellular cytotoxicity (ADCC) activity in NSCLC cells (Fig. 2) [154], which is well known in the anti-Her2 mAb trastuzumab for breast cancer [165] and in the anti-CD20 mAb rituximab for B-cell lymphoma [166].

In contrast to EGFR-TKIs, several basic and clinical reports have shown that the sensitivity to anti-EGFR mAbs is not associated with *EGFR* mutation status in NSCLC [143,144,153]. In colorectal cancer, *EGFR* copy number [167–169] and the grade of the skin rash [170–173] have emerged as an important predictive marker of response to anti-EGFR mAbs. In addition, the presence of *KRAS* mutation is negatively associated with the response to anti-EGFR mAbs in colorectal cancer patients [169,171,174]. Recent study indicated that both *EGFR* copy number [145] and skin rash of any grade [148] are also useful to predict the response to anti-EGFR mAbs in NSCLC patients. However, there is no evidence that *KRAS* is associated with resistance to anti-EGFR mAbs in NSCLC [146,148,153], suggesting that further analyses are required to identify predictive markers. Although there are few studies showing the mechanism of acquired resistance to cetuximab, PTEN instability [175], nuclear EGFR [176], and Src [176,177] are thought to be involved in the acquired resistance. In clinic, recent phase III FLEX trial [147,148] indicated that NSCLC patients treated with cetuximab plus platinum-based doublet survive longer than those treated with platinum-based doublet alone in the first-line chemotherapy, in line with several randomized phase II trials [149–151]. The BMS-099 phase III trial [152,153] did not show a significant difference in the primary end point PFS between NSCLC patients treated with cetuximab plus platinum-based doublet and those treated with platinum-based doublet alone. However, this trial showed OS which favored cetuximab as well as significant improvement in overall response rate (ORR) [152,153]. These results suggest that anti-EGFR mAbs are one of the strategies for NSCLC patients in the first-line chemotherapy.

8. Conclusion and clinical view of the molecular-targeted therapies in NSCLC

As we mentioned, recent phase III IPASS [17,18], WJTOG3405 [15], or NEJ002 trials [16] demonstrated that single agent EGFR-TKI could stand as the first-line chemotherapy for NSCLC by proper selection of the patients with *EGFR* mutation (Table 1a). EGFR-TKIs were also shown to be useful for the maintenance therapy [178–182] or the second line therapy [11,12,183] in several clinical trials for NSCLC, even though there are some negative studies [184–186] (Table 1c and d). On the other hand, recent phase III trials demonstrated the effectiveness of the anti-EGFR mAb cetuximab (FLEX study) [147,148] or the anti-VEGF mAb bevacizumab (ECOG4599 and AVAiL studies) [187,188] in combination with platinum-based doublets in the first-line chemotherapy for NSCLC, while EGFR-TKIs combined with platinum-based doublets did not show any additive effects (Table 1b) [189–192]. These results suggested that not only small molecule TKIs but also mAbs could be useful for the treatment of NSCLC patients. In addition, EML-ALK fusion genes were recently found in NSCLC patients with a fixed probability and expected to be a novel molecular target [193–200]. Together, it is critical to evaluate and compare these molecular-targeted therapies as well as to investigate potential biomarker for patient selection in NSCLC.

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