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REVIEW

Next-generation EGFR/HER tyrosine kinase inhibitors for the treatment of patients with non-small-cell lung cancer harboring *EGFR* mutations: a review of the evidence

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Abstract: Tyrosine kinase inhibitors (TKIs) against human epidermal growth factor receptor (EGFR/HER) family have been introduced into the clinic to treat cancers, particularly non-small-cell lung cancer (NSCLC). There have been three generations of the EGFR/HER-TKIs. First-generation EGFR/HER-TKIs, binding competitively and reversibly to the ATP-binding site of the EGFR TK domain, show a significant breakthrough treatment in selected NSCLC patients with activating *EGFR* mutations (act*EGFR*m) $EGFR^{L858R}$ and $EGFR^{Del19}$, in terms of safety, efficacy, and quality of life. However, all those responders inevitably develop acquired resistance within 12 months, because of the $EGFR^{T790M}$ mutation, which prevents TKI binding to ATP-pocket of EGFR by steric hindrance. The second-generation EGFR/HER-TKIs were developed to prolong and maintain more potent response as well as overcome the resistance to the first-generation EGFR/HER-TKIs. They are different from the first-generation EGFR/HER-TKIs by covalently binding to the ATP-binding site, irreversibly blocking enzymatic activation, and targeting EGFR/HER family members, including EGFR, HER2, and HER4. Preclinically, these compounds inhibit the enzymatic activation for act*EGFR*m, $EGFR^{T790M}$, and wt*EGFR*. The second-generation EGFR/HER-TKIs improve overall survival in cancer patients with act*EGFR*m in a modest way. However, they are not clinically active in overcoming $EGFR^{T790M}$ resistance, mainly because of dose-limiting toxicity due to simultaneous inhibition against wt*EGFR*. The third-generation EGFR/HER-TKIs selectively and irreversibly target $EGFR^{T790M}$ and act*EGFR*m while sparing wt*EGFR*. They yield promising efficacy in NSCLC patients with act*EGFR*m as well as $EGFR^{T790M}$ resistant to the first- and second-generation EGFR-TKIs. They also appear to have a lower incidence of toxicity due to the reduced inhibitory effect on wt*EGFR*. Currently, the first-generation EGFR/HER-TKIs gefitinib and erlotinib and second-generation EGFR/HER-TKI afatinib have been approved for use as the first-line treatment of metastatic NSCLC with act*EGFR*m. This review will summarize and evaluate a broad range of evidence of recent development of EGFR/HER-TKIs, with a focus on the second- and third-generation EGFR/HER-TKIs, in the treatment of patients with NSCLC harboring *EGFR* mutations.

Keywords: EGFR/HER, tyrosine kinase inhibitors, NSCLC, *EGFR* mutations, acquired resistance

Introduction

Lung cancer is currently the leading cause of cancer-related mortality worldwide, causing more than one-quarter of all cancer deaths (28% in males and 26% in females).[1] As of 2016, it is estimated that 224,390 new cases of lung cancer will be diagnosed in the US and 158,080 deaths will be caused from lung cancer.[2] Non-small-cell

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lung cancer (NSCLC), accounting for a high proportion (85%–90%) in lung cancer,[3] is subdivided histologically into adenocarcinoma, squamous-cell carcinoma, large-cell carcinoma, and other types.[4,5] In the last decade, the diagnosis and treatment of NSCLC has evolved dramatically from the traditional "one-size-fits-all" chemotherapeutic approach to new anticancer compounds molecularly targeting oncogenic driver mutations, due to the advances in cancer biology and technology. Various driver genomic alterations have been identified in oncogene-dependent NSCLC, especially two genes: the human epidermal growth factor receptor (*EGFR/HER*) and the anaplastic lymphoma kinase (*ALK*).[6]

The EGFR/HER family of receptor tyrosine kinases (TKs) has four members including EGFR (HER1, erbB-1), HER2 (erbB-2), HER3 (erbB-3), and HER4 (erbB-4), and their signaling pathways regulate cell growth, survival, adhesion, migration, and differentiation through three downstream pathways: RAS/RAF/mitogen-activated protein kinase, phosphoinositide 3-kinase/AKT, and Janus kinase/signal transducer and activator of transcription (JAK/STAT).[7,8] Dysregulated signaling of HER family has been associated with the development of several malignancies including NSCLC.[9] Many patients with NSCLC have somatic mutations of *EGFR*, first identified in 2004,[10,11] which lead to aberrant constitutive signaling via EGFR/HER family and their downstream protein markers. The *EGFR* mutations, including activating and resistant mutations, mostly occur in exons 18 to 21 of the *EGFR* gene encoding the ATP-binding pocket of the intracellular TK domain. The activating *EGFR* mutations (act*EGFR*m) have been reported in ~10%–15% of Caucasian patients but in up to 60% of selected Asian populations with NSCLC (female, never/light smoker, and adenocarcinoma).[12–14] The most frequent act*EGFR*m in NSCLC are in-frame deletions in exon 19 ($EGFR^{Del19}$, ≈60%) and L858R point mutation in exon 21 ($EGFR^{L858R}$, ≈30%).[12,15,16] These oncogenic mutations interact and generate stabilization with ATP, intrinsically stimulate phosphorylation of tyrosine residues, and then result in the intracellular signal transduction activation in a ligand-independent manner.[17,18] The NSCLC patients with act*EGFR*m become apparently dependent on EGFR activity to stimulate downstream signaling pathways to maintain the malignant phenotype ("oncogene addiction").[19,20] Therefore, blocking EGFR/HER family pathways with EGFR/HER TK inhibitors (TKIs) can suppress tumor cell proliferation and initiate apoptosis.

In the past decade, the EGFR/HER family has become a potential therapeutic target and has greatly changed the treatment paradigm for NSCLC patients, since the introduction of the first-generation EGFR/HER-TKIs gefitinib and erlotinib. Currently, these two agents and a second-generation EGFR/HER-TKI afatinib are approved for use in the first-line treatment of metastatic NSCLC with act*EGFR*m ($EGFR^{Del19}$ and $EGFR^{L858R}$), based on the outcomes of several clinical trials that demonstrate that these TKIs are superior to standard chemotherapy in terms of safety, efficacy, and quality of life.[21–23] However, despite a good initial response, the development of acquired resistance in most of the patients limits the long-term efficacy of TKI therapy. Therefore, extensive investigations on better understanding of the mechanisms of resistance are being undertaken in order to robust the benefit of EGFR/HER-TKIs in NSCLC. New generations of EGFR/HER-TKIs have been developed to improve cancer treatment efficacy, overcome resistance, and reduce side effects. These EGFR/HER-TKIs are listed in Table 1. In this review, we will provide a broad overview of recent development of EGFR/HER-TKIs, with a focus on second- and third-generation EGFR/HER-TKIs, in the treatment of patients with NSCLC harboring *EGFR* mutations.

First-generation EGFR/HER-TKIs

Gefitinib (AstraZeneca plc, London, UK) and erlotinib (Astellas Pharma Inc., Tokyo, Japan) are the first-generation EGFR/HER-TKIs approved to use in the first-line setting for the treatment of advanced NSCLC patients with act*EGFR*m ($EGFR^{Del19}$ and $EGFR^{L858R}$).[36,37] Both compounds (Figure 1) are orally active 4-anilino-quinzolines with antineoplastic activity and bind competitively and reversibly to the ATP-binding site of the TK domain of EGFR. This conformation of EGFR in this scenario prevents the autophosphorylation of the TK, blocks the activation of the EGFR signal transduction, inhibits tumor cell proliferation, and induces cell cycle arrest and apoptosis.[38] Among the diverse (activating and resistant) mutations clustering around the catalytic cleft of *EGFR* TK domain, it has been demonstrated that the act*EGFR*m leads to increased affinity for EGFR/HER-TKIs, thus conferring more sensitivity to this treatment.[13] Indeed, act*EGFR*m has been reported to bind 20-fold more tightly to TKIs than to the wild-type *EGFR* (wt*EGFR*).[39]

Clinically, lung cancer patients with $EGFR^{Del19}$and $EGFR^{L858R}$ show a striking response to gefitinib and erlotinib treatment. Retrospective analysis of associations between *EGFR* gene mutations and EGFR/HER-TKIs sensitivity has shown that 70% of act*EGFR*m NSCLC patients are responsive to TKIs compared with 10% of wt*EGFR* patients.[7,40] In the preselected subgroup of NSCLC patients

Table 1 Generations of EGFR/HER-TKIs for NSCLC

Generation	TKIs	Mechanism of action	Molecular targets	Clinical status	MTD/clinical dose	Most common adverse effects
First-generation EGFR/HER-TKIs	Gefitinib[22,24,25]	4-Anilino-quinazoline; reversible	*EGFR*^{L858R}, *EGFR*^{Del19}	Approved	700/250 mg once daily	Proteinuria, diarrhea, ALT increased, decreased appetite, AST increased, and skin reactions
	Erlotinib[24,25]			Approved	200/150 mg once daily	Skin rash and diarrhea
Second-generation EGFR/HER-TKIs	Afatinib[25,26]	Anilino-quinazoline (with acrylamide group); covalent; irreversible	wt*EGFR* (Cys-797), *EGFR*^{L858R}, *EGFR*$^{L858R/T790M}$, *EGFR*$^{L858R/T854A}$, wt*HER2* (Cys805), *HER2* amplification, *HER4* (Cys803)	Approved	50/40 mg once daily	Diarrhea, vomiting, dyspnea, fatigue, and hypokalemia
	Dacomitinib[25,27]	Anilino-quinazoline (with electrophilic motif); covalent; irreversible	*EGFR*^{L858R}, *EGFR*^{Del19}, *EGFR*^{T790M} wt*HER2*, mutant-*HER2*, *HER2* amplification, *HER4*	Phase III	45/45 mg once daily	Diarrhea, acne, and rash
	Neratinib[28–30]	Quinoline (with cyano group); covalent; irreversible	*EGFR*^{L858R}, *EGFR*^{T790M}, *HER2*, *HER4*	Phase III	320/240 mg once daily	Diarrhea, dyspnea, nausea, and vomiting
Third-generation EGFR/HER-TKIs	Osimertinib[25,31]	Mono-anilino-pyrimidine; covalent, irreversible	*EGFR*^{L858R}, *EGFR*^{Del19}, *EGFR*^{T790M}	Phase III	Not reached at 240/80 mg daily	Diarrhea, rash, nausea, and decreased appetite
	Rociletinib[32,33]	2,4-Disubstituted pyrimidine; covalent, irreversible	*EGFR*^{L858R}, *EGFR*^{Del19}, *EGFR*^{T790M}	Phase III	Not reached at 1,000/625 mg twice daily	Hyperglycemia, long QT interval, nausea, fatigue, and diarrhea
	HM61713[34,35]	Covalent, irreversible	*EGFR*^{L858R}, *EGFR*^{Del19}, *EGFR*^{T790M}	Phase II	800/800 mg once daily	Diarrhea, rash, skin exfoliation, nausea, and pruritus

Abbreviations: EGFR/HER-TKIs, human epidermal growth factor receptor tyrosine kinase inhibitors; NSCLC, non-small-cell lung cancer; TKI, tyrosine kinase inhibitor; MTD, maximal tolerated dose; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

with *EGFR*^{Del19}and *EGFR*^{L858R}, the first-generation reversible EGFR/HER-TKIs gefitinib and erlotinib as a first-line treatment can dramatically affect patient outcomes, showing superiority to traditional platinum-based chemotherapy in terms of objective response rate (ORR), progression-free survival (PFS), and quality of life, and an acceptable toxicity profile.[41–44] These studies resulted in their approval and widespread use for act*EGFR*m NSCLC patients.

On the other hand, resistant *EGFR* mutations (res*EGFR*m) either as primary or as secondary (acquired) events have also been reported, the most common being L747S and D761Y in exon 19, T790M and insertions in exon 20, and T854A in exon 21.[17,45] The primary resistance (initially refractory to EGFR/HER-TKIs treatment) is seen in ~30% of NSCLC patients with act*EGFR*m, involving coexistent genetic alterations: res*EGFR*m, *KRAS* mutations, *PTEN* losses, *PIK3CA* mutations, *BIM* deletion, and 60% unknown factors.[17,46–48] Additionally, although EGFR/HER-TKIs have great initial efficacy in 70% of patients with act*EGFR*m NSCLC, all those responders will inevitably develop acquired resistance (disease progression) within 1 year or 2 years.[47] Approximately 50%–60% of patients with acquired resistance develop a secondary mutation in *EGFR*, most commonly the substitution of threonine at the "gatekeeper" amino acid 790 to methionine (T790M) occurring within exon 20, causing a bulky methionine side chain in TK domain.[49,50] The *EGFR*^{T790M} mutation results in the receptor becoming refractory to these reversible EGFR/HER-TKIs through a steric hindrance that prevents drugs binding to ATP-pocket and results in restored affinity to ATP.[25,49,51,52] Preclinical modeling and analysis of tumor tissues obtained from patients after disease progression has also identified other less frequent mechanisms of acquired resistance, including bypass or alternative pathways (*HER2* amplification, *MET* amplification, *PIK3CA* mutation, *BRAF* mutation, *NF1* loss, and potentially FGFR signaling), histological/phenotypic transformation (small-cell lung cancer transformation or epithelial-to-mesenchymal transition), and unknown in 20%–30%.[4,17,25,41,53–55] Understanding the biological basis responsible for the acquired resistance has therapeutic implications, and several strategies are currently under investigation. Based on the aforementioned mechanisms, several combinations with other therapies targeting bypass or alternative activating pathways have been explored in preclinical models or clinical trials. The potential candidate partners include MET-TKI tivanitinib,[17] anti-MET antibody onartuzumab,[56] MET/VEGFR-TKI TAS-115,[57] anti-VEGF antibody bevacizumab,[58,59] and STAT3 inhibitor S3I-201.[60]

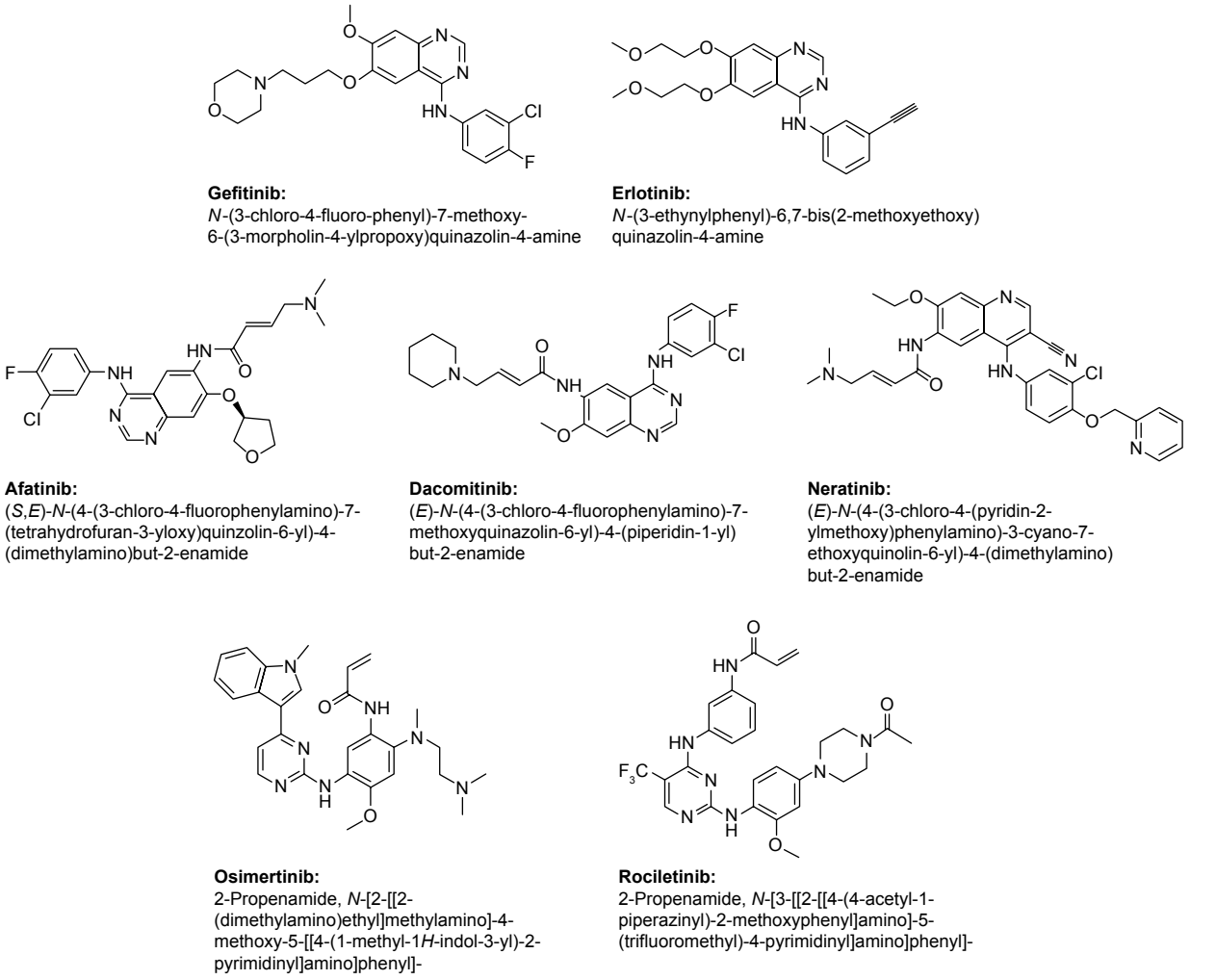

Figure 1 Structures and chemical names of EGFR/HER-TKIs.
Note: All structures are adapted from www.selleckchem.com.[101]
Abbreviation: EGFR/HER-TKIs, human epidermal growth factor receptor tyrosine kinase inhibitors.

Given the modest and nonoverlapping toxicities observed with EGFR/HER-TKIs compared with chemotherapy, a single Phase III trial demonstrated a significant improvement of intercalated chemotherapy and erlotinib in patients with advanced NSCLC harboring act*EGFR*m;[61] however, four large Phase III studies failed to show superiority of combination treatment to standard platinum doublet chemotherapy in unselected chemotherapy-naive advanced NSCLC patients, making the value of this approach uncertain.[62]

Considering that $EGFR^{T790M}$ mutation represents the most frequent acquired resistance mechanism, targeting this mutation by irreversible next-generation (second and third generations) EGFR/HER-TKIs, therefore, represents an attractive strategy to overcome treatment resistance to first-generation EGFR/HER-TKIs.

Second-generation EGFR/HER-TKIs

The poor survival rate for NSCLC with coexpression of EGFR and HER2[63] suggests that HER family members should be simultaneously targeted for treatment. Aiming for a prolonged and more potent response and overcoming the resistance to first-generation EGFR/HER inhibitors, the second-generation EGFR/HER-TKIs,[26] including afatinib (BIBW2992; Boehringer Ingelheim, Ingelheim am Rhein, Germany), dacomitinib (PF299804; Pfizer, Inc., New York, NY, USA), and neratinib (HKI272; Puma Biotechnology, Los Angeles, CA, USA), are designed to covalently bind to the ATP-binding site and irreversibly block enzymatic activation of EGFR and other HER family members including HER2 and HER4 (Table 1 and Figure 1). The second-generation irreversible EGFR/HER-TKIs were developed in part to inhibit the $EGFR^{T790M}$ mutation, in addition to the common act*EGFR*m.

Afatinib

In addition to gefitinib and erlotinib, a second-generation EGFR/HER-TKI afatinib is approved as a first-line treatment for the advanced NSCLC harboring act*EGFR*m in the US, Europe, Taiwan, Japan, and other countries.[23] Afatinib is an

ATP-competitive aniline-quinazoline derivate containing a reactive acrylamide group, covalently binding to EGFR, HER2, and HER4 and irreversibly inhibiting HER-family phosphorylation and signal transduction.[26] The theoretical advantages and promising preclinical data indicated that afatinib irreversibly inhibited the enzymatic activation of wt*EGFR*, *EGFR*^{L858R}, *EGFR*$^{L858R/T790M}$, *EGFR*$^{L858R/T854A}$, wt*HER2*, *HER2* amplification, and wt*HER4*, as well as showed antitumor activities in both EGFR/HER-TKI-naive and resistant tumor cells and xenograft models.[7,20]

As a first-line treatment for advanced *EGFR*-mutated NSCLC, the Lux-Lung 2 single-arm Phase II trial[64] showed that patients with common activating mutations (*EGFR*^{Del19} and *EGFR*^{L858R}) were most sensitive to afatinib monotherapy with an ORR of 66%, median overall survival (OS) of 24 months, and median PFS of 14 months (Table 2), as well as indicated 40 mg/d was preferable for subsequent studies. Two larger Phase III clinical trials (Lux-Lung 3 and 6),[65–67] comparing the second-generation TKI with the standard platinum-based chemotherapy, demonstrated that the first-line afatinib monotherapy for advanced act*EGFR*m lung adenocarcinoma had significantly prolonged median PFS and higher ORR as compared with up to six cycles of standard-of-care platinum-based (pemetrexed plus cisplatin or gemcitabine plus cisplatin) chemotherapy and improved quality of life (Table 2). The pooled analysis[68,69] of both trials mentioned previously confirmed that afatinib used as the first-line treatment improved OS compared with chemotherapy for patients with *EGFR*^{Del19} (regardless of race/ethnicity), but no difference in unselective *EGFR* mutations and even in *EGFR*^{L858R} subgroup. The improved OS has never been observed in the first-generation EGFR-TKIs, probably due to methodology differences in trial design. In April 2016, Lux-Lung 7 head-to-head international study,[70] comparing the first- versus second-generation EGFR/HER-TKIs, reported that irreversible afatinib is superior to reversible gefitinib in treatment-naive patients with act*EGFR*m NSCLC in terms of efficacy and safety (Table 2). The results showed that afatinib achieved significant but clinically minor improved outcomes but did so with an improved safety profile.

Similarly, as a second-line treatment in patients with advanced squamous NSCLC who progressed on platinum-based chemotherapy, Lux-Lung 8[71] demonstrated that afatinib improved the PFS and OS in a modest way compared with erlotinib, but again with an improved safety profile (Table 2). Exploring the efficacy of afatinib as salvage therapy for advanced NSCLC patients, who were pretreated with one or two chemotherapy regimens and acquired resistance to first-generation EGFR/HER-TKIs, both single-arm Lux-lung 4 and two-arm Lux-lung 1 (Table 2) were carried out. The single-arm Lux-lung 4 showed an ORR of 8.2% and median PFS of 4.4 months in patients treated with afatinib at 50 mg/d, and the two-arm Lux-lung 1 observed a longer PFS and higher ORR along with improvements in lung cancer-related symptoms for patients treated with afatinib compared with placebo, despite failure to show the different median OS (primary endpoint).[72,73] Both studies suggested that afatinib could overcome acquired resistance to gefitinib/erlotinib in some cases.

Dacomitinib

Dacomitinib (Figure 1) is an irreversible EGFR/HER-TKI against EGFR, HER2, and HER4 homodimers and heterodimers with a higher kinase inhibition than gefitinib/erlotinib, including act*EGFR*m (*EGFR*^{L858R} and *EGFR*^{Del19}), res*EGFR*m (*EGFR*^{T790M}), wt*HER2*, *HER2* amplification, and first-generation TKI-resistant *HER2* mutations.[27] Dacomitinib demonstrated activity in both gefitinib-sensitive and gefitinib-resistant NSCLC preclinical models.[74]

In relapsed/refractory setting, a Phase II study (NCT00769067) compared dacomitinib versus erlotinib as second- or third-line treatment in the EGFR/HER-TKI-naive NSCLC patients pretreated with chemotherapy.[75] Significant results favored a small superiority for dacomitinib over erlotinib in PFS (Table 2). However, two recent Phase III studies with dacomitinib in this setting failed to achieve their primary objectives (Table 2). This demonstrates the critical importance of well-powered randomized trials to establish the true impact of new therapies. The ARCHER 1009 study, which compared dacomitinib with erlotinib as salvage therapy in patients with advanced NSCLC who had disease progression after one or two chemotherapy regimens did not show superiority for dacomitinib to erlotinib in an unselected population or in patients with wt*KRAS*.[76] Another trial (BR.26), assessing dacomitinib versus placebo in patients pretreated with up to three-lines chemotherapy and a first-generation reversible EGFR/HER-TKI, showed similar OS regardless of *EGFR* mutation status, although improved OS was observed in wt*KRAS* and PFS that was significantly longer in dacomitinib group.[77]

In first-line setting, a Phase II study (NCT00818441) of dacomitinib in treatment-naive advanced NSCLC found an ORR of 73% and median PFS of 18.2 months in patients with act*EGFR*m (*EGFR*^{Del19} or *EGFR*^{L858R}; Table 2).[78] Based on these data, an ongoing randomized Phase III study (ARCHER 1050) has been designed to evaluate the efficacy of first-line dacomitinib versus gefitinib in locally advanced or metastatic NSCLC with act*EGFR*m.[74] The results (estimated study

Table 2 Treatment effectiveness and adverse events of second- and third-generation EGFR/HER TKIs

Drug/phase	Clinical trial	Patients selection	Case number	Dose	Treatment effectiveness	Adverse events (AE)
Afatinib/Phase II	Lux-Lung 2[64]	Lung adenocarcinoma with *EGFR* mutations	129 (99 with a starting dose of 50 mg; 30 with a starting dose of 40 mg)	50 mg or 40 mg daily	ORR: 61% of all 129 patients, 66% of the 106 patients with the two common act*EGFR*m; and 39% of 23 patients with less common mutations. mOS: 24.8 months (65 patients) mPFS: 13.7 months (96 patients)	Most common AE (grade 3): diarrhea 22% (50 mg) and 7% (40 mg); rash/acne 28% (50 mg) and 7% (40 mg) Serious AE: 14% (50 mg) and 7% (40 mg)
	Lux-Lung 4[73]	Advanced NSCLC who progressed during prior treatment with erlotinib, gefitinib or both	62	50 mg daily	ORR: 8.2% mPFS: 4.4 months mOS: 19.0 months	Most common AE: diarrhea 100% and rash/acne (91.9%)
	Lux-Lung 7[70]	Advanced NSCLC with common *EGFR* mutations	319 (160 to afatinib and 159 to gefitinib)	40 mg daily	Afatinib vs gefitinib: favors afatinib mPFS: 11.0 months vs 10.9 months; HR: 0.73 median time-to-treatment failure: 13.7 months vs 11.5 months; HR: 0.73	Afatinib vs gefitinib: most common AE (grade 3–4): diarrhea:13% vs 1%; rash/acne: 9% vs 3% Serious AE: 11% vs 4%
Afatinib/Phase III	Lux-Lung 1[72]	Advanced lung adenocarcinoma after failure of erlotinib, gefitinib or both, and one or two lines of chemotherapy	585 (390 to afatinib and 195 to placebo)	50 mg daily	Afatinib vs placebo: favors afatinib mOS: 10.8 months vs 12.0 months; HR: 1.08 mPFS: 3.3 months vs 1.1 months; HR 0.38	Most common AE (grade 3–4): diarrhea 17% and rash/acne 14% Serious AE: 10% (afatinib) vs <1% (placebo)
	Lux-Lung 3[65,66]	Stage IIIB/IV lung adenocarcinoma with *EGFR* mutations	345 (230 to afatinib and 115 to chemotherapy cisplatin/pemetrexed)	40 mg daily	Afatinib vs cisplatin/pemetrexed: favors afatinib mPFS: For all 345 patients: 11.1 months vs 6.9 months; HR: 0.58; for patients with common act*EGFR*m: 13.6 months vs 6.9 months; HR: 0.47	Most common AE (grade 3–4): rash/acne 16.2%, diarrhea 14.4%, and paronychia 11.4%
	Lux-Lung 6[67]	Asian patients with EGFR mutation-positive advanced NSCLC	364 (242 to afatinib and 122 to chemotherapy gemcitabine plus cisplatin)	40 mg daily	Afatinib vs gemcitabine plus cisplatin: favors afatinib mPFS: 11.0 months vs 5.6 months; HR: 0.28	Most common AE (grade 3–4): rash/acne 14.6%, diarrhea 5.4%, and stomatitis or mucositis 5.4% Serious AE: 6.3% (afatinib) vs 8.0% (gemcitabine plus cisplatin)
	Lux Lung 8[71]	Advanced squamous cell lung carcinoma	795 (398 to afatinib and 397 to erlotinib)	40 mg daily	Afatinib vs erlotinib: Favors afatinib mPFS: 2.6 months vs 1.9 months; HR: 0.81 mOS: 7.9 months vs 6.8 months; HR: 0.81 Disease control: 51% vs 40% Tumour shrinkage: 26% vs 23%	Afatinib vs erlotinib: Most common AE (grade 3–4): diarrhea 10% vs 2%; stomatitis 4% vs 0 and rash/acne 6% vs 10%

Dacomitinib/ Phase II	NCT00769067[75]	EGFR/HER-TKI-naive NSCLC patients pretreated with chemotherapy	188 (94 to dacomitinib and 94 to erlotinib)	45 mg daily	Dacomitinib vs erlotinib: Favors dacomitinib mPFS: 2.86 months vs 1.91 months; HR: 0.66 mOS: 9.53 months vs 7.44 months; HR: 0.80	Dacomitinib vs erlotinib: Most common AE (grade 3–4): diarrhea: 11.8% vs 4.3%; dermatitis acneiform: 10.8% vs 6.4%
	NCT00818441[78]	Treatment-naive advanced NSCLC	89	45 mg or 30 mg daily	PFS at 4 months: 76.8% in all population and 95.5% in the EGFR-mutation population ORR: 75.6% in the EGFR-mutation population	Most common AE (grade 3–4): diarrhea 15% and dermatitis acneiform 18%
Dacomitinib/ Phase III	ARCHER 1009[76]	Advanced NSCLC who had disease progression after one or two chemotherapy regimens	878 (439 to dacomitinib and 439 to erlotinib)	45 mg daily	Dacomitinib vs erlotinib: mPFS: 2.6 months vs 2.6 months; HR: 0.941 mOS: 7.9 months vs 8.4 months; HR: 1.08	Dacomitinib vs erlotinib: Most common AE (grade 3–4): diarrhea: 11% vs 2%; rash: 7% vs 3%; stomatitis: 3% vs $<$1% Serious AE: 12% vs 9%
	BR.26[77]	Advanced NSCLC pretreated with up to three-lines chemotherapy and a first-generation EGFR TKI	720 (480 to dacomitinib and 240 to placebo)	45 mg daily	Dacomitinib vs placebo: mPFS: 2.66 months vs 1.38 months; HR: 0.66 ORR: 7% vs 1% mOS: 6.83 months vs 6.31 months; HR: 1.00	Dacomitinib vs placebo: Most common AE (grade 3–4): diarrhea: 12% vs 0%; acneiform rash: 10% vs $<$1%; oral mucositis: 3% vs 0%
Neratinib/ Phase II	Clinical trial[30]	Advanced NSCLC	137	320 mg or 240 mg daily	ORR: 3% in patients with act*EGFR*m and 0% in patients with $EGFR^{T790M}$ and wt *EGFR*.	Most common AE (grade 3–4): diarrhea; 50% (320 mg) and 25% (240 mg)
Osimertinib/ Phase II	AURA2[47]	Advanced NSCLC with act*EGFR*m who had progressed after prior EGFR TKI therapy and had $EGFR^{T790M}$ mutation	210		ORR: 64% Disease control rate: 90%	Most common AE (all grades): diarrhea 34% and rash 40%.
Rociletinib/ Phase I/II	NCT01526928[32]	EGFR-mutated NSCLC who had disease progression during previous treatment with EGFR TKI	130	500, 625, or 750 mg twice daily	$EGFR^{T790M+}$ vs $EGFR^{T790M-}$: ORR: 59% vs 29% Disease control rate: 93% vs 59% mPFS: 13.1 months vs 5.6 months	Most common AE (grade 3): hyperglycemia 22%, nausea 2%, and fatigue 4%
HM61713/ Phase II	NCT01588145[35]	NSCLC with act*EGFR*m who had progressed on prior EGFR TKI therapy		650 mg daily	ORR: 58.8% in $EGFR^{T790M+}$ patients Disease control rate: 97.1% in $EGFR^{T790M+}$ patients	

Abbreviations: AE, adverse events; HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; ORR, objective response rate; EGFR/HER-TKIs, human epidermal growth factor receptor tyrosine kinase inhibitors; wt, wild type.

completion in 2017) may demonstrate a more substantial role for dacomitinib in the earlier setting.

Neratinib

Neratinib (Figure 1) is an irreversible EGFR/HER-TKI targeting EGFR, HER2, and HER4, which was found to be more effective at suppressing cell proliferation than gefitinib in lung cancer cells with both act*EGFR*m and *EGFR*^{T790M} in preclinical studies.[28,79] However, due to the limitations of clinical dosing, neratinib had modest clinical efficacy in a Phase II study for TKI-resistant and TKI-naive patients, showing only 3% ORR in mutated-*EGFR* arm and no response in patients with *EGFR*^{T790M} or wt*EGFR* (Table 2).[30]

In summary for the second-generation EGFR/HER-TKIs, in addition to inhibiting the two most common act*EGFR*m (*EGFR*^{L858R} and *EGFR*^{Del19}), these irreversible EGFR/HER-TKIs (afatinib, dacomitinib, and neratinib) demonstrated inhibitory activities in cells/tumors harboring *EGFR*^{T790M} in preclinical models.[80] However, they were most sensitive in patients with act*EGFR*m and were not clinically active in overcoming *EGFR*^{T790M} resistance, perhaps because of dose-limiting toxicity (narrow therapeutic window, such as the crucial gastrointestinal [diarrhea and mucositis] and dermatologic toxicity) due to simultaneous inhibition against wt*EGFR*.[66,67,72,73] Furthermore, the acquired resistance to these agents can develop due to *EGFR*^{T790M} amplification.[81,82]

In view of the low probability of drug–drug interactions, multiple treatment strategies have been clinically used, including the combination of EGFR/HER-TKI with chemotherapy or other targeted therapy.[20] The Phase III Lux-lung 5 study,[83] evaluating the efficacy and safety of continued irreversible HER-family blockade with afatinib plus paclitaxel versus investigator's choice of chemotherapy alone in patients with relapsed/refractory NSCLC following chemotherapy and acquired resistance to prior erlotinib/gefitinib and afatinib monotherapy, demonstrated that afatinib plus paclitaxel significantly improved PFS (5.6 months versus 2.8 months) and ORR (32.1% versus 13.2%) compared with single-agent chemotherapy. Another regimen that showed interesting clinical activity and a manageable safety profile is the combination of afatinib and cetuximab (anti-EGFR monoclonal antibody), which induced a PFS of 4.7 months and an ORR of 29% with median duration of response of 5.7 months in a small Phase IB trial for heavily pretreated patients with act*EGFR*m lung cancer and acquired resistance to erlotinib/gefitinib.[84] This study indicated that the dual vertical blockade of EGFR (in the intracellular domain of the HER-family members with afatinib and the extracellular domain of EGFR with cetuximab) was effective regardless of the *EGFR*^{T790M} status (ORR: 32% for *EGFR*^{T790M}-positive and 25% for *EGFR*^{T790M}-negative tumors, P=0.341). These combination studies on patients with acquired resistance to TKIs supported a focus upon rechallenging with EGFR/HER-TKI beyond disease progression in oncogene-addicted lung cancer, indicating that some tumor cells may remain dependent on HER signaling, due to either the type of acquired *EGFR* mutations, *EGFR* amplification, and/or *HER2* amplification.[84] However, current targeted therapeutic strategies for patients with *EGFR*^{T790M} are limited, and no approved treatment options are available.

This has led to the development of third-generation EGFR-TKIs that are designed to more effectively and selectively target *EGFR*^{T790M} and act*EGFR*m, while sparing the activity to wt*EGFR*.

Third-generation EGFR/HER-TKIs

Considering that the *EGFR*^{T790M} mutation represents the most dominant acquired resistance to first- and second-generation EGFR/HER-TKI therapy, specific drugs to target this mutation are recently under clinical development and may bring a breakthrough for the treatment of NSCLC patients. The third-generation EGFR/HER-TKIs (Table 1 and Figure 1), such as osimertinib (AZD9291; AstraZeneca plc), rociletinib (CO-1686; Clovis Oncology Inc., Boulder, Colorado, USA), HM61713 (Hanmi Pharmaceutical, Songpa-gu, Seoul, Korea), EGF816 (Novartis International AG, Basel, Switzerland), and ASP8273 (Astellas Pharma Inc.), selectively and irreversibly target *EGFR*^{T790M} and act*EGFR*m, and have the reduced affinity to wt*EGFR*.[52,85] Clinically, they have yielded promising results of favorable benefit for the act*EGFR*m patients who had disease progression (especially with *EGFR*^{T790M} mutation) following first-/second-generation-TKIs treatment, as well as showed very low inhibitory effect on wt*EGFR*, thus overcoming the toxicity limitation seen with earlier generation EGFR/HER-TKIs.[86–89] Unlike previous generation inhibitors, most adverse effects, such as diarrhea, rash, and nausea, were mild (grades 1 and 2). There were no DLTs at any dose level, and maximal tolerated dose was not defined.

Osimertinib

Osimertinib, a mono-anillino-pyrimidine compound (Figure 1), is a double-mutant selective third-generation irreversible EGFR/HER-TKI.[90] In cell culture and mouse models,[25] it potently inhibited signaling pathways and cellular growth in cells/tumors with both act*EGFR*m (*EGFR*^{Del19} and *EGFR*^{L858R}) and *EGFR*^{T790M}, with 200-fold less activity in

inhibiting wt*EGFR*, thus having the increased selectivity margin against wt*EGFR*.

As a second-line (or later) treatment, a global Phase I study (AURA)[31] demonstrated that osimertinib was highly active in lung cancer patients with the *EGFR*^{T790M} mutation who had disease progression during prior therapy with EGFR/HER-TKIs, showing a higher ORR (*EGFR*^{T790M} positive: 61%; *EGFR*^{T790M} negative: 21%) and longer median PFS (9.6 months versus 2.8 months, respectively) with low incidence of toxicity. AURA2, a global Phase II single-arm study[47] for patients with act*EGFR*m who had progressed after prior EGFR/HER-TKI therapy and had *EGFR*^{T790M} mutation, also showed antitumor efficacy of osimertinib suggesting it can overcome *EGFR*^{T790M}-mediated acquired resistance. The ORR was 64%, disease control rate (DCR) was 90%, and PFS was not reached (Table 2). In November 2015, osimertinib was granted accelerated approval by FDA for the treatment of metastatic *EGFR*^{T790M}-positive NSCLC who have progressed on or after EGFR/HER-TKI therapy.[91,92] In the same population, a Phase III AURA3 trial is ongoing to compare osimertinib monotherapy with pemetrexed plus platinum chemotherapy.

In a first-line expansion cohort of AURA trial for treatment-naive advanced NSCLC with act*EGFR*m, osimertinib achieved promising anticancer activity with an ORR of 70%, DCR of 97%, and PFS at 3 months and 6 months of 93% and 87%, respectively.[93] The ongoing Phase III FLAURA trial will assess the efficacy and safety of osimertinib versus gefitinib/erlotinib as the first-line treatment in patients with advanced NSCLC act*EGFR*m.

Rociletinib

Rociletinib, an oral 2,4-disubstituted pyrimidine covalent EGFR/HER-TKI (Figure 1), is a highly selective and irreversible inhibitor of both act*EGFR*m- and *EGFR*^{T790M}-resistance mutation. In a preclinical study, rociletinib demonstrated a significant growth inhibitory effect toward *EGFR*^{T790M} and act*EGFR*m cells/tumors with significantly less activity on wt*EGFR* in cell lines, xenograft, and transgenic mouse models.[33]

In a Phase I/II trial (TIGER X)[32] for 130 patients with act*EGFR*m and acquired resistance to EGFR/HER-TKIs, rociletinib showed promising activity for *EGFR*^{T790M}-positive patients, and to a lesser extent, to *EGFR*^{T790M}-negative populations (ORR was 59% versus 29% for patients with *EGFR*^{T790M} positive versus *EGFR*^{T790M} negative, respectively, DCR was 93% versus 59%, and PFS was 13.1 months versus 5.6 months; Table 2). The modes of action against *EGFR*^{T790M}-negative patients may include tumor heterogeneity, sensitivity of genotyping platform used, and activity against other resistant mechanisms.[47] Rociletinib had infrequent EGFR-related toxicity (due to the low affinity to wt*EGFR*) but had a tendency to other concerning safety issues such as hyperglycemia and long QT interval, because of a rociletinib metabolite (M502), which inhibited insulin-like growth factor receptor-1.[32] In May 2014, rociletinib was granted breakthrough therapy designation for NSCLC patients with *EGFR*^{T790M} after progression on a prior TKI. This has allowed investigation in several TIGER trials in various treatment lines, such as the randomized Phase II/III TIGER 1 study, comparing rociletinib versus erlotinib as the first-line monotherapy for advanced act*EGFR*m NSCLC regardless of *EGFR*^{T790M} status; the confirmatory Phase II single-arm TIGER 2 trial for advanced *EGFR*-mutated NSCLC progressed after previous EGFR/HER-TKI therapy and harboring *EGFR*^{T790M}; the randomized Phase III TIGER 3 trial, evaluating rociletinib versus platinum doublet chemotherapy for second-line treatment for patients with act*EGFR*m and *EGFR*^{T790M} after EGFR/HER-TKI failure.[85,87,88,94]

HM61713

HM61713 is another novel, oral mutant-selective inhibitor of act*EGFR*m and *EGFR*^{T790M}, but not wt*EGFR*, which demonstrated good efficacy in animal models, especially those with concurrent act*EGFR*m and *EGFR*^{T790M} mutations.[34] An open-label Phase I/II trial (NCT01588145) in Korea with act*EGFR*m NSCLC patients who had progressed on prior EGFR/HER-TKI therapy demonstrated promising antitumor activity of HM61713 (especially with *EGFR*T^{790M} mutation), showing an ORR of 21.7% and DCR of 67.5% in unselected population, an ORR of 29% and DCR of 75% in *EGFR*^{T790M}-positive patients, and an ORR of 12% in *EGFR*^{T790M}-negative group.[88,95] The Phase II cohort (Table 2) showed an ORR of 58.8% and DCR of 97.1% in patients with centrally confirmed *EGFR*^{T790M} who received HM61713 at a dose >650 mg/day, which also indicated that HM61713 had an encouraging clinical efficacy to overcome the EGFR/HER-TKI resistance.[35] HM61713 caused mild side effects and can be controlled easily. A Phase II trial is currently enrolling patients with act*EGFR*m treated with first-line HM61713.

Several other third-generation mutant-EGFR specific TKIs are currently being investigated in NSCLC patients with act*EGFR*m and *EGFR*^{T790M}, such as EGF816, ASP8273, AP26113, and poziotinib.[96–98] In addition, the activity of third-generation EGFR/HER-TKIs has been further investigated

in patients with central nervous system (CNS) metastases. The preliminary data[99,100] indicated that the response rate of these TKIs (such as osimertinib and rociletinib) was not affected by the history of CNS disease, showing that the ORR among patients with versus without CNS metastases was 56% versus 64%, respectively, for the treatment with osimertinib, and 58% versus 45%, respectively, for the treatment with rociletinib. In summary, because of highly mutant-selective targeting, the third-generation EGFR/HER-TKIs are potent for the treatment of prior EGFR/HER-TKI-resistant patients harboring the *EGFR*^{T790M} mutation, with probable lower EGFR treatment-related toxicity.

Although the efficacy of third-generation EGFR/HER-TKIs seems improved, responses are not durable, and disease progression still occurs. Possible mechanisms of acquired resistance have been described, including tertiary *EGFR* mutations (C797S, L844V, and L718Q), alternative/bypass signaling (increased RAS/RAF/ERK signaling by *NRAS*^{E63K0} mutation, *BRAF*^{V600E} mutation or increased MEK1 activity, *HER2* amplification, *MET* amplification, and *PIK3CA*^{E545K} mutation), and phenotypic alterations (epithelial–mesenchymal transition and small-cell lung cancer transformation).[4,47] Due to the diversity of resistance mechanisms, various therapeutic regimens are under investigation in preclinical and clinical settings. The medical model of combination therapy may be the main trend to enhance their effectiveness. Clinical trials of combination therapy using third-generation EGFR/HER-TKIs are in process, combining with selumetinib (MEK inhibitor), savolitinib (AZD6094 and MET-TKI), necitumumab (anti-EGFR antibody), or navitoclax (inhibitor of Bcl-xl, Bcl-2, and Bcl-w).[89]

Conclusion

By virtue of the extensive investigation and better understanding of the EGFR/HER-family signaling pathway, NSCLC diagnosis and treatment has evolved dramatically from the traditional one-size-fits-all chemotherapeutic approach to the new personalized molecular target therapy. The application of the first-generation EGFR/HER-TKIs in selected NSCLC patients with act*EGFR*m (*EGFR*^{L858R} and *EGFR*^{Del19}) showed a significant superiority over the standard chemotherapy in terms of safety, efficacy, and quality of life. The second-generation EGFR/HER-TKIs similarly improved act*EGFR*m patients OS but failed to overcome the acquired *EGFR*^{T790M} resistance. The third-generation EGFR/HER-TKIs selectively and irreversibly targeted *EGFR*^{T790M} mutation and act*EGFR*m and were sparing to wt*EGFR*. They seem efficacious for TKI-resistant patients with *EGFR*^{T790M} mutations. They have a lower incidence of toxicity due to the less inhibitory effect on wt*EGFR*. Currently, two first-generation EGFR/HER-TKIs gefitinib and erlotinib and one second-generation EGFR/HER-TKI afatinib have been used as the first-line treatment of metastatic NSCLC with act*EGFR*m (*EGFR*^{Del19} or *EGFR*^{L858R}). Recently, the third-generation EGFR/HER TKIs osimertinib and rociletinib have been granted accelerated approval and breakthrough therapy designation, respectively, by the FDA for patients with metastatic *EGFR*^{T790M} mutation-positive NSCLC. Their true place awaits definitive randomized trials.

Correct selection and use of these EGFR/HER-TKIs are mainly dependent upon identification of *EGFR* primary and secondary mutations. The pretreatment detection of *EGFR* mutations as predictive biomarkers maximizes the therapeutic index of personalized targeted therapy in lung cancer. Consequently, examining the genetic alterations is recommended for all new diagnosed NSCLC patients and when they experience disease progression, for better selection of the specific candidates for the targeted therapy. In addition, the rebiopsy will provide the genetic mechanisms for the development of acquired resistance and ultimately guide researchers to design the next generations of EGFR/HER-TKIs and strategies for cancer treatment.

Disclosure

The authors report no conflicts of interest in this work.

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