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Tyrosine kinase inhibitors for epidermal growth factor receptor gene mutation–positive non-small cell lung cancers: an update for recent advances in therapeutics

Clement Chung

Abstract

The presence of activating gene mutations in the epidermal growth factor receptor of non-small cell lung cancer patients is predictive (improved progression-free survival and improved response rate) when treated with small molecule tyrosine kinase inhibitors such as gefitinib, erlotinib and afatinib. The two most common mutations that account for greater than 85% of all *EGFR* gene mutations are in-frame deletions in exon 19 (LREA deletions) and substitution in exon 21 (*L858R*). Exon 18 mutations occur much less frequently at about 4% of all *EGFR* gene mutations. Together, exon 19 deletion and exon 21 *L858R* gene substitution are present in about 10% of Caucasian patients and 20–40% of Asian patients with non-small cell lung cancer. *T790M* gene mutation at exon 20 is associated with acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors. Early studies showed that activating *EGFR* gene mutations are most common in patients with adenocarcinoma histology, women, never smokers and those of Asian ethnicity. A recent multi-center phase III trial suggested that frontline epidermal growth factor receptor tyrosine kinase inhibitor therapy with afatinib is associated with improved progression-free survival compared to chemotherapy regardless of race. Moreover, guidelines now suggest *EGFR* gene mutation testing should be conducted in all patients with lung adenocarcinoma or mixed lung cancers with an adenocarcinoma component, regardless of characteristics such as smoking status, gender or race. The success of targeted therapies in non-small cell lung cancer patients has changed the treatment paradigm in metastatic non-small cell lung cancer. However, despite a durable response of greater than a year, resistance to epidermal growth factor receptor tyrosine kinase inhibitors inevitably occurs. This mini-review describes the clinically relevant *EGFR* gene mutations and the efficacy/toxicity of small molecule epidermal growth factor receptor tyrosine kinase inhibitors as targeted therapies for these gene mutations. Therapeutic strategies to overcome resistance, including emerging and novel therapies, are discussed.

Keywords

Epidermal growth factor receptor tyrosine kinase inhibitor, exon 19 deletion, exon 21 L858R gene substitution, acquired resistance, novel, therapy

Lung cancer is a leading cause of cancer death in the US. In 2014, an estimated 16,000 deaths are expected to occur because of the disease.[1] Lung cancer is usually diagnosed at an advanced stage and because of this, the overall 5-year survival is only 15%.[2] Primary tumor in clinical stages I to II is considered resectable. Treatment option generally consists of surgery with or without adjuvant chemotherapy.[3] Tumor in stage III is generally considered unresectable and the treatment option is chemoradiation,[4] whereas for stage IV, treatment options include chemotherapy or oral targeted therapies.[5] Chemotherapy typically consists of a platinum-based doublet therapy (i.e. cisplatin or carboplatin combined with agents such as gemcitabine, vinorelbine

Hematology/Oncology Clinical Pharmacist Specialist, Lyndon B Johnson General Hospital, Harris Health System, Houston, TX, USA.

Corresponding author:
Clement Chung, Hematology/Oncology Clinical Pharmacist Specialist, Lyndon B Johnson General Hospital, Harris Health System, Houston, TX, USA.
Email: clement_t_chung@yahoo.com

or taxanes and most recently, cisplatin or carboplatin with pemetrexed for non-squamous lung cancer). No regimen has proven superiority over another.[6] Non-small cell lung cancers (NSCLC) account for about 85% of all lung cancers and they can be squamous (epithermoid) or non-squamous (including adenocarcinoma, large cell and other subtypes). Adenocarcinoma is the most common lung cancer type in the US and in non-smokers.[2]

EGFR signaling pathway

First described in 1962,[7] epidermal growth factor receptor (EGFR; also known as HER1) is a 170-kDa transmembrane receptor tyrosine kinase (RTK) with an extracellular ligand-binding domain, a lipophilic transmembrane region and an intracellular regulatory domain with tyrosine kinase activity.[8] EGFR is found on the surface of epithelial cells and is often overexpressed in many malignancies.[9,10] In addition, somatic gene mutations in the intracellular kinase domain of the EGFR lead to ligand-independent activation of the signaling pathway, which causes constitutive activation of the tyrosine kinase that results in tumorigenesis.[11] In normal cells, the EGFR pathway is tightly regulated whereas loss of regulation leads to uncontrolled growth and oncogenesis.[12]

EGFR is a member of a family composed of four RTKs, EGFR (ERB-B1 or HER1), ERB-B2 (HER2/Neu), ERB-B3 (HER3) and ERB-B4 (HER4).[13] Multiple ligands activate different family members of EGFR. Ligand binding enables homo- or heterodimerization that results in intracellular tyrosine kinase domain activation and phosphorylation. This in turn creates docking sites for a diverse set of cytoplasmic signaling molecules and results in the activation of two key intracellular signaling pathways: the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathways. When stimulated, RAS protein, which is the first part of the MAPK pathway, exchanges GDP for GTP and sequentially activates RAF, followed by mitogen-activated extracellular signal-regulated kinase (MEK) and MAPK. Alternatively, ligand-bound EGFR can translocate PI3K to the cell membrane and activate AKT and other downstream molecules.[14] Tumor cells can upregulate the EGFR pathway through mechanisms such as EGFR overexpression, *EGFR* gene amplification, activating (also known as sensitizing) mutations of the receptor or any downstream proto-oncogene (e.g. *RAS*, *RAF*) that results in the constitutive activation of the pathway, leading to tumor growth and proliferation.[15,16]

In their normal (non-mutated) state, both *EGFR* and *K-RAS* are the so-called proto-oncogenes that regulate signal transduction, cell growth and other cellular processes. When mutated, they become hyperactivated and are involved in tumorigenesis. In fact, *EGFR* and *K-RAS* genes are two most common proto-oncogenes in lung adenocarcinoma. Of note, *K-RAS* gene mutations, which occur in about 30% of patients with lung adenocarcinoma and in smokers, are associated with resistance to EGFR tyrosine kinase inhibitors (TKI).[17] *EGFR* gene mutations are not found in tumors with *K-RAS* gene mutations.

EGFR gene mutations, observed in about 10% of Caucasians and 30–50% of Asians, first described in 2004,[18,19] now shifted the paradigm from using EGFR TKI agents initially in molecularly unselected patients[10–13] (patients who were not tested for *EGFR* gene mutations but had pathologic/clinical characteristics that are often associated with increased frequencies of *EGFR* gene mutations such as adenocarcinoma histology [40% vs. 3% in other histologies], East Asian descent [30% vs. 8% in non-Asians], female gender [42% vs. 14% in male patients] and never-smoking status [51% vs. 10% in current or ever-smokers]) to patients molecularly selected with *EGFR* gene mutations.

Activating or sensitizing mutations of the *EGFR* gene

In NSCLC patients, the most commonly found *EGFR* gene mutations (that account for more than 90% of all *EGFR* gene mutations) are present in the first four exons (i.e. exon 18–21) of the gene on chromosome 7 encoding for the tyrosine kinase domain which binds to the substrate ATP. First-generation EGFR TKIs such as erlotinib and gefitinib competitively prevent the binding of ATP to the intracellular kinase domain. Because these gene mutations result in the hyperactivation of the EGFR signaling pathway and they are also sensitive to targeted therapies with EGFR TKI, they are also termed sensitizing mutations. Second-generation EGFR TKI such as afatinib binds to both EGFR and HER2 through covalent bonds, resulting in irreversible and sustained target blockade. Third-generation EGFR TKI (currently all are investigational agents) circumvent the acquired resistance of tumors to the first and second-generation TKI.

The most common *EGFR* gene mutations (see Figure 1) are

(1) Exon 19 deletion (i.e. in-frame conserved deletions that encompass four amino acids on codons 747–750 or the "LREA" region) that occurs in 45% of patients with *EGFR* gene mutations. These four

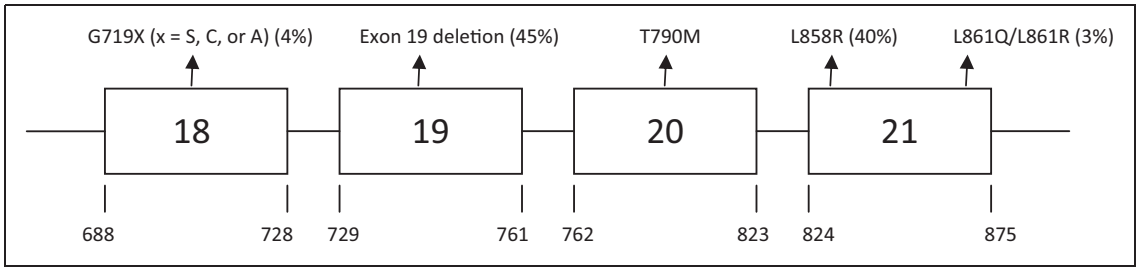

Figure 1. Frequency of gene mutations in exons 18–21 of the *EGFR* gene. The *EGFR* gene is located in the short arm of chromosome 7. It contains 28 exons. Exons 18–21 in the cytoplasmic tyrosine kinase domain of the EGFR receptor are commonly associated with sensitivity or resistance to EGFR TKIs when these genes are mutated.
The most prevalent *EGFR* gene mutations are Exon 19 deletion (45%), followed by the *L858R* mutation in exon 21 (40%). Exon 18 mutations (G719 S/C/A) account for approximately 4% of the overall gene mutations.
All gene mutations except T790M are associated with sensitivity and hence they are predictive biomarkers for response to EGFR TKIs. On the contrary, *T790M* gene mutation accounts for approximately 1% of primary resistance to EGFR TKIs. Another primary resistance to EGFR TKIs is due to insertions in exon 20 (about 4% of all gene mutations, not shown in diagram). The numbers below the vertical bar of each box refers to the amino acid number of each exon. G: Glycine; S: serine; C: cysteine; A: alanine; T: threonine; M: methionine; L: leucine; R: arginine; Q: glutamine.

amino acids are leucine (L), arginine (R), glutamic acid (E) and alanine (A).[16]

(2) Exon 21 *L858R* gene substitution (a mis-sense mutation that results in the substitution of leucine with arginine at codon position 858) that occurs in another 40% of patients with *EGFR* gene mutations.[17]

(3) Exon 18 *G719X* gene mutation (a mis-sense mutation that results in the substitution of glycine with cysteine, alanine or serine at codon position 719) that occurs in about 4% of all *EGFR* gene mutated patients. Other less-frequent drug-sensitizing gene mutations may include point mutations at exon 21.[18]

Resistance to small molecule EGFR TKI can be either primary or acquired. Patients with primary resistance are refractory to upfront TKI treatment, whereas acquired or secondary resistance occurs after an initial response to the frontline TKI therapy. Common acquired resistance mechanisms to EGFR TKIs are *T790M* (60% of cases with acquired resistance) mutation,[19] transformation of the NSCLC to small cell lung cancer (1–3% of cases with acquired resistance) and the mesenchymal-epidermal transition (MET) receptor overexpression or gene amplification (5–20% of cases with acquired resistance).[19] *MET* is a proto-oncogene that encodes a transmembrane tyrosine kinase receptor which binds to a ligand called the hepatocyte growth factor (HGF). The ligand-bound receptor induces receptor dimerization, phosphorylation and PI3K activation, resulting in persistent activation of the downstream pathway that overcomes the inhibition by EGFR TKI.[20] Amplification of the *MET* gene is involved in the invasion, metastasis and angiogenesis of tumors.[21]

In addition, less common somatic gene mutations, such as *HER2*[22], *HER4*[23], *BRAF*[24] and *PIK3CA*[25] are also found in the EGFR signaling pathway. Other receptor tyrosine kinases such as AXL[26] are also implicated in the acquired resistance to EGFR TKI. Taken together, these rare gene mutations activate the EGFR signaling pathway and promote EGFR-mediated pro-survival and anti-apoptotic signals through the downstream targets. However, whether these gene mutations represent predictive biomarkers of interest and promising therapeutic targets in patients with *EGFR*-mutation positive NSCLC remain an area of ongoing research. It is possible that future targeted therapies can take advantage of these additional gene mutations in the EGFR signaling pathway. However, currently there is no US Food and Drug Administration (FDA)-approved targeted therapies for these rare gene mutations in the EGFR signaling pathway for NSCLC.

To summarize, both exon 19 gene deletions and exon 21 *L858R* gene substitutions result in the activation of the tyrosine kinase domain. These mutations are associated with sensitivity (i.e. sensitizing mutations) to small molecule EGFR TKI, but these mutations seldom occur simultaneously. Despite the high response rate and prolonged progression-free survival (PFS) in patients with *EGFR* gene mutations treated with first-generation EGFR TKI, about 50% of these lung adenocarcinoma patients will develop the acquired *T790M gene* mutation,[19] a secondary point mutation (developed after initial therapy with TKIs) located at exon 20 that results in substitution of methionine (T)

for threonine (M) at codon position 790. The presence of a de novo *T790M* mutation (i.e. primary resistance to first-line EGFR TKI therapy) is predictive for poor survival outcome associated with all first-generation EGFR TKIs.[17–19] In other words, the *T790M* substitution is a "gatekeeper" mutation that interferes with drug–target interaction, resulting in the failure of EGFR TKIs to compete for ATP binding at the transmembrane receptor.[19]

EGFR TKI therapy and molecular selection of patients

Current FDA-approved EGFR TKIs for advanced NSCLC patients with activating or sensitizing *EGFR* gene mutations include gefitinib (Iressa ®, AstraZeneca Inc.), erlotinib (Tarceva ®, Genentech, US) and afatinib (Gilotrif ®, Boehringer Ingelheim, US). The comparison of each EGFR TKI in terms of FDA-approved indications, adverse effects, drug/food interactions is summarized in Table 1.

According to current US National Comprehensive Cancer Network (NCCN) guidelines,[27] first-line treatment for advanced or metastatic non-squamous NSCLC in patients with Eastern Cooperative Oncology Group (ECOG) performance status 0–1 (0 = asymptomatic; 1 = symptomatic but completely ambulatory) and negative or unknown *EGFR* gene mutation is a platinum-based two-drug combination regimen. On the other hand, in non-squamous NSCLC patients who have known or documented activating (or sensitizing) *EGFR* gene mutations, they benefit from first-line EGFR TKI therapy rather than chemotherapy.

Historically, high levels of *EGFR* gene expression were initially observed in metastatic NSCLC across all histology types and provided the initial impetus for early lung cancer trials targeting the EGFR pathway.[13,15–18] *EGFR* gene mutations were subsequently identified in selected patients after clinical benefit to EGFR TKI was observed in 2004.[11,28] Evaluation of tumor specimens in these patients led to the identification of two common mutations in the *EGFR* gene, namely the exon 19 deletion and the exon 21 *L858R* substitution, both of which can be readily targeted by first-generation reversible EGFR TKI such as gefitinib and erlotinib and second-generation TKI such as afatinib.[28–30] Of important note, these early trials (see Table 2) were not powered to demonstrate improvement in overall survival in molecularly unselected patients with advanced NSCLC randomly assigned for chemotherapy plus TKI or chemotherapy. Overall survival was similar to standard platinum-based chemotherapy doublet.

In absence of patient selection for *EGFR* gene mutations (e.g. when genetic testing is not performed or not available at time of therapy initiation), it is hypothesized that EGFR TKI can still be offered to unselected patients as front-line therapy (and even second or third line of therapy) for advanced NSCLC, with no adverse effect on the outcome of overall survival. To date, however, data[31,32] seem to indicate that EGFR TKI cannot be used as frontline therapy over chemotherapy in molecularly unselected patients. More studies are needed to evaluate whether EGFR TKI can be preferentially given to unselected patients with advanced NSCLC that are not fit for chemotherapy without a negative impact on overall survival.

Erlotinib is the current EGFR TKI agent of choice in US for patients with sensitizing *EGFR* gene mutations because of the restricted access of gefitinib. Of note, although gefitinib was shown to delay disease progression over placebo in the second and third-line settings (3.0 vs. 2.6 months, $p = 0.0006$) in two phase II trials, IDEAL-1[29] and IDEAL-2,[33] the lack of overall survival benefit (5.6 vs. 5.1 months, $p = 0.087$) in the confirmatory phase III ISEL trial[34] prompted the FDA's withdrawal of gefitinib's accelerated approval. Gefitinib is now restricted to patients already on this medication and continue to benefit from it (enrollment through the Iressa ® Access program) whereas in Europe, both gefitinib and erlotinib are approved for patients with locally advanced and metastatic NSCLC with activating *EGFR* mutations. In US, no new patients can be initiated with gefitinib unless they are enrolled in clinical trials.

Afatinib is a newly FDA-approved second-generation reversible oral TKI agent that inhibits EGFR (HER1), HER2 and HER4 (HER3 has no intrinsic tyrosine kinase activity). It is FDA approved for the first-line treatment of metastatic non-squamous NSCLC patients with sensitizing *EGFR* mutations. It had been evaluated as a first-line agent in patients with *EGFR* gene mutations in LUX-Lung 2,[35] 3,[30] 6,[36] and 7[37] trials (see Table 3). LUX-Lung 8[38] is a recently completed phase III randomized trial that compared head to-head afatinib with erlotinib, in advanced squamous cell lung cancer patients. Moreover, afatinib was evaluated as a second- or third-line agent in patients who had previously been treated with other EGFR TKIs (LUX-Lung 1,[39] 4[40] and 5[41] trials). In fact, the efficacy of afatinib in overcoming acquired resistance to first-generation TKI such as gefitinb or erlotinib was confirmed in the LUX-Lung 1, 4 and 5 trials. The treatment setting and molecular selection for NSCLC patients of the LUX-Lung trials are summarized in Table 3.

Preliminary results from the ongoing LUX-Lung 5 trial[41] showed an improvement in PFS when continuing treatment with afatinib in combination with chemotherapy after disease progression with afatinib. Tumor

Table 1. Summary of current FDA-approved small molecule tyrosine kinase inhibitors (TKIs) against mutation-positive epidermal growth factor receptor (EGFR) in advanced or metastatic non-small cell lung cancers.

EGFR TKI/dosing	FDA-approved indications	Interaction with PPI or H2A	Drug-food interaction	Adverse effects (all grades)	Hepatic or renal adjustment
Gefitinib 250 mg p.o. qday	First-line therapy in metastatic NSCLC with EGFR exon 19 deletions or exon 21 (*L858R*) substitution mutations US patients must be enrolled in IRESSA program	PPI or H2A: may decrease serum conc. of gefitinib. Monitor therapy	Give with or without food	Dermatologic (including pustular rash, dry skin, paronychia): 58% Diarrhea: 35–47% Fever: 9% Ocular: 7%	No adjustment needed
Erlotinib 150 mg p.o. daily	First-line therapy in metastatic NSCLC with EGFR exon 19 deletions or exon 21 *(L858R)* substitution mutations. Maintenance therapy in metastatic NSCLC after four cycles of platinum-based first-line chemotherapy Second- or third-line therapy in metastatic NSCLC	H2A: May decrease the serum conc. of erlotinib Avoid H2A concurrently in pts receiving erlotinib. Administer erlotinib 10 h after the H2A and at least 2 h prior to next dose of H2A PPI: May decrease the serum conc. of erlotinib, avoid PPI	Give without food Avoid concomitant PPI	Skin rash: 49%–85% Paronychia: 4%–16% Diarrhea: 20%–62% Fever: $\leq$11% Weakness: $\leq$53%, Back pain: 19%, Arthralgia: $\leq$13% Musculoskeletal pain: 11% Conjunctivitis: 12%–18% Keratoconjunctivitis sicca: 12%	No information If total bilirubin >3 times ULN and/or transaminases >5 times ULN during use: consider discontinuing
Afatinib 40 mg p.o. daily	First-line therapy for patients who have metastatic NSCLC tumors with EGFR exon 19 deletions or exon 21 *L858R* substitution mutations	No information for H2A or PPI	Give without food	Acneiform eruption: 90% Paronychia: 58% xeroderma: 31% pruritus: 21% Conjunctivitis: 11% Fever:12%	CrCl > 60 mL/min: dose adjustment not necessary CrCl < 60 mL/min: caution and adjust if necessary. Withhold therapy for $\geq$grade 3 hepatic dysfunction Child-Pugh class A or B: no dosage adjustment

Conc: concentration; CrCl: creatinine clearance; H2A: H2 antagonist; PPI: proton pump inhibitor; pts: patients; ULN: upper limit normal.

Table 2. Summary of major clinical trials to test clinical efficacy of chemotherapy and EGFR TKIs in different treatment sequences in advanced NSCLC patients not molecularly selected for *EGFR* gene mutations.

Trial	Dosing schedule/clinical efficacy	Adverse effects
Chemotherapy + EGFR TKI		
INTACT I[52] (Iressa NSCLC Trial Assessing Combination Treatment) I Phase III randomized, double-blind, placebo-controlled, multicenter trial n = 1093 Chemotherapy-naive patients with unresectable stage III or IV NSCLC End points included OS (primary), TTP, RR and safety evaluation	Up to six cycles of cisplatin 80 mg/m^2 i.v. on day 1 and gemcitabine 1250 mg/m^2 i.v. on day 1 and 8 q3 weeks plus either gefitinib 500 mg p.o. daily, gefitinib 250 mg p.o. daily or placebo Daily gefitinib or placebo continued until disease progression No difference in efficacy end points between the treatment groups (gefitinib 500 mg p.o. daily, gefitinib 250 mg p.o. daily and placebo, respectively) Median survival times were 9.9, 9.9 and 10.9 months, respectively Median TTP: 5.5, 5.8 and 6.0 months, respectively RR: 49.7%, 50.3%, and 44.8% respectively	No significant unexpected adverse events were seen
INTACT II[53] Phase III, randomized, placebo-controlled, double-blind trial in chemotherapy-naive patients with advanced NSCLC n = 1037 End points included OS, TTP, response rate, and safety evaluation	Patients received paclitaxel 225 mg/m^2 i.v. and carboplatin AUC 6 q 3 wks plus gefitinib 500 mg p.o. daily, gefitinib 250 mg p.o. daily or placebo. After a maximum of 6 cycles, gefitinib or placebo continued until disease progression No difference in OS (median, 8.7, 9.8 and 9.9 months for gefitinib 500 mg p.o. daily, 250 mg p.o. daily and placebo respectively), TTP or RR between arms	Dose-related diarrhea and skin toxicity in gefitinib-treated pts No significant/unexpected safety findings from combination with chemotherapy
TRIBUTE[54] Phase III, randomized, double-blind, multicenter trial in previously untreated patients with advanced NSCLC n = 1059	Pts received either erlotinib or placebo in combination with paclitaxel 200 mg/m^2 i.v. over 3 h and carboplatin AUC 6 i.v. Median survival for pts treated with erlotinib was 10.6 vs. 10.5 months for placebo (hazard ratio, 0.99; 95% CI: 0.86 to 1.16; $p = 0.95$) No difference in OR or median TTP	Erlotinib and placebo arms were equivalent in adverse events (except rash and diarrhea)
Chemotherapy followed by EGFR TKI (EGFR → TKI)		
SATURN[55] Sequential Tarceva in Unresectable NSCLC (SATURN) study n = 1949 (enrolled) Multi-center, randomized, double-blind phase III trial in pts with unresectable or metastatic NSCLC Pts were not allowed to have been	Following completion of four cycles of standard chemotherapy (cisplatin/carboplatin plus another agent), pts (n = 889) without disease progression, intolerable toxicity or poor PS (ECOG ≤ 2) were randomized to receive erlotinib 150 mg p.o. daily (n = 438) or placebo and standard	65% of patients receiving erlotinib and 20% of patients receiving placebo had adverse effects Most events on the erlotinib arm: < = grade 2 rash (60%) or diarrhea (18%) No difference in overall QOL between the two groups.

(continued)

Table 2. Continued

Trial	Dosing schedule/clinical efficacy	Adverse effects
previously treated with chemotherapy or EGFR TKIs or have uncontrolled brain metastases. In the erlotinib and placebo-treated groups, most patients were male (73 and 75%, respectively), Caucasian (84 and 83%, respectively), performance status 1 (69 and 68%, respectively), current or former smokers (83 and 83%, respectively).	supportive care (n = 451) until disease progression or intolerable toxicity Pts that received maintenance erlotinib had significantly prolonged PFS compared with patients treated with placebo (12.3 vs. 11.1 weeks; HR: 0.71; 95% CI: 0.62–0.82; $p < 0.0001$). The few pts with documented EGFR-activating mutations that received erlotinib had a more impressive median PFS (~44 vs. 14 weeks; HR: 0.10; 95% CI: 0.04–0.25; $p < 0.0001$) than pts without activating mutations (HR: 0.78; 95% CI: 0.63–0.96; p = 0.0185) Median OS was significantly prolonged in the group receiving erlotinib (12 months) vs. placebo (11 months; HR: 0.81; 95% CI: 0.70–0.95; $p = 0.0088$)	
Chemotherapy with intermittent TKIs		
FAST-ACT trial[57] Multicenter trial n = 154 (median age: 57, 94% Asians) chemonaïve stage IIIB/IV PS = 0/1 and adequate organ function	Intervention arm: Erlotinib150 mg p.o. daily + chemotherapy Comparator arm: Placebo p.o. days 15–28 + chemotherapy Chemotherapy: Gemcitabine 1,250 mg/m^2 i.v. days 1, 8 + cisplatin 75 mg/m^2 i.v. or carboplatin AUC 5 i.v. day 1 for a maximum of six cycles (cycle to repeat q 4 weeks) Responding pts continued to receive erlotinib or until disease progression or intolerable toxicity Primary endpoint was non-progression rate (=CR + PR + SD) Median number of treatment cycles received: 6 for chemo + erlotinib; 5 for chemo + placebo Statistically significant improvement in PFS ($p = 0.005$) was observed in the erlotinib + chemotherapy arm	Rash-like events: 66% in chemo + erlotinib arm; 35% in chemo + placebo arm Diarrhea: 24% chemo + erlotinib arm; 18% in chemo + placebo arm Most common grade 3–5 adverse events (chemo + erlotinib vs. chemo + placebo): neutropenia (20% vs 15%) anemia (8% vs 6%) thrombocytopenia (5% vs 5%) vomiting (3% vs 8%) Overall safety profiles were similar between the two arms

AUC: area under concentration/time curve; CR: complete response; ECOG: Eastern Cooperative Oncology Group (ECOG); PFS: progression-free survival; PR: partial response; PS: performance status; Pts: patients; RR: response rate; SD: stable disease; TTP: time to disease progression

growth was delayed by 5.6 months and 2.8 months, respectively, in patients who continued on afatinib while on chemotherapy versus patients who were only on chemotherapy ($p = 0.003$). This corresponded to a 40% reduction in risk of disease progression (HR = 0.60). Most common adverse events in patients treated with afatinib and chemotherapy versus chemotherapy were diarrhea (53.8% vs. 6.7%), hair loss or alopecia (32.6% vs. 15%) and weakness or asthenia (27.3% vs. 28.3%).

Despite the clinical efficacy of these EGFR TKIs, almost all patients who initially responded to EGFR TKI treatment (duration of response may last for 10–14 months) will inevitably experience disease progression and become refractory to TKI therapy.[21,30] Preclinical studies of afatinib demonstrated that it was more effective than erlotinib and gefitinib in inhibiting the tumors harboring the *L858R and T790M* mutants.[42] Additionally, it had significant in vitro and in vivo activity against the *T790M*

Table 3. Summary of major LUX trials to test clinical efficacy of chemotherapy and/or EGFR TKIs in different treatment sequences in advanced NSCLC patients.

EGFR TKI as first-line agents in patients with EGFR gene mutation	
Trial	Clinical efficacy
LUX Lung 2[35] Phase II trial enrolled Pts (n = 129) with *EGFR*-mutated lung cancer who were untreated or progressing after chemotherapy	ORR (defined as PR + CR) was 60% with afatinib 40 mg once daily; 62% with 50 mg per day. Median PFS reached 12 months for treatment-naïve pts and 8 months for EGFR TKI-naive pts pretreated with chemotherapy. Median PFS was 12 months for treatment-naive pts and 8 months for EGFR-TKI-naive pts pretreated with chemotherapy Median PFS in the first-line setting of patients with common mutations (i.e. exon 19 deletions and exon 21 L858R gene mutations) reached 13–14 months
LUX Lung 3[30] Pts (n = 345) with untreated lung cancer and *EGFR*-mutated tumors were randomly assigned in a 2:1 ratio to receive afatinib (n = 230) or pemetrexed/cisplatin (n = 115)	PFS was significantly longer in afatinib-treated pts (11.1 vs. 6.9 months; HR = 0.58, 95% CI: 0.43–0.78; $p = 0.0004$). Statistically improvement in OS for pts harboring exon 19 deletion (HR = 0.59; 95% CI: 0.45–0.77; $p < 0.001$). No OS difference was noted in exon 21 *L858R* substitution In pts with common *EGFR* mutations (both 19 deletion and *L858R*), PFS was 13.6 months (longest to date in cross-trial comparison)
LUX Lung 6[36] Asian pts (n = 364) with untreated lung cancer and *EGFR*-mutated tumors were randomly assigned 2:1 ratio to receive afatinib (n = 242) or gemcitabine-cisplatin (n = 122)	Median PFS was significantly longer in the afatinib group (11.0 months, 95% CI 9·7–13.7) than in the gemcitabine-cisplatin group (5.6 months, 5.1–6·7; hazard ratio 0.28, 95% CI 0.20–0.39; $p < 0·0001$) Statistical improvement in overall survival for pts harboring deletion 19. No overall survival difference was noted in exon 21 *L858R* substitution
LUX Lung 7[37] Phase IIb head-to-head study of afatinib vs. gefitinib in *EGFR*-mutated NSCLC pts (ongoing)	Results not available at time of manuscript preparation Results likely to assist selectin of EGFR TKI in the first-line treatment setting of *EGFR* gene mutation-positive pts
EGFR TKI as second- or third-line agent in patients who had previously been treated with other EGFR TKIs	
LUX Lung 1[39] Randomized, double-blind, multicenter, phase IIb/III trial comparing afatinib (n = 390) plus best supportive care vs. placebo (n = 195) in three continents (Asia, Europe, North America) for pts (n = 585) with stage IV adenocarcinoma of lung, failed one or two lines of chemotherapy, had disease progression with erlotinib or gefitinib Study started before routine *EGFR* genotyping (pts were not required to harbor *EGFR* gene mutations)	Median PFS was longer in the afatinib group (3.3 months, 95% CI 2.79–4.40) than the placebo group (1.1 months, 0.95–1.68; hazard ratio 0.38, 95% CI 0.31–0.48; $p < 0.0001$). No CR to treatment 29 (7%) pts had a PR in the afatinib group, as did one pt in the placebo group
LUX Lung 4[40] To evaluate the efficacy of afatinib in Japanese pts (n = 62) who progressed on gefitinib or erlotinib and chemotherapy 45 pts (73%) had *EGFR* gene mutation and 51 (82%) had acquired resistance to gefitinib or erlotinib	In the 61 evaluable pts, 4 pts had PR, median PFS and OS was 4.4 (95% CI: 2.8 to 4.6 months) and 19.0 months (95% CI: 14.9 months to not achieved), respectively 2 pts had acquired *T790M* gene mutations: they had stable disease for 9 months and 1 month, respectively
LUX Lung 5[41] Randomized, open-label, active-controlled, multi-center	ORR (defined as PR + CR) higher in combination group vs. chemotherapy alone (32.1% vs. 13.2%; $p = 0.005$)

(continued)

Table 3. Continued

EGFR TKI as first-line agents in patients with EGFR gene mutation	
Trial	Clinical efficacy
trial to determine the efficacy of afatinib as an add-on agent (40 or 50 mg p.o. daily) to paclitaxel 80 mg/m^2 i.v. weekly in pts (n = 202; 134 pts on afatinib plus paclitaxel; 68 pts on paclitaxel alone) with NSCLC Stage IIIb or IV progressing after afatinib monotherapy compared to chemotherapy alone	OS was similar in both groups (12.2 vs 12.2 months; HR = 1.00; 95% CI: 0.70–1.43; $p = 0.994$) Afatinib plus paclitaxel significantly improved both PFS and RR vs chemotherapy alone
LUX Lung 8[38]	Interim results showed improved progression-free survival (2.4 months vs. 1.9 months), disease control rate (46% vs. 37%) of afatinib compared to erlotinib
Head-to-head studies of afatinib vs. erlotinib in second-line therapy in pts with advanced NSCLC squamous cell histology or wild type EGFR pts (ongoing)	

CI: confidence interval; CR: complete response; HR: hazard ratio; ORR: objective response rate; OS: overall survival; PFS: progression-free survival; PR: partial response; PS: performance status; Pts: patients; RR: response rate.

mutations.[43] However, the concentration of afatinib in overcoming the *T790M* gene mutation may not be achievable in human subjects due to the non-selective dose-limiting toxicities in patients with wild type EGFR genes.[44]

While first-generation reversible TKIs do not bind to EGFR receptors with secondary *T790M* gene mutations, secondary or acquired resistance may still occur in patients who have not received prior TKI therapy. These patients will not respond to initial treatment with gefitinib or erlotinib and are deemed to have primary or de novo resistance.[45] However, some studies[45–47] suggest that the presence of *T790M* gene mutations may not necessarily imply a worse treatment outcome compared to patients without the *T790* gene mutations. At present, afatinib does not have the FDA labeled indication for use in patients with *T790M* gene mutations. Moreover, *T790M* gene mutation should not be regarded as a predictive biomarker for afatinib.

Clinical efficacy of TKI therapy in metastatic NSCLC

The place of therapy for EGFR TKIs underwent major changes in the last decade. Initial studies with gefitinib and erlotinib as single agents demonstrated biologic and clinical activity in only a relatively limited subset of molecularly unselected NSCLC patients in the second- or third-line setting after failure of first-line platinum-based chemotherapy.[48] For instance, erlotinib monotherapy was shown to improve PFS (2.2 vs. 1.8 months, $p < 0.001$) and overall survival over best supportive care (6.7 vs. 4.7, $p < 0.001$) in unselected NSCLC patients with advanced disease who had failed one or two prior lines of chemotherapy (BR 21 trial).[49]

Studies[28–34] showed that activating *EGFR* gene mutations are most common in patients with adenocarcinoma histology, women, never or light smokers and those of Asian ethnicity. As a result, these patients exhibited increased response to EGFR TKIs. The overall response rate may be as high as 80% in molecularly selected patients with gene mutations and 10–20% in unselected populations. The prevalence of sensitizing *EGFR* mutations (mainly exon19 deletion and exon 21 *L858R* substitutions) is approximately 20–40% among Asians and 10% among Caucasians to treatment with first-generation, reversible EGFR TKIs such as gefitinib or erlotinib. Of important note, molecular selection of patients with *EGFR* gene mutations upfront is necessary to maintain efficacy of TKIs as the first-line therapy in metastatic setting. In molecularly unselected patients in early clinical trials, EGFR TKIs did not show additional survival benefit when added to platinum-doublet chemotherapy nor have they shown superiority to single-agent chemotherapy in the salvage treatment setting in unselected patients.[30,36,49]

Recently, the American Society of Clinical Oncology (ASCO)[50] endorsed the consensus guideline of several professional organizations for *EGFR* mutational or genetic testing be performed in all patients (i.e. molecularly selected patients) with lung adenocarcinoma regardless of patient's characteristics (ethnic status, gender, smoking status). In patients with non-adenocarcinoma histology, it is not routinely recommended except for non-smokers, mixed tumors or small biopsy specimens, when an adenocarcinoma component

cannot be completely excluded. Two randomized trials helped to cement this rationale: the recently completed LUX-Lung 3 study[30] suggested that frontline or initial EGFR TKI therapy with afatinib is associated with improved PFS compared to cisplatin-pemetrexed chemotherapy doublet, regardless of race. In addition, in the European Tarceva versus chemotherapy (EURTAC)[51] study, erlotinib is associated with improved survival outcome in European patients.

Current NCCN guidelines[27] recommend erlotinib as a first-line therapy agent for advanced or metastatic NSCLC patients with sensitizing mutations. Erlotinib should not be given as a first-line therapy to patients negative for these mutations or with unknown *EGFR* status. Afatinib is also recommended as a first-line agent for selected patients with sensitizing mutations. Interestingly, NSCLC patients with deletion 19 consistently demonstrated improved outcomes with EGFR TKIs compared to patients with exon 21 *L858R* gene substitutions.[30,36] The cause of this difference in response is not known. But subgroup analysis suggested that afatinib significantly improved the overall survival (compared to chemotherapy) in patients with deletion 19 whereas for patients with *L858R* substitution, there was only improvement in PFS and response rate but not overall survival. Further studies on different populations (deletion 19 vs. *L858R*) with EGFR TKI agent is likely to elucidate whether difference in gene mutations will result in differences in disease outcome, and hence data on exon 19 deletion and exon 21 *L858R* gene substitution patients should not be pooled together.

In patients who have experienced disease progression either during or after first-line therapy, single-agent docetaxel, pemetrexed or erlotinib are established second-line agents. Erlotinib is superior to best supportive care and afatinib may also be used in selected patients with sensitizing *EGFR* gene mutations. Erlotinib is also recommended as third-line agent. In general, erlotinib, gefitinib, afatinib are recommended for continuation after disease progression in patients with sensitizing *EGFR* mutations. Erlotinib has a category two NCCN recommendation for maintenance therapy in patients without disease progression after 4–6 cycles of first-line platinum-based chemotherapy.[27]

Sequencing of TKI therapy with or without chemotherapy

There was initial interest in whether combination of EGFR TKI and chemotherapy can enhance patient survival after their FDA approval in the last decade. However, four large front-line trials[52–55] (see Table 2 for details) failed to demonstrate a survival advantage with the first-line use of either gefitinib or erlotinib in combination with chemotherapy. Based on the survival benefit of erlotinib in previously treated patients, there was interest in determining whether erlotinib treatment is more effective immediately following the completion of first-line chemotherapy. The Sequential Tarceva in Unresectable NSCLC (SATURN) trial[55] was designed to investigate the efficacy of maintenance erlotinib treatment until the time of progression. Erlotinib demonstrated significant improvement in overall survival in maintenance therapy.[56]

A recent study[57] of intermittent TKI therapy with chemotherapy had suggested its preliminary efficacy but since the current standard of care still favors EGFR TKI for maintenance therapy, its role requires validation in long-term studies. Another study[58] from a single institution suggested that when patients with *EGFR* mutations progressed on erlotinib and when progression occurred in only a limited number of sites (<4), the same therapy or local disease control (e.g. stereotactic body radiation therapy in CNS disease) may be offered. Patients with *EGFR* gene mutations who have disease progression often experience disease flare-up when the EGFR TKI is discontinued.[59]

In addition, studies[60–62] suggest that instead of first-line chemotherapy, erlotinib or gefitinib or afatinib should be the first-line systemic therapy in patients with *EGFR* gene mutations documented before starting first-line therapy. PFS (overall survival is not statistically significant) is improved with the use of these EGFR TKIs in patients with sensitizing or activating *EGFR* mutations compared to standard chemotherapy. In the recent LUX-Lung 3 trial, afatinib improved the quality of life compared to those who received cisplatin/pemetrexed chemotherapy. However, in the trial, afatinib was associated with four deaths (see discussion under section ''Toxicities of EGFR TKIs'') whereas chemotherapy had no treatment-related deaths.[45]

To summarize, *EGFR* gene mutations of NSCLC patients are predictive (improved PFS and response rate) when treated with EGFR TKIs such as gefitinib and erlotinib in the first-line therapy of metastatic disease compared to conventional platinum-based chemotherapy. EGFR TKIs are also used in second-, third-line or maintenance therapy.

Toxicities of EGFR TKIs

The most frequent adverse events in LUX trials of the second-generation EGFR TKI afatinib were generally diarrhea, rash or acne. In the LUX-Lung 1 and 3 trials,[30,39] most common adverse reactions associated with afatinib were diarrhea (95% for all grades, 14.4% for grade ≥3) and rash (89.1% for all grades and 16.2% grade ≥3). Stomatitis and nail effects also

appeared frequently. These toxicities were similarly observed in erlotinib and gefitinib trials but gefitinib[60,61] and erlotinib[62,63] typically had a less frequency in diarrhea (25–57% for erlotinib vs. gefitinib 34–54%) and rash (73–80% for erlotinib vs. 66–85% for gefitinib). Overall treatment-related adverse events that were ≥grade 3 occurred in 49% of patients.

Fatal adverse reactions in afatinib-treated patients were rare. There were four deaths due to potential treatment-related complications (two respiratory, decompensations, one sepsis and one unknown).[30] Toxicities of afatinib could be managed by dose reductions to 40 mg or 30 mg, and only less than 10% of patients (8% in LUX-Lung 1[39] and 9% in LUX-Lung 2[35]) required afatinib discontinuation due to drug-related adverse events.

In the LUX-Lung 3 trial (n = 230),[30] most common treatment-related grade 3 or 4 adverse events associated with afatinib were rash or acne (16%; n = 37), diarrhea (14%; n = 33) and paronychia (11%; n = 26). Treatment-related adverse events that led to drug discontinuation in more than one patient included diarrhea (1%; n = 3), paronychia (1%; n = 2) and interstitial lung disease (1%; n = 2). On the other hand, in the LUX-Lung 6 trial (n = 239),[36] most common treatment-related grade 3 or 4 adverse events were rash or acne (15%; n = 35), diarrhea (5%, n = 13) and stomatitis or mucositis (5%; n = 13). Treatment-related adverse events that led to drug discontinuation in more than one patient included rash (2%; n = 5). To summarize, toxicities (diarrhea, stomatitis and paronychia) of afatinib were more frequent and serious than those reported for first-generation reversible EGFR TKIs (gefitinib and erlotinib). There was no clear difference observed in skin rash incidence on erlotinib or afatinib.

On the other hand, side effects of gefitinib and erlotinib are usually mild to moderate, and most commonly manifest as dose-dependent skin rash and diarrhea. Rash can be seen in more than 50% up to 100% of patients, but only a small percentage (less than 5%) of the patients experience grade 3 reactions.[64,65] Of note, EGFR is present in keratinocytes in epidermis and hair follicles. Stimulation of the EGFR pathway promotes the survival of the keratinocytes whereas inhibition of the EGFR pathway leads to secondary inflammation that is manifested as acneiform eruption.[66] Other cutaneous toxicities include paronychia (nail fold inflammation), hair/nail changes and xerosis. Because of the efficacy of EGFR TKI, it is important to treat patients through skin eruptions while minimizing the cutaneous side effects by supportive care (e.g. sunscreen, moisturizing cream, skin cleanser, topical steroids, oral antibiotics).[67] Interstitial lung disease, a potential life-threatening event, is uncommon in patients treated with EGFR TKIs and is rarely lethal.

Genetic testing recommendation

In the setting of lung cancer resection specimens, *EGFR* gene testing is recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade. *EGFR* gene testing is not recommended in lung cancers that lack any adenocarcinoma component. In squamous NSCLC, *EGFR* gene mutation testing is generally not required, but can be considered in never smokers, small biopsy specimen or mixed histology. If *EGFR* gene mutation is confirmed during first-line chemotherapy, patient may either (1) complete chemotherapy or (2) interrupt chemotherapy, start erlotinib or afatinib or (3) add erlotinib or afatinib to chemotherapy (NCCN category 2B recommendation).[27]

Various DNA mutational analyses can be used to determine the *EGFR* mutation status in tumor cells: direct sequencing of DNA corresponding to exon 18–21, PCR-based mutational screening assays and next generation sequencing can be used.[68] A number of central or reference laboratories offer *EGFR* genotyping of exons 18–21. Typical examples of FDA-approved qualitative PCR testing include: cobas® EGFR Mutation Test (Roche Molecular Diagnostics, Pleasanton, CA), therascreen EGFR RGQ PCR kit (Qiagen, Valencia, CA) and some others.

Future of anti-EGFR TKI therapy with or without chemotherapy

At present, combination therapy of anti-EGFR TKIs with chemotherapy in molecularly unselected NSCLC patients have not resulted in improved survival outcome. In selected patients with sensitizing *EGFR* gene mutations, combination therapy of anti-EGFR TKIs with chemotherapy has been shown to improve survival outcome.[69] Combination therapy may be necessary in patients with large tumor burden. In addition, there is reported efficacy with combination therapy of anti-EGFR TKI and chemotherapy for treatment beyond disease progression after TKI failure, necessitating more studies into the novel combination therapy to address these acquired mechanisms of resistance.[70] In addition, outcome of the head-to-head trials[37,38] of afatinib with gefitinib and erlotinib may help define which TKI agent is the choice for first-line treatment of patients with metastatic *EGFR* gene mutation-positive NSCLC, as well as comparing the toxicities between the reversible and irreversible TKIs.

Recently, a small molecule TKI, tivantinib, and the monoclonal antibody, onartuzumab, have both

been evaluated in the second-line setting in EGFR-TKI-naïve patients after chemotherapy failure. In the phase 3 trial, combination therapy of onartuzumab and erlotinib was not shown to improve PFS (2.7 vs. 2.6 months, $p = 0.92$) or objective response rate 8.4% vs. 9.6%, $p = 0.63$).[42] Despite this negative finding, many ongoing trials are likely to shed some light in elucidating the additional roles of EGFR TKIs with other agents and how these agents could be sequenced to optimized treatment outcome.

The role of antiangiogenesis is investigated in an open-label, randomized phase II Japanese study.[43] Chemotherapy-naïve patients (n = 154) with stage IIIB/IV non-squamous NSCLC with activating *EGFR* gene mutation either received erlotinib 150 mg orally once-a-day plus bevacizumab 15 mg/kg i.v. every 3 weeks (n = 77) or erlotinib 150 mg orally once-a-day monotherapy (n = 77) as first-line therapy until disease progression or intolerable toxicity. Median PFS was 16.0 months (95% CI 13.9–18.1) with erlotinib plus bevacizumab and 9.7 months (5.7–11.1) with erlotinib monotherapy (hazard ratio 0.54, 95% CI 0.36–0.79; $p = 0.0015$), suggesting that erlotinib plus bevacizumab combination could be a new first-line regimen in *EGFR* mutation-positive NSCLC. Further study of the regimen is warranted.

Combination of EGFR TKI (e.g. erlotinib) and anti-EGFR monoclonal antibody (e.g. cetuximab) did not seem to result in survival benefit in patients with acquired resistance to first-generation EGFR TKIs,[71] while in another phase Ib study,[72] the combination of afatinib and cetuximab resulted in response rate in about 30% of NSCLC patients who developed *T790M* gene mutations. Apparently, more studies will be needed to validate the role of this dual "EGFR blockage."

Recently, two randomized trials published their preliminary results on whether EGFR TKI should be continued during disease progression. In the phase III IMPRESS trial,[73] 265 patients from 71 centers in Europe and Asia were enrolled and randomly assigned to cisplatin/pemetrexed plus gefitinib versus cisplatin/pemetrexed plus placebo; 65% of patients were female and mean age was about 60. Overall response rate was 31.6% for gefitinib vs 34.1% for chemotherapy, and the disease control rate was 84.2% vs 78.2%, respectively. Overall survival data have not reached during the study cut-off date. The study demonstrated that EGFR TKI should not be continued beyond progression. The standard treatment at progression remains platinum-based chemotherapy.

On the other hand, another phase II study (Aspiration)[74] evaluated the safety and efficacy of erlotinib before and after disease progression in untreated Asian patients with *EGFR*-mutated NSCLC in 150 patients; 81 of those received erlotinib with a median 1-year PFS of 9.3 months. In patients who did not receive erlotinib after disease progression, median 1-year PFS was 7.2 months. Patients with exon 19 deletion and exon 21 *L858R* substitutions had more favorable PFS than those without. Among the 207 patients evaluated for safety, 45.4% reported grade ≥ 3 adverse events. The study suggested that even though there is a slight increase in the tumor on the assessment, if the treatment is well-tolerated, and if the patient remains asymptomatic, patient should not be switched to chemotherapy immediately but continue therapy until there is clear clinical progression. More studies will be needed to address the place of therapy for EGFR TKI in the disease progression setting.

Novel EGFR TKI agents are now being developed after initial resistance (i.e. secondary or acquired resistance) to traditional TKI agents in advanced NSCLC patients. Rociletinib (CO-1686), an investigational agent that covalently binds to the conserved cysteine residue 797 in the ATP-binding pocket of the EGFR kinase domain, is a third-generation irreversible TKI which targets *EGFR* sensitizing as well as *T790M* gene mutations. Preclinical studies of rociletinib suggested inhibitory activity in both *T790M* gene mutation-positive and *EGFR* gene mutation-positive NSCLC animal models. Efficacy and acceptable toxicity were also demonstrated in a study by Sequist and colleagues.[75] A phase I/II study is now under way to evaluate the pharmacokinetics, safety and efficacy of oral rociletinib.[76]

Similarly, AZD9291 is another third-generation EGFR TKI investigational agent for patients who have developed acquired resistance. In a phase I multicenter trial,[77] nine out of 18 patients with *T790M* gene mutations had confirmed or unconfirmed partial responses with tolerable adverse effects. Of note, it is important to rebiopsy the patient at the acquired resistance setting since researchers currently do not know if the third generation TKI is still effective for patients who do not harbor this second site mutation.[78] Both Rociletinib and AZD9291 have been designated the breakthrough status by the FDA.

Blockade of immune checkpoints with monoclonal antibodies has received attention for advanced NSCLC. T-cells play a significant role in the immune recognition of tumor cells and the generation of cytotoxic T cells that can kill tumor cells. Check point of T-cell activation refers to the inhibitory pathway that is important for the maintenance of self-tolerance and avoidance of physiologic immune response to the host tissue.[79] Cancer cells can evade host immune systems by expressing certain ligands to down-regulate cytotoxic T cells through these inhibitory pathways, which are usually initiated by ligand-receptor interactions. Programmed death 1 protein (PD1) is a transmembrane protein found in T cells that regulates T-cell activation and proliferation. It has two ligands, programmed death–ligand 1 (PD-L1) and programmed death–

ligand 2 (PD-L2). Overexpression of PD-L1 is frequently associated with many human malignancies. A recent clinical trial[80] showed that inhibition of the PD-L1/PD1 interaction with antibodies resulted in antitumor efficacy in patients with various malignancies, including NSCLC. In March 2015, nivolumab (Opdivo®, Bristol-Myers Squibb Inc. USA), an anti-PD1 agent, gained U.S. FDA approval for the treatment of patients with previously treated (e.g. with docetaxel) metastatic squamous cell NSCLC. In another study by Azuma and colleagues,[81] they found that the presence of *EGFR* gene mutations and adenocarcinoma histology were significantly associated with increased PD-L1 expression. Down-regulation of PD-L1 expression and consequent activation of antitumor immune response may contribute to the durable therapeutic response of *EGFR* mutation-positive NSCLC patients to EGFR TKIs. More upcoming clinical studies will likely delineate the role of EGFR TKI in combination with immune checkpoint inhibitors in advanced NSCLC.

Can EGFR TKI be used in squamous cell NSCLC?

Limited treatment options exist for patients with advanced NSCLC of squamous histology, which represents approximately 30% of all NSCLC cases. Less than 15% of patients survive for 5 years or longer.[82] Current standard therapy for patients with advanced NSCLC of squamous histology and good performance status is generally a platinum-based chemotherapy doublet.[27] Recently, Interim results[83] of the LUX-Lung 8 trial,[38] a study that compared the efficacy of two EGFR TKI head-to-head in patients with advanced squamous cell carcinoma of the lung, showed that there was improved PFS (2.4 months vs. 1.9 months), disease control rate (46% vs. 37%) of afatinib compared to erlotinib. Severe adverse events were 50.2% in patients treated with afatinib compared to 49.1% with erlotinib. A higher incidence of ≥grade 3 diarrhea and stomatitis were observed in patients treated with afatinib compared to erlotinib (≥grade 3 diarrhea: 9% vs. 2%; stomatitis: 3% vs. 0%). There was a higher incidence of ≥grade 3 rash/acne observed with erlotinib compared to afatinib (9% vs. 6%).

Conclusion

The success of targeted agents in molecularly defined subsets of NSCLC patients has radically changed the treatment paradigm of metastatic lung adenocarcinoma. It is becoming clinically relevant to re-biopsy tumor at disease recurrence that helps to define what therapeutic options are considered appropriate. To date, the most significant progress is for metastatic NSCLC patients whose tumors harbor *EGFR* mutations, in whom first-line treatment with EGFR TKIs led to improvement in survival outcomes compared to standard chemotherapy. Early clinical trials for EGFR TKIs demonstrated improved PFS in molecularly unselected metastatic NSCLC patients or they have crossed over to receive other lines of therapies. In patients who are properly selected for EGFR-positive gene mutations, EGFR-TKIs have been shown to improve symptom control, and quality of life, especially in frail elderly patients who desire to avoid the systemic side-effects of cytotoxic chemotherapy while achieving a certain level of clinical efficacy. As more clinical trials for novel third-generation EGFR TKIs and other alternative therapies mature, better understanding may be gained through the use of these agents in improving treatment efficacy in adenocarcinoma or even squamous cell histology of metastatic NSCLC.

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Conflict of interest

None declared.

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