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Tyrosine Kinase Inhibitors for EGFR Gene Mutation-Positive Non-Small Cell Lung Cancers: An Update for Recent Advances in Therapeutics

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Abstract

The presence of activating gene mutations in the epidermal growth factor receptor (EGFR) of non-small cell lung cancer (NSCLC) patients is predictive (improved progression-free survival and improved response rate) when treated with small molecule tyrosine kinase inhibitors (TKIs) 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 point mutations in exon 21 (*L858R*). Exon 18 mutations occur much less frequently at about 4% of all *EGFR* gene mutations. Together, deletion19 and *L858R* gene mutations are present in about 10% of Caucasian patients and 20–40% of Asian patients with NSCLC. *T790M* gene mutation at exon 20 is associated with acquired resistance to EGFR TKIs. 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 EGFR TKI therapy with afatinib is associated with improved progression-free survival compared to chemotherapy regardless of race. Moreover, guidelines suggest EGFR 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 NSCLC patients has changed the treatment paradigm in metastatic NSCLC. However, despite a durable response of greater than a year, resistance to EGFR TKIs inevitably occurs. This mini-review describes the clinically significant *EGFR* gene mutations and the efficacy of small molecule EGFR TKIs as targeted therapies for these gene mutations. Therapeutic strategies to overcome resistance, including selected emerging and novel therapies are discussed.

Keywords: EGFR; TKI; Afatinib; Lung adenocarcinoma; Targeted therapy; Exon 19 deletion; Exon 21; L858R; Gene mutations

Introduction

Lung cancer is a leading cause of cancer death in the U.S. 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 five-year survival is only 15% [2]. Primary tumor in anatomic/clinical stages I to IIIA is considered respectable. Treatment option generally consists of surgery with or without adjuvant chemotherapy [3]. Tumor in the stage IIIB is no longer resectable and the treatment option is chemoradiation [4] whereas for stage IV, treatment options include chemotherapy or oral targeted therapy agents [5]. Chemotherapy typically consists of a platinum-based doublet therapy (i.e. cisplatin or carboplatin combined with agents such as gemcitabine, vinorelbine or taxanes and most recently, cisplatin-pemetrexed for non-squamous lung cancer). No regimen has proven superiority over another [6]. Common symptoms of lung cancer include weight loss, cough, dyspnea and chest pain. Symptomatic patients are more likely to have chronic obstructive pulmonary disease. 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 U.S. and in non-smokers [2].

EGFR Signaling Pathway

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 [7]. EGFR is normally found on the surface of epithelial cells and is often over-expressed in many malignancies [8,9]. In addition, somatic gene mutations in the intracellular kinase domain of the EGFR lead to ligand-independent activation of the signaling pathway, leading to constitutively activated tyrosine kinase that results in tumorigenesis [10]. In normal cells, the EGFR pathway is tightly regulated whereas loss of regulation leads to uncontrolled growth and oncogenesis [11].

EGFR is the prototypical member of a family of four RTKs, EGFR (ERB-B1, HER1), ERB-B2 (HER2/Neu), ERB-B3 (HER3) and ERB-B4 (HER4) [12]. 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 MEK (mitogen-activated, extracellular signal-regulated kinase) and MAPK. Alternatively, ligand-bound EGFR can translocate PI3K to the cell membrane and activate AKT and other downstream molecules [13]. Tumor cells can upregulate the EGFR pathway through mechanisms such as EGFR over expression, **EGFR** gene amplification, activating also (known as sensitizing) mutations of

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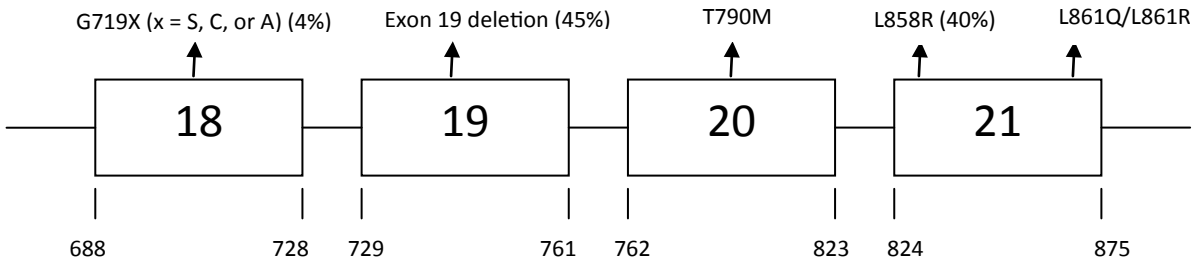

The *EGFR* gene is located in the short arm of chromosome 7. It contains 28 exons. Exons 18-21 are in the cytoplasmic tyrosine kinase domain of the EGFR receptor are commonly associated with sensitivity or resistance to EGFR TKIs when these genes are mutated.
The most prevalent *EGFR* gene mutations 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 shown above are associated with sensitivity and hence they are predictive biomarkers for response to EGFR TKIs. *T790M* 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).
Key: G= Glycine; S=serine; C=cysteine; A=alanine; T=threonine; M=methionine; L=leucine; R=arginine; Q= glutamine. The numbers below the vertical bar of each box refers to the amino acid number of each exon.

Figure 1: Frequency of gene mutations in exons 18-21 of the *EGFR* gene.

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 [14].

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 encoding the tyrosine kinase domain which binds to the substrate ATP. These **EGFR** gene mutations (Figure 1) are:

- Exon 19 deletion (i.e. in-frame conserved deletions that encompass 4 amino acids on amino acid positions 747–750 or the "LREA" region) that occurs in 45% of patients with **EGFR** gene mutations. These 4 amino acids are: leucine (L), arginine (R), glutamic acid (E) and alanine (A) [15].
- Exon 21 **L858R** gene mutation (a mis-sense mutation that results in a substitution of leucine with arginine at amino acid position 858) that occurs in another 40% of patients with **EGFR** gene mutations [16].
- Exon 18 **G719X** gene mutation (a mis-sense mutation that results in the substitution of glycine with cysteine, alanine or serine at amino acid position 719) that occurs in about 4% of all **EGFR** gene mutated patients. Other drug-sensitizing gene mutations include point mutations at exon 21 [17].
- **T790M** mutation, 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 amino acid position 790, is associated with acquired resistance to TKI therapy in 50-60% of patients with adenocarcinomas of the lung [18].

Resistance to small molecule EGFR tyrosine kinase inhibitors (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. Common acquired resistance mechanisms to EGFR TKIs are **T790M** mutation, transformation of the NSCLC to small cell lung cancer (SCLC) and the mesenchymal-epidermal transition (MET) receptor overexpression or gene amplification. Of interest, **MET** gene amplification is observed in 20% of resistance cases in NSCLC patients treated with gefitinib or erlotinib [18]. **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 that induces receptor dimerization, phosphorylation and PI3K activation, resulting in persistent activation of the downstream pathway that overcomes the inhibition by EGFR TKI [19]. Amplification of the **MET** gene is involved in the invasion, metastasis and angiogenesis of tumors [20].

In addition, less common somatic gene mutations, such as **HER2** [21], **HER4** [22], **BRAF** [23] and **PIK3CA** [24] are also found in the EGFR pathways. Other receptor tyrosine kinases such as AXL [25] are also implicated in the acquired resistance to EGFR TKI. Taken together, some of these **EGFR** gene mutations activate the EGFR signaling pathway and promote EGFR-mediated pro-survival and anti-apoptotic signals through the downstream targets. However, whether these less common 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. For purpose of simplicity, these downstream gene mutations are not addressed in this mini-review. Interested readers are encouraged to consult further references.

In summary, both exon 19 gene deletions and exon 21 **L858R** gene mutations result in the activation of the tyrosine kinase domain. These mutations are associated with sensitivity (i.e. sensitizing mutations) to small molecule EGFR TKIs. These four major types of gene mutations seldom occur simultaneously. Despite the high response rate and prolonged progression-free survival in patients with **EGFR** gene mutations treated with first-generation EGFR TKIs (such as gefitinib and erlotinib), about 50% of these patients will develop the acquired **T790M** mutation [5,12,15]. 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 EGFR TKIs [16-18].

Targeted Therapy against EGFR Protein and *EGFR* Gene Mutations

There are two types of EGFR targeted therapy: anti-EGFR monoclonal antibodies that bind to the extracellular domain of the EGFR protein and the small molecule TKIs that bind to the intracellular

EGFR TKI/dosing	FDA approved indications	Interaction with PPI or H2A	Drug-food interaction	Adverse effects	Hepatic or renal adjustment
Gefitinib 250 mg po qday	First-line therapy in metastatic NSCLC with EGFR exon 19 deletions or exon 21 (*L858R*) substitution mutations	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 po daily	First-line therapy in metastatic NSCLC with EGFR exon 19 deletions or exon 21 *(L858R)* mutations. Maintenance therapy in metastatic NSCLC after 4 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 hrs after the H2Aand at least 2 hours 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: ≤11% Weakness: ≤53%, Back pain: 19%, Arthralgia: ≤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 po daily	First-line therapy for patients who have metastatic NSCLC tumors with EGFR exon 19 deletions or exon 21 *L858R* 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 ≥ grade 3 hepatic dysfunction Child-Pugh class A or B: no dosage adjustment

Key: Conc= concentration; **H2A=** H2 antagonist; **PPI=** proton pump inhibitor; **pts=** patients; **ULN=** upper limit normal

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.

tyrosine kinase domain with activating gene mutations. Together, these inhibitors act on a series of signaling pathways that mediate cell survival, proliferation, metastasis and angiogenesis. Of note, there is a fundamental clinical difference between anti-EGFR monoclonal antibodies and EGFR TKIs: anti-EGFR monoclonal antibodies such as cetuximab (Erbitux®, Eli Lilly and Bristol-Myers Squibb, U.S.) and panitumumab (Vectibix®, Amgen, U.S.) currently are not approved by U.S. Food and Drug Administration (FDA) indicated in the treatment of advanced or metastatic NSCLC. On the contrary, current FDA-approved EGFR TKIs for advanced NSCLC patients with activating or sensitizing **EGFR** gene mutations include gefitinib (Iressa®, AstraZeneca Inc.), erlotinib (Tarceva®, Genentech, U.S.) and afatinib (Gilotrif®, Boehringer Ingelheim. U.S). 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 U.S. National Comprehensive Cancer Network (NCCN) guidelines [26], 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 epidermal growth factor receptor (*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 [26].

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 [12,14-17]. *EGFR* gene mutations were subsequently identified in select patients after clinical benefit to EGFR TKI was observed in 2004 [10,27]. Evaluation of tumor specimens in these patients led to the identification of two common mutations in *EGFR* gene, the exon 19 deletion and the exon 21 *L858R* missense mutation, both of which can be readily targeted by first-generation reversible EGFR TKIs such as gefitinib and erlotinib and second-generation TKI such as afatinib [27-29].

Erlotinib is the current EGFR TKI agent of choice in U.S. 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 [28] and IDEAL-2 [30], the lack of overall survival benefit (5.6 vs. 5.1 months, P = 0.087) in the confirmatory phase III ISEL trial prompted the FDA's withdrawal of gefitinib's accelerated approval. Gefitinib is now restricted in patients already on this medication and continue to benefit from it (enrollment through the Iressa® Access program) whereas in Europe and Asia, it is still approved or used for locally advanced and metastatic NSCLC with activating *EGFR* mutations. In U.S., 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 is worth pointing out that 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 [20,29]. Preclinical studies of afatinib demonstrated that it was more effective than erlotinib and gefitinib in inhibiting the tumors harboring the *L858R and T790M* mutants [31]. Additionally, it retained significant in vitro and in vivo activity against the *T790M* mutations [32]. However, how much of this pharmacologic activity translates to clinical benefit currently remains unknown.

It is important to point out that the first-generation reversible TKIs such as gefitinib and erlotinib do not bind to EGFR receptors with *T790M* gene mutations which may also occur in patients who have not

Treatment options	Trial	Dosing schedule/clinical efficacy	Adverse effects
Chemotherapy + EGFR TKI			
	INTACT I [40] **Iressa NSCLC Trial Assessing Combination Treatment** Phase III randomized, double-blind, placebo-controlled, multicenter trial n=1,093 Chemotherapy-naive patients with unresectable stage III or IV NSCLC End points included OS (primary), TTP, RR and safety evaluation	Up to 6 cycles of cisplatin 80 mg/m^2 i.v. on day 1 and gemcitabine 1,250 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 po daily, gefitinib 250 mg po 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 [41] Phase III, randomized, placebo-controlled, double-blind trial in chemotherapy-naive patients with advanced NSCLC n=1,037 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 po daily, gefitinib 250 mg po 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 po daily, 250 mg po 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 [42] Phase III, randomized, double-blind, multicenter trial in previously untreated patients with advanced NSCLC n = 1,059	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 v 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)
	TALENT (Tarceva Lung Cancer Investigation trial) [43] Phase III, randomized, double-blind, placebo-controlled, multicenter trial Primary end point was OS Secondary end points included TTP, RR, duration of response, and QOL n =1,172 Baseline demographic and disease characteristics were well balanced	Intervention arm: erlotinib 150 mg p.o. daily Comparator arm: placebo, combined with up to six 21-day cycles of chemotherapy (gemcitabine 1,250 mg/m^2 i.v. on days 1 and 8 and cisplatin 80 mg/m^2 on day 1 No differences in OS, TTP, RR, duration of response, and QOL In a small group of patients who had never smoked, OS and PFS were increased in the erlotinib group; no other subgroups were found more likely to benefit	Erlotinib with chemotherapy was generally well tolerated Incidence of adverse events was similar between arms, except for an increase in rash and diarrhea with erlotinib (generally mild)
Chemotherapy ➔ EGFR TKI			
	SATURN **Sequential Tarceva in Unresectable NSCLC (SATURN) study** [44] n=1,949(enrolled) Multi-center, randomized, double-blind phase III trial in pts with unresectable or metastatic NSCLC Pts were not allowed to have been 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	Following completion of 4 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 po daily (n = 438) or placebo and standard 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)	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.

Treatment options	Trial	Dosing schedule/clinical efficacy	Adverse effects
Chemotherapy with intermittent EGFR TKI			
	FAST- ACT trial [45] Multicenter trial n= 154 (median age: 57, 94% Asians) chemonaïve stage IIIB/IV PS = 0/1 and adequate organ function	Intervention arm: Erlotinib150mg 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. day1 for a maximum of 6 cycles (cycle to repeat q4 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

Key: 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

Table 2: Summary of major clinical trials to test clinical efficacy of chemotherapy and EGFR TKIs in different treatment sequences in advanced NSCLC patients.

received TKI therapy. These patients will not respond to initial treatment with gefitinib or erlotinib and are deemed to have primary or de novo resistance [33]. However, some studies suggest that the presence of *T790M* gene mutations may not necessarily imply a worse treatment outcome compared to patients without the *T790* gene mutations [33-35]. 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 unselected NSCLC patients in the second or third line setting after failure of first-line platinum-based chemotherapy [36]. For instance, erlotinib monotherapy was shown to improve progression-free survival (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) [37].

Early studies 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 selected patients with gene mutations and 10-20% in unselected populations. The prevalence of sensitizing *EGFR* mutations (mainly exon19 deletion and exon 21 *L858R* mutations) 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, selection of the patient population with *EGFR* gene mutations upfront is necessary to maintain efficacy of TKIs as the first line therapy in metastatic setting. In unselected patients in the 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 [29,37-39].

More questions now arise as to whether the survival benefit is restricted to Asian patients or non-smokers alone. The recent randomized, multicenter, international LUX-Lung 3 study [29] suggested that frontline or initial EGFR TKI therapy with afatinib is associated with improved progression-free survival compared to cisplatin-pemetrexed chemotherapy doublet, regardless of race. In addition, in the EURTAC [38] (European Tarceva versus chemotherapy) study, erlotinib is associated with improved survival outcome in European patients. Recently, the American Society of Clinical Oncology (ASCO) endorsed the consensus guideline of several professional organizations for *EGFR* gene testing to all patients with lung adenocarcinoma (or mixed lung cancers with an adenocarcinoma component), regardless of characteristics such as smoking status, gender or race [39].

Current NCCN guidelines [26] 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 select patients with sensitizing mutations. 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 select patients with sensitizing EGFR 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 [26].

Combination of TKI Therapy with 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 [40-43] (Table 2) 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 [44] 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.

A recent study [45] 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 [46] 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 [47].

In addition, studies [48-50] 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. Progression-free survival (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 received cisplatin/pemetrexed chemotherapy. However, in the trial, afatinib was associated with 4 deaths whereas chemotherapy had no treatment-related deaths [29].

To summarize, *EGFR* gene mutations of non-small cell lung cancer (NSCLC) patients are predictive (improved progression-free survival 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 clinical trials of afatinib were diarrhea, rash or acne (78% -97%of patients treated in the LUX-Lung trials). Stomatitis and nail effects also appeared frequently. These toxicities were similar to those observed in erlotinib and gefitinib trials. 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 [51], and 9% in LUX-Lung 2 [52]) required afatinib discontinuation due to drug-related adverse events. Side effects of gefitinib and erlotinib are usually mild to moderate, and most commonly manifest as dose-dependent skin rash and diarrhea.

Genetic Testing

In the setting of lung cancer resection specimens, *EGFR* testing is recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade. *EGFR* 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) [26].

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 [53]. 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 Chemotherapy

At present, combination therapy of anti-EGFR TKIs with chemotherapy in unselected NSCLC patients have not resulted in added value. In selected patients with sensitizing *EGFR* gene mutations, combination therapy of anti-EGFR TKIs with chemotherapy has been shown to improve survival outcome [54]. Combination therapy may be necessary in patients with a large tumor burden. In addition, treatment beyond disease progression after TKI failure with combination therapy of anti-EGFR TKI and chemotherapy has been reported, necessitating more studies into the novel combination therapies to address these acquired mechanisms of resistance [55].

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. 2.6months, **P**=0.92) or objective response rate 8.4% vs. 9.6%, **P**=0.63) [56]. Despite this negative finding, many ongoing trials will likely shed some light to elucidate 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 2 Japanese study. 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 progression-free survival 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 [57].

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 who acquired resistance to first-generation EGFR TKIs [58], while in another phase Ib study [59], the combination of afatinib and cetuximab resulted in response rate in about 30% of NSCLC patients who developed *T790M* gene mutations. More study is apparently needed to validate the role of this dual "EGFR blockage".

Recently, two interesting trials published their preliminary results on whether EGFR TKI should be continued during disease progression. In the phase III IMPRESS trial, 265 patients from 71 centers in Europe and Asia were enrolled and randomly assigned to cisplatin/pemetrexed plus gefitinib vs. 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 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 [31].

On the other hand, another phase II study (Aspiration) 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 progression-free

survival (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* mutations 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 continues until there is clear clinical progression [32]. More studies will be needed to address the place of therapy for EGFR TKI in disease progression.

Conclusion

The success of targeted agents in molecularly defined subsets of patients has radically changed the treatment paradigm of metastatic lung adenocarcinoma. It is becoming clinically relevant to re-biopsy tumor at recurrence and defines 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. As more clinical trials for EGFR TKI mature, better understanding may be gained through the use of these agents either alone or in combination with different therapeutic agents (e.g. chemotherapy) and in different sequences in improving treatment efficacy of metastatic lung adenocarcinoma.

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