ADMET

Review

Tyrosine kinase inhibitors for EGFR- and ALK-mutated non-small cell lung cancer

Jonathan R.Thompson[1], Smitha P. Menon[2] and Grace K. Dy[3]*

[1]*Mazie Froedtert Willms &Sue Froedtert Cancer Fellow at Froedtert Hospital, Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI*
[2]*Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI*
[3]*Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY*

*Corresponding author. E-mail: Grace.Dy@RoswellPark.org.

Abstract

Discovery of the epidermal growth receptor (EGFR) activating mutations and anaplastic lymphoma kinase (ALK) rearrangements has expanded the therapeutic landscape in non-small cell lung cancer (NSCLC). Survival outcomes for patients with these mutations have improved dramatically with EGFR and ALK tyrosine kinase inhibitors (TKIs). Multiple generations of EGFR and ALK TKIs have been rapidly developed, and patients and clinicians now have several options for first- and second-line treatments. While these small molecule TKIs have some similarities in therapeutic and pharmacologic profiles, the differences can be clinically substantial, allowing tailored treatment for each unique patient. This review details the clinical efficacy, pharmacology, safety profiles, CNS penetration, and mechanisms of resistance of the four EGFR TKIs and three ALK TKIs that are currently approved by the United States Food and Drug Administration (US FDA).

Keywords

Epidermal growth factor receptor; Anaplastic lymphoma kinase; targeted therapy

Introduction

Lung cancer is the leading cause of cancer mortality worldwide, leading to 1.6 million deaths annually [1]. Non-small cell lung cancer (NSCLC) is a heterogeneous disease that accounts for 85 % of all lung cancer diagnoses [2]. Most patients are diagnosed at an advanced stage, where 5-year survival is less than 5 % [3]. Within the past decade, numerous somatic molecular mutations have been discovered that drive oncogenesis in NSCLC. Protein kinase inhibitors that target these mutations improve response rates and survival compared to standard systemic chemotherapy. The most common clinically relevant "driver" mutations encountered are the epidermal growth factor receptor (EGFR) activating mutations, which are present in 30-40 % of Asian NSCLC patients and 10 % of Caucasian patients with NSCLC [4,5]. Rearrangements in the anaplastic lymphoma kinase (ALK) gene lead to fusion proteins, which drive cellular proliferation. The ALK gene rearrangement occurs in 3 to 7 % of all NSCLC patients [6].

EGFR activating mutations and ALK gene rearrangements create constitutively active protein kinases for which there are multiple highly active tyrosine kinase inhibitors (TKIs) available for treatment. These TKIs have dramatically impacted clinical outcomes for patients with NSCLC who previously carried poor

prognoses. The standard overall survival for all patients with advanced NSCLC ranges from 4 to 12 months [7-10], but median overall survival in patients with EGFR- or ALK-mutated NSCLC now approaches 3 to 4 years or more [11-15]. Currently, there are four EGFR TKIs and three ALK TKIs that are widely approved for clinical use. The first-generation EGFR TKIs, gefitinib and erlotinib, reversibly inhibit the intracellular catalytic domain of the EGFR tyrosine kinase. Afatinib is a second-generation EGFR TKI that irreversibly inhibits the EGFR tyrosine kinase domain. Osimertinib is a third-generation EGFR TKI specifically designed to inhibit T790M-mutated EGFR as well as other EGFR activating mutations. Crizotinib was the first TKI to show activity in ALK-positive NSCLC, however, it is more potent at inhibiting MET than ALK. Ceritinib and Alectinib are TKIs that are more potent and specific for the ALK tyrosine kinase.

In this article, the relevant EGFR and ALK mutations and the TKIs which inhibit these mutated proteins will be reviewed. The clinical efficacy, safety, and pharmacology of these TKIs will be detailed in order to promote understanding of this promising and rapidly evolving field.

EGFR tyrosine kinase inhibitors

EGFR protein and activating mutations

EGFR is a tyrosine kinase receptor of the ErbB receptor family. It is composed of an extracellular domain (N-terminus) that binds ligands including EGF and TGF-α, a transmembrane domain, and an intracellular domain (C-terminus) that is responsible for intracellular signaling through tyrosine kinase activity [16,17]. Upon binding to its ligand on the extracellular domain, the receptor dimerizes, leading to autophosphorylation of the intracellular tyrosine-kinase domain. Autophosphorylation of the intracellular domain is followed by binding of adaptor proteins and signaling through down-stream pathways. These pathways include the Ras/Raf/MEK/ERK, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), and janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathways, which contribute to cell migration, survival, and proliferation [18,19]. Activating mutations in the EGFR gene were originally reported in 2004, and were associated with female sex, Asian descent, absence of tobacco exposure and adenocarcinoma histology [4,20,21]. The EGFR gene exists on chromosome 7, and activating mutations are predominantly located on exons 18 to 22. At diagnosis, 90 % of patients with an activating EGFR mutation will have either an in-frame deletion in exon 19 or a L858R missense mutation on exon 21. These mutations all cause prolonged, ligand-independent signaling of the EGFR [22]. Due to the dependence on the constitutively activated EGFR, these malignant clones are particularly susceptible to EGFR TKIs. The receptor targets, clinical indications, pharmacokinetics, pharmacodynamics and CNS penetration of the FDA approved EGFR TKIs are summarized in Table 1.

Erlotinib

<u>Clinical efficacy</u>

Prior to the widespread knowledge of EGFR activating mutations as predictive biomarker of treatment sensitivity to EGFR TKIs, erlotinib gained FDA approval in November 2004 for treatment of advanced NSCLC, regardless of tumor mutation status, after failure of first- or second-line chemotherapy. In a phase III, placebo-controlled randomized study, erlotinib provided advantages in progression-free survival (PFS) (2.2 months vs 1.8 months, hazard ratio, 0.61;P<0.001) and overall survival (OS) (6.7 months vs 4.7 months, hazard ratio, 0.71; P<0.001) compared to placebo [25]. Erlotinib was next FDA-approved for maintenance treatment of NSCLC in April 2010 for patients whose disease had not progressed after four cycles of platinum-based chemotherapy based on the SATURN trial. The randomized, double-blind,

placebo-controlled study of 889 patients with stage IIIB/IV NSCLC who received erlotinib or placebo after receiving first-line platinum-based chemotherapy demonstrated improvements in PFS (12.3 weeks vs 11.1 weeks, HR 0.71; P<0.001) as well as OS (12 months vs 11.1 months, HR 0.81; P=0.0088) for erlotinib maintenance. Patients with activating mutations of EGFR experienced more dramatic PFS and OS benefits [26].

Table 1. EGFR TKI characteristics

EGFR TKI Name	Erlotinib	Gefitinib	Afatinib	Osimertinib
Primary Targets	Wild type EGFR Deletion 19 L858R	Wild type EGFR Deletion 19 L858R	Wild type EGFR Deletion 19 L858R T790M HER2/3/4	Deletion 19 L858R T790M
Type of inhibition	Reversible	Reversible	Irreversible	Irreversible
Clinical approval	First-line treatment of advanced EGFR-mutated NSCLC	First-line treatment of advanced EGFR-mutated NSCLC	First-line treatment of advanced EGFR-mutated NSCLC	Second-line treatment of advanced EGFR-mutated NSCLC
EGFR mutation IC_{50} (in vitro)[23]	23 nM (del 19) 39 nM (L858R)	30 (del 19) 100 nM (L858R)	0.2 nM (del19) 0.2 nM (L858R)	9 nM (del 19) 12 nM (L858R)
T790M IC_{50} (in vitro) [22,24]	1600 nM (del 19) >10000 nM (L858R)	>5000 nM	141 nM (del 19) 196 nM (L858R)	3 nM (del 19) 13 nM (L858R)
Drug Interactions	Proton pump inhibitors H_2 antagonists CYP3A4 inducers/inhibitors	Proton pump inhibitors H_2 antagonists CYP3A4 & CYP2D6 inducers/inhibitors	P-gp inducers/inhibitors	CYP3A4 inducers/inhibitors P-gp inducers/inhibitors
Food interactions	Food increases bioavailability. Take on empty stomach.	None	High-fat meals decrease bioavailability. Take on empty stomach.	None
Metabolism	Liver CYP3A4	Liver CYP3A4 CYP2D6	Non-enzyme catalyzed Michael adduct formation to electron-rich small molecules and proteins	Liver CYP3A4
Method of Elimination	83 % feces (1 % unchanged drug) 8 % urine	86% feces 4% urine	85% feces (89% unchanged) 4% urine	68 % feces (2 % unchanged) 14 % urine
CNS penetration	~5 % CNS response achieved in patients refractory to gefitinib.	~1 %	CSF concentration of 1nM in single case report	0.2-1 % in case reports. Higher clinical response witnessed than first generation TKIs.

Abbreviations. IC_{50}: concentration at which drug inhibits 50 % of receptor's activity; nM: nanomolar; CYP: cytochrome P450; P-gp: P-glycoprotein

As knowledge of the EGFR activating mutations developed, development of companion diagnostic assays for a predictive biomarker of treatment sensitivity utilized in registrational studies facilitated FDA-approval of this agent for first-line treatment of patients with metastatic NSCLC whose tumors harbor EGFR exon 19 deletions or the L858R mutation in May 2013. EURTAC, the open-label, randomized phase 3 trial demonstrated improvement in PFS (9.7 months vs 5.2 months, HR 0.37; p<0.001) for erlotinib compared

with standard first-line chemotherapy. A benefit in OS was not demonstrated, but most patients in the chemotherapy group crossed over to receive erlotinib upon disease progression [27]. Multiple subsequent phase III trials have shown that erlotinib improves PFS compared to conventional platinum-based chemotherapy in first-line treatment of NSCLC [28,29].

Clinical safety

Skin rash is the most common toxicity encountered with erlotinib use. All-grade rash occurs in 61 to 80 % and grade 3 or 4 rash occurs in 2 to 13 % of patients. The classic erlotinib (or any EGFR-inhibitor) rash is an acneiform rash that occurs on the scalp, cheeks, nose, perioral area, and upper trunk. The rash typically manifests within the first 2 weeks of treatment but can occur as late as 2 months after starting therapy [30]. Less commonly, bullous eruptions can occur. Toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome(SJS) have developed in patients taking erlotinib.

Diarrhea is another common adverse event caused by erlotinib. Any grade diarrhea occurs in 21 to 57 % and grade 3 or 4 diarrhea occurs in 1-6 %. Rarely (<5 %), patients can develop cerebrovascular events, gastrointestinal perforation, fatal pneumonitis, liver/renal failure, or corneal perforation. Patients on warfarin should have INR monitored closely as fatal hemorrhage has occurred in patients on both erlotinib and warfarin. Dose reduction is necessary in 19 to 21 % of patients (7-12 % rash, 5-7 % diarrhea), and treatment interruption occurs in 27 % (14 % rash, 6 % diarrhea). Treatment discontinuation is rarely necessary, occurring in only 3 to 6 % of patients [11,25,27,28].

Pharmacology

Erlotinib hydrochloride, 6, 7-bis(2-methoxyethoxy)-*N*-(3-ethynyl-phenyl) quinazolin-4-amine [CP-358774 , OSI-T774, Tarceva, (Genentech)] reversibly inhibits the EGFR tyrosine kinase at the ATP binding domain with high selectivity and potency. Erlotinib inhibits purified EGFR kinase with an IC_{50} of 2 nanomolar (nM) and inhibits EGFR autophosphorylation with an IC_{50} of 20 nM in intact tumor cells. Erlotinib binds more strongly to EGFR harboring L858R or del 19 than it does to wild-type EGFR [31], but it binds to several off-target kinases, including cyclin G-associated kinase (GAK), Ste20-like kinase (SLK) and serine/threonine kinase 10 (STK10). The inhibition of STK10 enhances lymphocyte migration and cytokine release, which contributes to the dermatologic toxicity of the medication [32]. Erlotinib carries a recommended oral starting dose of 150 mg daily. The drug reaches peak plasma levels approximately 4 hours after administration, and the volume of distribution is 232 L [33]. Bioavailability of erlotinib is 60 % but increases to nearly 100 % if administered with food [34]. The effect of food on erlotinib absorption appears to have inter-and intra-individual variability, and erlotinib is to be taken at least 1 hour prior to or at least 2 hours after eating [35]. Erlotinib has multiple drug interactions, and van Leeuwen et al. have previously reviewed common TKI drug-drug interactions in detail [170]. Gastric pH is a significant determinant in erlotinib absorption. As a weak base, the drug can exist in either ionized or non-ionized forms. At an elevated pH, the drug shifts to its less-soluble, non-ionized form, and drug absorption decreases. Therefore, proton pump inhibitors and other drugs that increase gastric pH lead to significantly decreased serum levels of erlotinib and can decrease erlotinib AUC by 46 % and maximum erlotinib serum concentration by 61 %. Interestingly, this can be overcome by administering erlotinib with acidic beverages like cola [36].

Upon absorption, erlotinib is heavily protein-bound (93-95 %) to albumin and α-1 acid glycoprotein, and its median half-life is 36 hours [37]. There is no significant association with drug clearance and gender, weight, or age [33]. Erlotinib is primarily metabolized to its active metabolite, desmethyl erlotinib, in the liver through CYP3A4 and to a lesser degree by CYP1A2 and extrahepatic CYP1A1. Therefore, induction and

inhibition of CYP3A4 activity through medications like rifampin and ketoconazole, respectively, impacts serum erlotinib concentrations. Tobacco is a well-known CYP inducer and increases erlotinib clearance by 24 % [37]. Serum erlotinib levels have been successfully monitored in patients who are on concomitant medications that interact with CYP3A4, and levels correlate with disease response [38]. Caution is recommended when erlotinib is used in patients with hepatic failure; however, PK and safety profiles are similar between patients with liver impairment and normal liver function [39]. Erlotinib is a substrate for P-glycoprotein (P-gp/ABCB1) and breast cancer resistance protein (BRCP/ABCG2). P-gp and BRCP are efflux transporters that negatively regulate intestinal absorption of erlotinib [40]. In patients with genotypes that produce low P-gp or BRCP expression, higher trough concentrations of erlotinib and higher rates of clinical toxicity exist [41,42]. After metabolism to desmethyl erlotinib, oxidation and glucuronidation occur [43].

Erlotinib and its metabolites are eliminated predominantly in the feces (83 %) and less than 2 % of the recovered drug is unchanged, indicating extensive metabolism prior to excretion [44]. Given the limited role of renal metabolism and excretion of erlotinib, it can be safely administered in patients who have chronic renal failure and who are undergoing hemodialysis [45].

CNS penetration

Central nervous system (CNS) metastases occur commonly in NSCLC. Erlotinib crosses the blood-brain-barrier (BBB) to a low degree. The efflux transporter proteins P-gp and BRCP are expressed on the BBB and decrease CNS accumulation of the erlotinib. The BRCP 421A polymorphism leads to decreased efflux of erlotinib and higher CNS penetration [42]. In a small study of NSCLC patients with brain metastases who received erlotinib, the mean CSF concentration of erlotinib was 54 ng/ml. The mean CSF penetration rate of erlotinib was 5.1 %. Even at these concentrations, however, disease response in the CNS was witnessed [46]. In a case series of 23 Korean patients with untreated brain metastases who were treated with erlotinib 150 mg daily or gefitinib 250 mg daily, intracranial disease response was achieved in 69.6 % and intracranial disease control was witnessed in 82.6 % of patients [47]. High dose erlotinib given at 600 mg every 4 days or 300 mg every other day has demonstrated efficacy in controlling CNS metastases with tolerable side effects in 2 case reports [46,48,49]. Pulsatile dosing of erlotinib has also been investigated. In a retrospective analysis of 9 patients with EGFR-mutated NSCLC and brain metastases, a median erlotinib dose of 1500 mg weekly was utilized. Partial CNS response was witnessed in 67 % of patients with a median time to CNS progression of 2.7 months and median OS of 12 months [50].

Mechanisms of resistance

The most common mechanism of resistance to erlotinib is a secondary mutation in the EGFR gene at exon 20 that leads to substitution of methionine for threonine at position 790 (T790M) causing a mutation in the EGFR kinase domain. T790M occurs as the method of erlotinib resistance in approximately 50 % of patients upon disease progression [51,52]. Erlotinib forms an important hydrogen bond with the threonine residue at position 790, which is positioned in the hydrophobic ATP-binding pocket of the catalytic region [53]. Erlotinib-resistance with T790M-mutated EGFR is driven by increased binding affinity for ATP at the ATP binding pocket. The L858R mutation reduces EGFR affinity for ATP, thereby increasing susceptibility to erlotinib inhibition, but the T790M mutation restores EGFR ATP affinity to near wild-type levels ($K_{m[ATP]}$: L858R 148 µM, T790M 5.9 µM, WT EGFR 5.2 µM), and decreases the competitive advantage of the ATP competitive inhibitor erlotinib. Irreversible inhibitors of the EGFR ATP binding domain, such as afatinib, can overcome resistance to T790M in vitro because they are not in competitive equilibrium with ATP [51]. *MET* amplification (see gefitinib-resistance below), small cell transformation and *HER2* amplification have all

been less commonly observed as causes for erlotinib resistance [54]. A case report of a patient with EGFR-mutated NSCLC on erlotinib, who developed *MET* amplification as a resistance mechanism, showed tumor response with the addition of crizotinib [55].

Gefitinib

Clinical efficacy

Gefitinib was the first EGFR TKI to reach the market, and originally received accelerated approval by the FDA for marketing in May 2003 for patients with NSCLC who were refractory to platinum- and docetaxel-based chemotherapy [56]. The approval was based on a randomized, double-blind, phase II trial of 216 patients comparing gefitinib 250 mg/day with gefitinib 500 mg/day. The overall response was 12 % in the 250 mg dose and 9 % in the 500 mg dose with a 1 year OS of 25 % [57]. However, a survival benefit in this patient population was unable to be demonstrated and AstraZeneca suspended promotion of gefitinib. Access to the drug was limited to patients who were previously responding to gefitinib. In September 2011, per FDA request, AstraZeneca voluntarily withdrew gefitinib from the US market due to failure to demonstrate a survival benefit for the drug.

While gefitinib did not appear to have meaningful activity in an unselected NSCLC patient population, multiple subsequent large phase III studies performed in Asian populations consistently demonstrated clinical benefit for first-line gefitinib for patients with exon 19 and L858R mutations. In these studies, gefitinib improved PFS by 3 to 5 months compared with conventional platinum-based chemotherapy [58-61]. Given this data, gefitinib attained widespread approval outside of the United States.

In July 2015, the FDA once again approved gefitinib under orphan product designation for first-line treatment of patients with advanced NSCLC with the exon 19 or L858R EGFR-activating mutations. The approval was based on a phase IV study of gefitinib for first-line treatment of Caucasian patients with exon 19 or L858R EGFR mutations. In the 106 patients in this study, the overall response rate was 69.8 % with a median PFS of 9.7 months and median OS of 19.2 months [5].

Clinical safety

Gefitinib causes less dose-dependent dermatologic toxicity than erlotinib. This is likely due to the comparatively lower standard therapeutic dose of gefitinib commonly administered to patients compared to other EGFR TKIs. The approved clinical dose of gefitinib (250 mg daily) is only 33 % of the maximum tolerated dose, whereas erlotinib is clinically dosed at its maximum tolerated dose of 150 mg daily. There does not appear to be a difference in PFS or OS for lower dose gefitinib (250 mg daily) vs higher dose gefitinib (500 mg daily) [62].For gefitinib, all-grade rash ranges from 44 to 77 % but grade 3 to 5 rash is very rare (<5 %) with gefitinib. Skin rash on gefitinib manifests similarly compared to erlotinib, and TEN and SJS have occurred in patients on gefitinib as well. Like erlotinib, diarrhea with gefitinib is relatively frequent but not typically severe (all-grade: 32-47 %, grade 3-5: 1-4 %). Grade 3 or 4 transaminitis occurs in up to 11 % of patients. Rare fatal hepatotoxicity and interstitial pneumonitis have occurred with gefitinib [59,60,63,64].

Pharmacology

Gefitinib, 4-quinazolinamine,*N*-(3-Chloro-4-flurophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy] [ZD-1839, Iressa (AstraZenaca)] reversibly inhibits EGFR by competing with ATP at the tyrosine kinase domain. The recommended starting dose is 250 mg orally daily. Gefitinib has an IC_{50} of 0.03-0.1μM for EGF, but demonstrates similar IC_{50} values for other growth factors, including PDGF and IGF [56]. Maximum plasma

levels are achieved between 3 and 7 hours after administering gefitnib with oral bioavailability of approximately 60 % [65,66]. Unlike erlotinib, absorption of gefitnib is not impacted by food [66]. However, the drug has significantly decreased solubility and absorption at higher gastric pH, therefore, use of proton pump inhibitors and H_2-receptor antagonists should be avoided when possible. Upon absorption, gefitnib is 90 % protein bound to alpha$_1$-acid glycoprotein and albumin and the volume of distribution is about 1700 L [67]. Gefitnib is metabolized predominantly in the liver by CYP3A4 and CYP2D6 and to a lesser degree by CYP3A5. Therefore, gefitnib interacts with similar CYP3A4 inducers and inhibitors as erlotinib as well as CYP2D6 inhibitors/inducers [68]. Although there is no specific dose adjustment recommended for patients with hepatic impairment, caution is recommended, as serum gefitnib levels increase in this setting. The primary metabolite of gefitnib, O-desmethyl gefitnib, is inactive [69]. Only 4 % of the drug is renally excreted, and no dose-adjustments are recommended for patients with renal failure.

CNS penetration

Gefitnib does not penetrate the BBB well. In a study of 8 patients with EGFR activating mutations with CNS metastases, the CSF gefitnib concentration was 3.7+/- 1.9 ng/mL and the CSF penetration of the drug was 1.13+/-0.36 %. Despite only 1 % of the drug penetrating into the CSF, a partial response in CNS metastases was demonstrated in one patient. Erlotinib does have superior CNS penetration to gefitnib, and erlotinib has successfully induced CNS response to previously gefitnib-refractory CNS metastases [70]. High-dose gefitnib has been attempted in order to improve control of CNS disease. In a case report, gefitnib was increased to 1000 mg daily. This resulted in higher CSF concentration of the drug as well as radiographic and symptomatic response in the patient. However, transaminitis and somnolence developed due to the high dose of the drug. Of note, at autopsy, the patient's extracranial tumors all harbored T790M mutation, but the brain metastases did not carry this mutation [71].

Mechanism of resistance

Like erlotinib, the main mechanism EGFR of resistance to gefitnib is through the secondary T790M mutation at the intracellular catalytic domain [52,72]. In a small series of 18 patients, 4 (22 %) developed gefitnib resistance through *MET* amplification. This leads to ErbB3 (HER3)-dependent PI3K/AKT activation, which bypasses the need for signaling through the inhibited EGFR. The T790M mutation was present in about half of the samples in which *MET* amplification occurred [54,73].

Afatinib

Clinical efficacy

Afatinib was the first irreversible EGFR TKI available on the market. The drug was first FDA approved on July 2013 for first-line treatment of patients with metastatic NSCLC whose tumors harbor exon 19 or L858R activating EGFR mutations. The approval was based on results from the LUX-Lung 3 trial, a randomized phase III study comparing afatinib with cisplatin plus pemetrexed in patients with metastatic EGFR-mutated NSCLC. A total of 345 patients were randomized in this trial. Overall response rate was significantly higher in patients receiving afatinib (56 % vs 23 %; P=.001). Median PFS was 13.6 months with afatinib and 6.9 months with chemotherapy (HR 0.47; 95 % CI, 0.34 to 0.65; P=.001). Both Asian and non-Asian patients were enrolled in the study, but the study was underpowered to detect a PFS difference in non-Asian patients [74]. Similarly, the LUX-Lung 6 trial demonstrated a significant PFS benefit for first-line afatinib versus cisplatin and gemcitabine in Asian patients [75]. A combined analysis of LUX-Lung 3 and LUX-Lung 6 demonstrated an overall survival benefit for patients with exon 19 deletion but not with the L858R point mutation [76]. In the randomized phase IIB LUX-Lung 7 trial, which compared first-line afatinib vs gefitnib

in EGFR-mutated NSCLC, afatinib produced superior PFS at 12, 18 and 24 months [77]. Afatinib also recently gained FDA approval in April 2016 for second-line treatment of advanced squamous cell NSCLC. Compared with erlotinib, afatinib prolonged both PFS and OS in patients with advanced squamous cell lung cancer [78]. Patients who develop acquired resistance to erlotinib or gefitinib have little response (8 %) to single-agent afatinib [79]. However, dual inhibition of EGFR with afatinib and cetuximab induced responses in patients who previously progressed on erlotinib or gefitinib. Responses of 32 % and 25 % were witnessed in T790M-positive and T790M-negative tumors, respectively [80].

Clinical safety

Diarrhea occurs commonly with afatinib. All-grade diarrhea has been reported as high as 95 % with grade 3 to 5 diarrhea of 14 % in clinical trials. Rash/acne (all-grade: 89 %, grade 3-5: 16 %) and stomatitis (all-grade: 51-72 %, grade 3-5: 8 %) are frequently encountered [74,75]. Rare adverse effects include decrease in left ventricular ejection fraction, interstitial lung disease and fatal hepatotoxicity.

Pharmacology

Afatinib, (E)-N-[4-(3-chloro-4-fluoroanilino)-7-[(3S)-oxolan-3-yl]oxyquinazolin-6-yl]-4-dimethylami-no)-but-2-enamide [BIBW 2992, Gilotrif, (Boehringer Ingelheim)], irreversibly inhibits ErbB1 (EGFR), ErbB2 (HER2), and ErbB4 (HER4). As opposed to first-generation EGFR TKIs, afatinib forms an irreversible covalent bond at the ATP-binding site of EGFR. The acrylamide group permits afatinib to form a covalent bond at the tyrosine kinase site and is important in its activity against T790M, HER2, and ErbB4 [81]. Afatinib demonstrates a similar IC_{50} for wild-type EGFR and L858R-mutated EGFR, but it is 100-times more potent against T790M-mutated EGFR. However, clinically meaningful inhibition of T790M with higher dose afatinib is unable to be achieved without substantial toxicity [80]. Afatinib also inhibits HER3 activity. HER3 activates the PI3K/AKT survival pathway in NSCLC. This receptor lacks intrinsic kinase activity and its activity depends upon transphosphorylation by heterodimerization with EGFR, HER2 and MET. Outside of the ErbB family of receptors, afatinib does not significantly inhibit other tyrosine or serine/threonine kinases [82].

The recommended starting dose of afatinib is 40 mg administered orally daily, and peak plasma concentrations are reached 2 to 5 hours later. Absorption is decreased with high-fat meals and the drug should not be taken with food. Solubility of afatinib is not affected by physiologic gastric pH levels; therefore, antacids do not affect absorption. Upon absorption, afatinib is heavily protein bound (~95 %), bioavailability is 92 %, and half-life is 37 hours [83]. Unlike the reversible EGFR TKIs, afatinib is not significantly metabolized through the CYP pathway, and therefore, CYP inhibitors and inducers have little impact on plasma levels of afatinib. Metabolism of afatinib is primarily mediated by non-enzyme catalyzed Michael adduct formation to nucleophilic, electron-rich small molecules and proteins. However, 89 % of afatinib is excreted as the unchanged parent compound (85 % feces, 4 % urine) [84].

Afatinib serves as a substrate and inhibits both P-gp and BRCP. Inhibitors of P-gp, such as ritonavir can increase concentrations of afatinib, while inducers of P-gp reduce afatinib levels. The clinical consequences of concurrent use of afatinib with P-gp inducers/inhibitors are questionable, and these drugs can be administered 6-12 hours apart to avoid significant interactions [85].

In patients with renal impairment with eGFR of 15-29 mL/minute/1.73 m^2, a dose reduction to 30 mg daily is recommended. Canadian labeling recommends against use of afatinib if CrCl is less than 30 mL/min. The drug has not been well-studied in patients undergoing hemodialysis. Caution is advised in patients with hepatic impairment, but no specific dose adjustments are recommended. Canadian labeling advises against

afatinib use in patients with Child-Pugh class C cirrhosis.

CNS penetration

The LUX-Lung trials allowed enrollment of patients with stable brain metastases. The LUX-lung 3 trial enrolled 35 patients with brain metastases who were treated with either first-line afatinib or cisplatin and pemetrexed. The median PFS was 11.1 months in the afatinib group and 5.4 months in the chemotherapy group (HR, 0.52; p=0.13). In an analysis 541 patients treated on the afatinib compassionate use program, 100 patients who had brain or leptomeningeal metastases were identified (74 % had documented EGFR activating mutation). Thirty-five percent of evaluable patients developed a CNS response with median duration response of 120 days. One patient who developed a response on 40 mg of oral afatinib, achieved a CSF afatinib concentration of about 1 nM [86].

Mechanisms of resistance

A study evaluating tumor samples from 42 patients who experienced disease progression on afatinib shed light on acquired resistance mechanisms to afatinib. T790M EGFR mutation was detected in 47.6 % of specimens. There was no association with previous first-generation EGFR TKI use and detection of T790M after resistance to afatinib. There were no mutations detected in *PIK3CA, HER2, BRAF, KRAS, NRAS, MEK1, AKT2, JAK2* or *LBK1* [87]. Pre-clinical models have suggested that *MET* amplification, upregulation of the STAT3 pathway, and FGFR1-induced survival signals contribute to afatinib resistance [88-90].

Osimertinib

Clinical efficacy

Osimertinib was the first "third-generation" EGFR TKI to make it to market. The third generation TKIs are designed to irreversibly target mutant EGFR (exon 19 deletion, L858R and T790M), while sparing wild-type EGFR. In mouse models of EGFR-mutated NSCLC, osimertinib had similar antitumor activity as afatinib against L858R but more activity against T790M NSCLC [91]. The phase I component of the AURA trial had dose escalation and expansion cohorts, which enrolled 253 patients with locally advanced or metastatic EGFR-mutated NSCLC who had disease progression on an EGFR TKI. A total of 127 (50.2 %) patients were confirmed to have the T790M mutation. In all patients, the response rate to osimertinib was 51 %. In patients harboring a T790M mutation, the response rate to osimertinib was 61 %, but in patients without detectable T790M, the response rate was only 21 %. The median PFS in T790M-positive patients was 9.6 months and 2.8 months in T790M-negative patients [12]. Preliminary data from the 201 patients enrolled in the phase II extension of AURA demonstrated an ORR of 58 %. The phase II AURA2 trial also has preliminary data demonstrating a 64 % ORR with osimertinib. Both phase II trials have not yet reached sufficient maturity to determine a median PFS [92]. In November 2015, osimertinib was granted accelerated FDA approval for treatment of EGFR-mutated NSCLC that has progressed on a previous EGFR TKI. Osimertinib is currently in phase III development for adjuvant treatment as well as first- and second-line treatment of advanced EGFR-mutated NSCLC.

Clinical safety

Overall, osimertinib is well-tolerated. All-grade diarrhea occurs in 42 % of patients, with only 1 % experiencing grade 3 or 4 diarrhea. Similarly 41 % of patients will experience a rash but only 0.5 % will develop grade 3 or 4 rash. Dose reductions are necessary in only 4 % of patient on osimertinib. Prolongation of the QTc interval was the most common reason for dose reduction, and occurred in 2.2 % of patients. As with other EGFR TKIs, fatal pneumonitis has occurred in patients with osimertinib but is rare

(1 %). Fatal stroke and pneumonias have also occurred in patients on the drug. Cardiomyopathy, with a decline in left ventricular function by >10 % was also rarely encountered. Discontinuation of osimertinib, due mostly to pneumonitis and stroke, was necessary in 5.6 % of patients enrolled in the key clinical trials to date [12,92,93].

Pharmacology

Osimertinib, N-[2-[2-(dimethylamino)ethyl-methylamino]-4-methoxy-5-[[4-(1-methylindol-3-yl)-pyrimi-din-2-yl]amino]phenyl]prop-2-enamide [AZD-9291, Tagrisso, (AstraZeneca)], irreversibly inhibits mutated EGFR by covalently bonding with the cysteine-797 residue in the ATP binding site. The drug's affinity for T790M may be related to hydrophobic interactions between the methionine moiety on T790M-mutated EGFR and the pyrimidine component of osimertinib. It has nearly 200-fold greater affinity for EGFR with L858R/T790M mutation than wild-type EGFR in vitro [91]. Osimertinib also inhibits HER2, HER3, HER4, ACK1 and BLK in vitro [94]. The manufacturer-recommended oral dose of osimertinib is 80 mg daily. Antacids do not impact drug absorption. Intake of high fat foods prior to osimertinib administration mildly increases osimertinib plasma concentration by about 14 % [95]. After administration, maximum plasma concentration is reached in approximately 6 hours, the volume of distribution is 986 L, and the half-life is 48 hours [12,94]. Metabolism primarily occurs via oxidation in the liver through CYP3A4. The two active metabolites of osimertinib are AZ7550 and AZ5104, the latter of which causes more wild-type EGFR inhibition than the parent drug [91]. There is no difference in absorption or metabolism of osimertinib based on age, ethnicity, or tobacco use. Osimertinib is a substrate for P-gp and BRCP, and it inhibits BRCP but not P-gp. Osimertinib is eliminated in feces (68 %) and in the urine (14 %) with only 2 % of the drug being unchanged at elimination. Moderate renal impairment (creatinine clearance >30 mL/min) and mild hepatic impairment do not appear to impact drug levels or toxicity. Osimertinib use in severe renal impairment (CrCl<30 mL/min) or moderate to severe hepatic impairment has not been studied [94].

CNS penetration

In a mouse model, osimertinib's distribution to the brain was 10-times higher than gefitinib and response in brain metastases were witnessed with osimertinib. In AURA and AURA2, the CSF concentrations of osimertinib in two patients were 0.2 and 1 % of the predicted plasma concentrations [94]. Case reports of two patients T790M-mutated EGFR and untreated brain metastases showed that osimertinib 80 mg daily induced sustained partial responses to both CNS and systemic disease [96]. The phase I BLOOM study evaluated osimertinib 160 mg daily for patients with EGFR-mutated NSCLC with leptomeningeal disease. All patients had previously received an EGFR TKI. Preliminary data from this trial showed that in 12 patients who had 12-week imaging assessment, high-dose osimertinib produced radiologic improvement in CNS disease in 7 patients (58.3 %), stable disease in 2 patients (16.7 %) and 3 patients were not evaluable. Overall, the higher dose of osimertinib was well-tolerated, but 5 of 20 patients required dose interruptions and only 2 required dose reductions to 80 mg daily. Grade 3 neutropenia was observed in one patient on the higher dose of osimertinib, but this resolved after 3 days of holding the drug and did not recur after reducing the dose to 80 mg [97].

Resistance mechanisms

Currently, the most commonly reported acquired resistance mutation to osimertinib is the missense mutation of cysteine for serine at position 797 (C797S). This substitution prevents osimertinib from forming a covalent bond at the kinase-binding site [98]. The C797S mutation accounts for up to 40 % of acquired resistance to osimertinib in the limited data on this topic to date [99]. Clinically, loss of the T790M

mutation has been observed after treatment with osimertinib [99]. In PC9 and NCI-H1975 cell lines, acquired NRAS and KRAS mutations were noted after exposure to osimertinib. A number of these resistant cell lines were sensitive to combination of osimertinib and the MEK inhibitor selumetinib [100].

Investigational agents (Table 2)

Another third generation EGFR TKI, olmutinib (BI 1482694/HM61713), gained FDA breakthrough therapy designation in December 2015. Like osimertinib, olmutinib targets mutant EGFR and T790M while sparing wild-type EGFR. In the phase I/II HM-EMSI-101 trial, patients with EGFR-mutated NSCLC who progressed on a previous EGFR TKI received olmutinib. As of June 2016, 76 patients had received the drug at the recommended phase II dose of 800 mg daily. The drug produced an ORR of 62 % with a DCR of 91 % by independent assessments. Few grade 3 or 4 toxicities were observed, including a 5 % risk of grade 3 or 4 skin rash [101]. The phase II ELUXA 1 (HM-EMSI-202) trial is open for patient recruitment and is investigating the efficacy of olmutinib in patients who have developed T790M after exposure to first-line EGFR TKIs [102]. Multiple monotherapy trials of olmutinib are being planned, including phase III studies comparing olmutinib with platinum-based chemotherapy in a second-line setting and comparing olmutinib with afatinib in the first-line treatment of EGFR-mutated NSCLC. Investigators are also planning on trials evaluating olmutinib in combination with various agents, including pembrolizumab, bevacizumab, nintedanib, afatinib, and the IGF ligand-neutralizing antibody BI836845.

Table 2. EGFR TKIs in development

Drug Name	Targets	Clinical Activity	Select ongoing/future trials
Olmutinib (BI 482694/HM61713) [102]	Mutant EGFR T790M	Phase I/II (n=76) ORR 62%, DCR 91%	Ongoing Phase II ELUXA 1 (NCT02485652) Planned: Olmutinib vs Platinum regimen in 2nd line; Olmutinib vs afatinib in 1st line; Combinations with: pembrolizumab, bevacizumab, nintedanib, afatinib, BI836845
ASP8273 [103]	Mutant EGFR T790M	Phase I ORR 50% (n=36); In T790M ORR 80% (n=15)	Ongoing phase II (NCT02192697); Ongoing 1st line study (SOLAR) (NCT02588261) ASP8273 vs erlotinib or gefitinib
EGF816 [104]	Mutant EGFR T790M	Phase I/II (n=22) ORR 55%, DCR 86%	Ongoing phase I/II (NCT02108964)
AZD3759 [106]	Mutant EGFR High CNS penetration	Phase I (n=2) 1 intracranial PR and 1 intracranial SD	Ongoing phase I NCT02228369)
Epitinib (HPML-813) [105]	Mutant EGFR High CNS penetration	Phase I (n=12) 71% TKI naïve pts achieved PR in CNS; 100% pts w/previous TKI exposure achieved SD in CNS	Ongoing phase I (NCT02590952)
Tarloxotinib bromide (TH-4000) [107]	Wild-type EGFR Mutant EGFR (non-T790M) HER2	N/A	Ongoing phase II (NCT02454842)

Other third-generation EGFR TKIs in development, including ASP8273 and EGF816, have selective activity against mutant EGFR (L858R, del exon 19) as well as T790M without affecting wild-type EGFR. These compounds have produced ORRs of 35.5 to 44 %, DCRs of 65 to 91 % and median PFS of 6.7 months to 9.2 months, respectively [103,104].

Epitinib (HMPL-813) is an EGFR TKI that was specifically designed for high CNS penetration. In a phase I study (NCT02590952), the drug was well-tolerated, with grade 3 elevation in liver function tests in 2 to 5 % of patients. In the dose expansion arm of the study, 12 patients were evaluable as of October 2015. Of these, 5 patients who were TKI naïve achieved a PR both systemically and in the CNS. In 5 patients who had previous TKI exposure, all had stable disease in the brain [105]. AZD3759 is another EGFR TKI designed to have high BBB penetration. In a phase I trial (NCT02228369) of patients with EGFR-mutated NSCLC and brain metastases, of the 2 evaluable patients with brain metastases reported to date, 1 patient achieved an intracranial PR and the other had stable CNS disease. The trough CSF concentration of AZD3759 was between 6 and 7.7 nM, which is close to the mutant EGFR IC_{50} of AZD3759 [106].

Tarloxotinib bromide (TH-4000) is a prodrug that is converted to an irreversible TKI of wild-type EGFR, mutant EGFR and HER2 under hypoxic conditions. As the tumor environment is typically hypoxic, it is hypothesized that greater intra-tumoral TKI exposure and a greater therapeutic index will be achieved with this drug, and EGFR TKI resistance has been overcome with TH-4000 in animal models. It is currently being investigated in a phase II study (NCT02454842) for patients with EGFR-mutated NSCLC who have non-T790M mediated resistance to first-line EGFR TKI [107].

ALK fusion protein

In 2007, another key molecular driver of NSCLC was identified. An inversion within chromosome 2p causes the fusion of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*) gene [6,108]. At least 8 different *EML4-ALK* fusion variants have been identified. All variants harbor a breakpoint at intron 19 of *ALK* and variable intron breakpoints in *EML4* [109,110]. The *EML4-ALK* oncogene generates the EML4-ALK fusion protein and all variants of this protein contain an EML4 transmembrane domain and an intracellular ALK tyrosine kinase domain. The EML4 domain promotes self-association of the ALK tyrosine kinase domain driving autophosphorylation and downstream signaling, promoting oncogenesis through PI3K/AKT and JAK/STAT pathways [6,111]. The EML4-ALK fusion mutation occurs in approximately 3 to 7 % of NSCLC (typically adenocarcinoma) with a predilection for younger patients with low tobacco exposure [112]. Currently, there are three ALK TKIs, which have widespread clinical approval for the treatment of ALK-positive NSCLC (Table 3).

ALK TKIs

Crizotinib

Clinical efficacy

Crizotinib first gained accelerated FDA approval in August 2011 for all patients with advanced *ALK*-positive NSCLC based on two small phase I/II clinical trials, which demonstrated response rates of 50 to 60 % and PFS of over 9 months [113,114]. Regular approval for the same indication was granted by the FDA in November 2013 due to evidence presented in the phase III, open label PROFILE 1007 trial that compared crizotinib to docetaxel or pemetrexed for use in advanced *ALK*-positive NSCLC. In 347 previously treated *ALK*-positive patients, crizotinib produced superior response rates compared with chemotherapy (ORR 65

vs 20 %, respectively). The median PFS with crizotinib was 7.7 months vs 3.0 months with chemotherapy (HR for progression or death with crizotinib, 0.49; 95 % CI, 0.37-0.64; p<0.001) [115]. In the first-line treatment setting, PROFILE 1014 compared crizotinib to pemetrexed plus platinum demonstrating superior ORR (74 % vs 45 %; p<0.0001) and PFS (10.9 vs 7 months; HR 0.45;95 % CI, 0.35-0.60) with crizotinib [116].

Table 3. ALK TKI characteristics

ALK TKI Name	Crizotinib	Ceritinib	Alectinib
Primary Targets	ALK c-MET ROS1	ALK (including crizotinib resistance mutations) IGF-1R INsR ROS1	ALK (including crizotinib resistance mutations) RET
Type of inhibition	ATP-competitive	ATP-competitive	ATP-competitive
Clinical approval	First-line treatment of advanced ALK-positive NSCLC	Second-line treatment of advanced ALK-positive NSCLC	Second-line treatment of advanced ALK-positive NSCLC
ALK IC_{50} (cell free assay)[23]	3.6 nM	0.15 nM	1.9 nM
Drug Interactions	CYP3A4 inducers/inhibitors P-gp inducers/inhibitors	CYP3A4 inducers/inhibitors P-gp inducers/inhibitors	CYP3A4 minor substrate, no significant interactions with inducers/inhibitors
Food interactions	Take with or without food.	High fat meal increases bioavailability. Take on empty stomach.	High fat meal increases bioavailability. Take with food.
Metabolism	Liver CYP3A4/5	Liver CYP3A4	Liver CYP3A4
Method of Elimination	63% feces (53% unchanged) 22% urine (2% unchanged drug)	92% feces (68% unchanged) 1% urine	98% feces (84% unchanged) <1% urine
CNS penetration	0.06-0.26% CNS penetration	Intracranial disease control 80%. CSF concentrations unpublished to date.	86% CNS penetration in 4 patient study

Clinical safety

Crizotinib produces unique visual disturbances in up to 70 % of patients, though these are rarely severe. The visual disturbances are pronounced with changes in ambient lighting and can present as floaters, trails or flashes of light in the peripheral vision. Vision changes tend to be mild, brief, and self-limited and no associated ophthalmologic changes were identified in patients on the PROFILE 1005 study. Dose adjustment is rarely needed for vision changes [113,116,117]. Diarrhea, peripheral edema, and vomiting occur in approximately 50 to 60 % of patients, but reach grade 3 or 4 toxicity in less than 5 % of patients. Grade 3 or 4 elevations of aminotransferase levels occur in up to 15 % of patients receiving crizotinib and can be managed with dose interruptions or reductions. Treatment discontinuation is rarely necessary for liver function abnormalities [113,116]. Symptomatic hypogonadism has been linked to crizotinib use in male patients. In one study, 84 % of male patients were found to have low free testosterone levels while taking crizotinib with concomitant decline in FSH and LH, suggesting that crizotinib may induce secondary hypogonadism [118]. Therefore, monitoring of free testosterone may be necessary in males taking

crizotinib, particularly in patients with symptoms suggestive of androgen deficiency. Rare fatal interstitial lung disease/pneumonitis has been reported [119]. Bradycardia and Q-T prolongation can occur in about 5 % of patients taking crizotinib [120].

Pharmacology

Crizotinib, 3-[(1R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridine-2-amine [PF-2341066, Xalkori, (Pfizer)] competes with ATP at the catalytic domain of the ALK tyrosine kinase and mesenchymal-epithelial transition factor (c-Met) kinase [111]. Identified as a potent inhibitor of ALK and c-MET in biochemical enzymatic screens, crizotinib inhibits tyrosine phosphorylation of NPM-ALK (a fusion protein present in some cases of anaplastic large cell lymphoma) with mean IC_{50} values between 25 to 50 nM and c-MET with mean IC_{50} values of 5 to 20 nM [121-123]. The EML4-ALK fusion protein is inhibited less potently by crizotinib, with IC_{50} values between 250 to 340 nM [124,125]. The recommended dose of crizotinib is 250 mg administered twice daily. Food does not have a significant impact on absorption. Bioavailability of crizotinib is 43 % and peak plasma concentration is reached at a median of 4 hours. The drug is heavily protein bound (91 %) with a volume of distribution of 1772L and a terminal half-life of 42 hours [126]. Crizotinib undergoes extensive hepatic metabolism through the CYP3A4/5 pathway, and CYP3A4 inducers and inhibitors interact with crizotinib [126,127]. Steady-state is achieved within 15 days, and crizotinib exhibits a non-linear PK, meaning the rate of clearance decreases with multiple dosing. The geometric mean values for CL/F declined from 100 L/hr after a single dose of crizotinib to 64.5 L/hr and 60.1 L/hr after 15 and 28 days of dosing, respectively. This phenomenon may be due to autoinhibition of CYP3A4. Age, gender, race and body weight do not appear to influence the PK of crizotinib [128]. 63 % of the drug is eliminated via the feces (53 % unchanged) and 22 % in the urine (2.3 % unchanged) [126]. Given the extensive hepatic metabolism of crizotinib, caution is advised in patients with liver dysfunction, though no specific dose adjustments are recommended. Likewise, crizotinib has not been well-studied in patients with renal impairment, and close monitoring is advised in this setting.

CNS penetration

Overall, crizotinib has poor CNS penetration. In a 29-year-old patient with metastatic *ALK*-positive NSCLC, new brain and leptomeningeal metastases developed 7.5 months after starting crizotinib. His plasma crizotinib concentration was 237 ng/mL, but his CSF concentration was only 0.616 ng/mL, with a CSF-to-plasma crizotinib ratio of 0.0026 [125]. Despite poor CNS penetration, crizotinib has been reported to induce responses in previously untreated CNS disease. In a 58-year-old patient with an asymptomatic solitary brain metastasis and leptomeningeal carcinomatosis, crizotinib was initiated, and a complete CNS response was achieved despite a crizotinib CSF-to-serum ratio of 0.0006 [129]. A retrospective analysis of *ALK*-positive patients with asymptomatic CNS metastases who received crizotinib on the PROFILE 1005 and 1007 trials showed that 275 (31 %) of the 888 had asymptomatic brain metastases upon enrollment on these trials. In patients with untreated brain metastases, crizotinib produced intracranial disease control in 56 % at 12 weeks. The median intracranial time to progression was 7 months in this population. In patients who had received brain radiotherapy prior to enrollment in these trials, CNS disease control was attained in 62 % with median time to progression of 13.2 months. However, due to poor CNS penetration of crizotinib, among patients without brain metastases at enrollment, 20 % developed CNS disease while taking crizotinib [130]. Second-generation ALK inhibitors have better CNS penetration and activity.

Resistance mechanisms

ALK-positive tumors can develop *ALK* dependent and *ALK* independent mechanisms of crizotinib

resistance. Crizotinib resistance predominantly occurs through acquired mutations in the ALK kinase domain at the ATP-binding pocket, which prevents binding of the drug. These mutations include the L1196M point mutation, which is homologous to the TKI resistance mutations T790M in EGFR-mutated NSCLC and T315I in chronic myelogenous leukemia [131,132]. Other acquired ALK kinase mutations that confer resistance to crizotinib include C1156Y, L1152R, I1171T, S1206Y, F1174L and G1269A [131,133-136]. The G1202R crizotinib-resistance mutation also confers resistance to second generation ALK TKIs [137]. Increase in *ALK* copy number has also been demonstrated in patients who develop crizotinib resistance [132].

In humans, *ALK* independent mechanisms of resistance include *KRAS* mutations and development of EGFR mutation without evidence of persistent *ALK* gene rearrangement [132]. In a mouse model, crizotinib resistance was induced through activation of the EGFR signaling pathway, which could be overcome by combination therapy with crizotinib and the heat shock protein 90 (Hsp90) inhibitor ganetespib. Hsp90 is a chaperone protein that regulates the folding and stability of multiple "client proteins," including EGFR, EML4-ALK, HER2, and BRAF and can therefore target multiple pathways of drug resistance [138]. Insulin-like growth factor 1 receptor activation has been identified as a possible resistance mechanism to crizotinib, and serves as a target of the second-generation ALK inhibitor ceritinib (detailed below) [139].

Ceritinib

<u>Clinical efficacy</u>

Ceritinib was the first second-generation ALK TKI approved for clinical use in the US. Preclinical data demonstrated that certinib overcomes crizotinib-resistance mutations, particularly L1196M, G1269A, S1206Y and I1171T but not G1202R and F1174C [136]. In April 2014, the FDA granted accelerated approval to ceritinib for the treatment of patients with metastatic *ALK*-positive NSCLC who were intolerant to or experienced disease progression on crizotinib. This approval was based on the single-arm, multicenter phase I ASCEND-1 trial that enrolled a total of 130 patients with advanced *ALK*-rearranged NSCLC, including patients who had experienced disease progression during crizotinib treatment. The ORR for patients who received at least 400 mg of ceritinib daily was 58 %, while the response rate for patients who had previously received crizotinib was 56 %. Median PFS was 7.0 months (95 % CI, 5.6 to 9.5) [14]. In this trial, tumor response was noted regardless of the underlying mechanism of acquired crizotinib resistance, and patients with *ALK* gene amplification and ALK tyrosine kinase domain mutations developed responses to ceritinib. Given the excellent responses witnessed in crizotinib-treated patients, the main mechanism of crizotinib resistance appears to be ALK dependent, and may be overcome with a more potent ALK inhibitor, such as ceritinib [14]. Updated data from this trial, including 246 patients, showed an ORR of 58.5 % in all patients, with a median duration of response of 9.7 months, time to first response of 6.1 weeks and median PFS of 8.2 months [140]. ASCEND-2 was a single-arm, multicenter phase II trial of 140 patients that evaluated efficacy and safety of ceritinib in *ALK*-positive NSCLC who progressed after chemotherapy and crizotinib. The ORR was 38.6 % with median PFS of 5.7 months. The trial also allowed patients with untreated brain metastases to participate (71.4 % had baseline brain metastases, 28 % had untreated brain metastases). Median PFS was 5.4 months in patients with brain metastases versus 11.3 months in patients without brain metastases [141]. ASCEND-3 examined ceritinib use for metastatic, *ALK*-positive NSCLC patients who were ALK TKI-naïve, including those with brain metastases. At presentation, 124 patients had enrolled in the trial. In this study population, the ORR was 63.7 % with disease control rate of 89.5 %, and the median PFS was 11.1 months [142].

Clinical safety

Like many other TKIs, ceritinib induces nausea, diarrhea, and vomiting. While 60 to 80 % of patients experience these symptoms on ceritinib, only 5 to 7 % of patients experience grade 3 or 4 nausea, diarrhea or vomiting. Approximately 58 % of patients taking ceritinib at the recommended starting dose of 750 mg daily required at least one dose reduction [143]. Ceritinib was discontinued entirely in only 7 to 8 % of patients on clinical trial due to adverse events [14,140-142]. Severe interstitial lung disease, which may be related to ceritinib has also been reported, but the incidence is less than 5 %. Grade 3 or 4 transaminitis occurs in approximately 21 % of patients. Like crizotinib, low risks of bradycardia and Q-T prolongation have been observed. Ceritinib also affects the insulin-like growth factor 1 receptor (IGF-1R) and insulin receptor (InsR), and grade 3 or 4 hyperglycemia was witnessed in up to 13 % of patients. Underlying diabetes may exacerbate this effect. Elevated lipase and fatal pancreatitis have been reported in clinical trials [14,140].

Pharmacology

Ceritinib, 5-chloro-2-N-(5-methyl-4-piperidin-4-yl-2-propan-2-yloxyphenyl)-4-N-(2-propan-2-ylsulfo-nyl-phenyl)-pyrimidine-2,4-diamine [LDK378, Zykadia, (Novartis)] is a second-generation ALK inhibitor that competitively inhibits the ALK tyrosine kinase domain through phosphorylation. It also inhibits IGF-1R, INsR and the ROS1 receptor. Unlike crizotinib, it does not inhibit c-MET [136,143]. Ceritinib more potently inhibits ALK than crizotinib, as ceritinib produces IC_{50} values of 0.15 nM in enzymatic assays (crizotinib IC_{50} 3.6 nM) and IC_{50} of 25nM in cell lines [136,144]. The recommended starting dose is 750 mg administered orally once daily. Its absorption is increased when administered with a meal, with peak plasma activity increasing by 41 to 43 % compared to a fasting state. The drug should, therefore, be taken on an empty stomach. Oral bioavailability is unknown, but peak plasma levels are achieved at 4 to 6 hours after dosing. Ceritinib is 97 % protein-bound upon absorption, has a volume of distribution of 4230L, and reaches steady state at 15 days. It is primarily metabolized in the liver by CYP3A4 and serves as a substrate for P-glycoprotein. The drug may inhibit CYP3A and CYP2C9 at the recommended dosing. Ceritinib has a terminal half-life of 41 hours, and like crizotinib, has a nonlinear PK over time, with a CL/F of 88.5 L/hr after one dose and a CL/F of 33.2L/hr at steady state. Following administration, 92.3 % of the drug is eliminated in the feces (68 % unchanged) [143,144]. No dose adjustments for hepatic or renal failure have been recommended.

CNS penetration

The ASCEND trials allowed patients with untreated, asymptomatic brain metastases to enroll. In ASCEND-2, intracranial disease control was attained in 80 % of patients with brain lesions. In 5 of the 6 patients with brain lesions that had not previously been treated, 2 complete responses and 3 partial responses were achieved with ceritinib [141]. Likewise, in ASCEND-3, the intracranial disease control rate was 80 %, and 2 partial responses were achieved with ceritinib. All responses in the brain matched or exceeded the responses achieved systemically [142]. To date, CSF measurements of ceritinib after oral administration are unavailable.

Resistance mechanisms

In 10 patients who developed disease progression while taking ceritinib (median duration of treatment 7.5 months), tumor analysis revealed several novel ALK resistance mutations in the tyrosine kinase domain. A total of 11 samples were obtained in the 10 patients, and in 5 of 11 samples, secondary mutations in the ALK tyrosine kinase domain were identified. Of these mutations, 3 were G1202R, 1 was F1174C, and 1 was

F1174V. Two of the patients had S1206Y and G1269A mutations prior to ceritinib, which were no longer present on post-ceritinib tumor samples. *ALK* amplification was not witnessed in this population [145].

Alectinib

Clinical efficacy

Alectinib is another potent, second-generation oral ALK TKI that, like ceritinib, inhibits wild-type ALK as well as the crizotinib resistance mutations L1196M, L1152R, G1296A and C1156Y [146,147]. In a phase I/II study performed on a Japanese population of ALK TKI naïve patients, alectinib at a dose of 300 mg twice daily produced an ORR of 93.5 % [148]. Another phase I/II study examining alectinib 600 mg twice daily for *ALK*-positive patients who had previously progressed on or were intolerant to crizotinib showed ORR of 55 % [149]. Based on these data, in December 2015, the FDA granted accelerated approval to alectinib for treatment of *ALK*-positive NSCLC patients who experience disease progression on or intolerance to crizotinib. Subsequently, a phase II trial of 87 patients evaluating alectinib for ALK-positive NSCLC patients who had progressed on either chemotherapy or crizotinib showed an ORR of 48 [150]. Recently, results of the J-ALEX study, a randomized open-label phase III trial were presented at the American Society of Clinical Oncology annual meeting. This trial randomized 207 Japanese patients with advanced ALK-positive NSCLC who were ALK TKI naïve to alectinib 300 mg twice daily or crizotinib 250 mg twice daily. The primary endpoint of the study was PFS, and alectinib produced a statistically superior PFS with a PFS HR of alectinib compared to crizotinib of 0.34 (99.6826 % CI: 0.17-0.70, stratified log-rank p<0.0001). The median PFS of alectinib was not reached (95 % CI: 20.3-not estimated) and the median PFS of crizotinib was 10.2 months (95 % CI: 8.2-12) [151]. Based on these results, enthusiasm exists for moving alectinib to front-line treatment for ALK-positive NSCLC, and a global, randomized phase III trial comparing alectinib to crizotinib is underway (NCT02075840).

Clinical safety

Alectinib carries a favorable side effect profile with significantly less GI toxicity than crizotinib. Fatigue is the commonest adverse event encountered and occurs in approximately 30 % of patients (all grade 1-2). Grade 1-2 myalgias, peripheral edema, increased creatine kinase, nausea, elevated aminotransferases, constipation and photosensitivity occur in 13-36 % of patients. Grade 3 or 4 events are rare and occur at an incidence of less than 5 % with the exception of aminotransferase abnormalities occurring in 5 to 6 % of cases. Fatal hemorrhage was witnessed in one patient who was on anticoagulants. In the phase II trial, dose interruption was needed for 36 % of patients and dose reduction occurred in 16 % of patients. The medication was discontinued in 2 to 9 % of patients due to adverse events [148-150].

Pharmacology

Alectinib, 9-ethyl-6,6-dimethyl-8-(4-morpholin-4-ylpiperidin-1-yl)-11-oxo-5H-benzo[b]carbazole-3-carbonitrile [CH5424802, Alecensa, (Roche)] competitively inhibits the tyrosine kinase domain of the ALK fusion protein. It is a more potent ALK inhibitor than crizotinib with an in vitro kinase IC_{50} of 1.9 nM and IC_{50} of 3.0 nM in cell lines. It demonstrates high target selectivity with weak or no inhibition of over 20 other kinases, including ROS1 and MET [152]. However, recently, it was discovered that alectinib potently inhibits RET kinase activity with an IC_{50} of 4.8 nM, and is active against oncogenic RET-rearrangements observed in NSCLC [153]. The recommended dose is 600 mg twice daily with food. It is metabolized to its major active metabolite, M4, in the liver by CYP3A4 [154]. The maximum plasma concentration at steady-state is 665ng/mL (44 %) and for M4 is 246 ng/mL (45 %), and the drug reaches steady-state after 7 days. Under fed conditions, the bioavailability of alectinib is 37 %, and a high-calorie, high-fat meal increased exposure

of the drug by 3-fold [154]. The volume of distribution for alectinib is 4,016 L and 10,093L for the M4 metabolite. Alectinib also demonstrates nonlinear PK with apparent clearance of 81.9L/hr for alectinib and 217 L/hr for M4. After administration, 84 % of the drug is eliminated in the feces as unchanged alectinib and 6 % as M4 [148,149,154]. Despite its metabolism by CYP3A, co-administration with strong CYP3A inhibitors and inducers have not been found to significantly alter alectinib levels. Alectinib does inhibit P-gp and BRCP. No dose adjustments have been recommended for hepatic or renal impairment [154].

CNS penetration

Alectinib has higher CNS responses than crizotinib. In one of the phase I/II studies, 21 patients were enrolled with CNS metastases. Of these patients, 12 (57 %) had progressive CNS disease at enrollment. In the 21 patients with CNS disease, alectinib produced a CNS ORR of 52 % with six (29 %) complete responses and five (24 %) partial responses. Overall CNS disease control rate was 90 %. In the four patients who had not received prior brain radiotherapy, a complete response was achieved in two patients and one had a partial response. In this study, at the 600 mg twice daily dose of alectinib, the CSF trough concentration of alectinib was 2.69 nM/L, which is nearly the same as the unbound systemic trough of alectinib of 3.12 nM/L, and exceeds the IC_{50} for ALK inhibition for alectinib in cell-free assays (1.9 nM/L) [149]. In the Japanese phase I/II alectinib study, 15 patients had known brain metastases, 12 of whom had previous brain radiation. No CNS disease progression was witnessed on alectinib. Of the 3 patients who did not receive radiation to the brain, 2 continued on study for more than 300 days without CNS disease progression [148]. In the phase II study, 16 patients had measurable CNS disease at baseline. Overall, 100 % of patients achieved disease control in the CNS and 75 % of patients achieved intracranial disease response. Four (25 %) of these patients achieved complete CNS response and eight (50 %) developed partial response in the CNS. The median duration of CNS response was 11.1 months [150]. Therefore, durable CNS control and even complete CNS responses are attainable with alectinib.

Resistance mechanisms (Table 4)

In a cell line study, an acquired V1180L mutation was inducible with alectinib exposure. This mutation exists in the ALK tyrosine kinase domain at the back of the ATP binding pocket, and sterically interferes with the ability of both alectinib and crizotinib to bind effectively to the kinase and mediates a high-level resistance. An I1171T mutation was isolated in a tumor specimen in a patient who had relapsed on alectinib. This mutation is thought to disrupt the hydrogen bond between alectinib and E1167. Compared with V1180L, the I1171T mutation confers an intermediate level of alectinib resistance [155]. However, ceritinib inhibits I1171T [136]. G1202R is an acquired mutation that drives crizotinib resistance but has also been demonstrated to confer high-level in vitro and in vivo resistance to alectinib and ceritinib [137]. A case of *MET* amplification has been reported in an alectinib-resistant tumor. It is unclear if *MET* was the driver of resistance, however, the patient did have a 5 month duration of response to the c-MET inhibitor crizotinib [156]. Given the variety of secondary ALK mutations encountered, and the varying susceptibilities to second generation and investigational ALK TKIs, it is important to consider serial tumor molecular profiling at each step of disease progression.

Table 4. Selected ALK Resistance Mutations [157-162]

ALK Mutation	Treatment for mutation
L1196M	Alectinib, Ceritinib, Brigatinib, Lorlatinib, JH-VIII-157-02*
G1269A	Alectinib, Ceritinib, Brigatinib, Lorlatinib, JH-VIII-157-02*
1151Tins	Alectinib, Lorlatinib
I1171T/N/S	Ceritinib, Brigatinib
S1206Y	Alectinib, Ceritinib, Brigatinib, Lorlatinib, JH-VIII-157-02*
S1206F	Brigatinib
C1156Y	Alectinib, Ceritinib, Lorlatinib, JH-VIII-157-02*
L1152R	Alectinib, Lorlatinib
L1198F	Crizotinib
F1174C	Brigatinib
F1174L/V	Alectinib, Brigatinib, Lorlatinib, JH-VIII-157-02*
R1275Q	Alectinib, Brigatinib
G1202R	Brigatinib, Lorlatinib, JH-VIII-157-02*

*Structural analogue of alectinib, not currently in clinical testing

Investigational agents (Table 5)

Brigatinib (AP26113) is a second-generation ALK TKI that also inhibits EGFR as well as various ALK mutations resistant to crizotinib, alectinib and ceritinib, including the G1202R mutation. Preclinically, it has demonstrated inhibition of EGFR T790M resistance mutation as well as potent activity against ROS1 with IC50 of 1.9 nm [161]. Early clinical data showed disappointing activity for brigatinib in EGFR-mutated patients, and it has been predominantly developed as an ALK inhibitor [163]. A phase I trial investigating brigatinib for advanced ALK-positive patients who had disease progression after crizotinib or chemotherapy showed an ORR of 74 % and median PFS of 13.4 months [163]. The phase II study (NCT02094573) is ongoing, but preliminary results were recently presented. As of December 2015, 222 patients were randomized to either 90 mg/day or 180 mg/day of brigatinib for the treatment of crizotinib-resistant ALK-positive NSCLC. The ORR was 46 % and the median PFS was 8.8 months for those who received 90 mg/day of brigatinib versus an ORR of 54 % and median PFS of 11.1 months for those who received 180 mg/day. In the higher dose group, grade 3 or higher toxicity for increased CPK (8 %) and pneumonitis (3 %) was noteworthy. Many of the pulmonary events occurred within 7 days of treatment initiation. A phase III study is planned comparing brigatinib versus crizotinib for TKI-naïve, advanced ALK-positive NSCLC [162].

Lorlatinib (PF-06463922) is a third-generation ALK/ROS1 TKI with activity against most ALK crizotinib-resistance mutations that was designed to have high CNS penetration. In an ongoing phase I/II study (NCT01970865), 54 patients who had advanced ALK or ROS1 positive NSCLC received lorlatinib, producing an ORR of 50 % with an intracranial ORR of 44 % (22 % CR). Response was witnessed in patients with the G1202R mutation [164]. Grade 3 or 4 hypercholestrolemia occurred in 9 % of patients. The phase II component of the trial is currently enrolling patients. The L1198F mutation confers resistance to lorlatinib. Interestingly, this mutation resensitizes the ALK kinase domain to crizotinib, and a clinical response with crizotinib has been witnessed in a patient with a L1198F lorlatinib-resistance mutation [160].

X-396 is another TKI that potently inhibits ALK, including the crizotinib-resistance mutations, L1196M and C1156Y. In a multicenter phase I/II trial, among 6 ALK-positive NSCLC patients who received at least 200 mg of X-396, PR was achieved in 83 % with the remainder achieving stable disease. Enrollment for this

trial is ongoing (NCT01625234) [165]. ASP3026 is another ALK inhibitor that achieved an ORR of 44 % in 15 ALK-positive patients who progressed on crizotinib in a phase I trial (NCT01401504) [166].

Table 5. ALK TKIs in clinical development

Drug Name	Targets	Clinical Activity	Select ongoing/future trials
Brigatinib (ASP26113) [162,163]	ALK EGFR ALK L1196M EGFR T790M	Phase I: ORR 74%, median PFS 13.4 mo; Phase II: ORR 46-54% Median PFS 8.8-11.1 mo	Ongoing phase II (NCT02094573); Planned phase III trial Brigatinib vs crizotinib in TKI naïve patients
Lorlatinib (PF-06463922) [164]	ALK ROS1 Crizotinib-resistance mutations	Phase I/II: ORR 50% systemic Intracranial ORR 44%	Ongoing phase II (NCT01970865)
X-396 [165]	ALK Crizotinib-resistance mutations	Phase I/II (n=6) ORR 83%	Ongoing phase II (NCT01625234)
ASP3026 [166]	ALK Crizotinib-resistance mutations	Phase I (n=15) ORR 44%	No currently active trials
Entrectinib (RXDX-101, NMS-E628) [169]	ALK ROS1 TrkA, TrkB, TrkC	Phase I 1 of 1 pt with ALK+ NSCLC developed PR	Phase I/II STARTK-1 (NCT02097810)
TSR-011 [168]	ALK TrkA, TrkB, TrkC	Phase I/II 120 mg: ORR 3 of 3 pt <120 mg: ORR 5 of 9 pt	Ongoing phase I/II (NCT02048488)

Entrectinib (RXDX-101, NMS-E628) and TSR-011 are inhibitors of ALK as well as the tropomysin receptor kinases (Trk) TrkA, TrkB and TrkC. Recently, gene fusions between the kinase domain of the *NTRK1* gene and other genes, including *MPRIP and CD74* have been discovered in up to 3 % of lung cancer patients, and appear to be independent of other driver mutations. These fusions create constitutively active Trk proteins that drive tumorigenesis and serve as targets for Trk inhibitors [167]. A phase I/II trial (NCT02048488) is ongoing for TSR-011, but preliminary results in ALK-positive patients showed 3 of 3 evaluable patients developed a response at the 120mg or above daily dose and 5 of 9 patients achieved stable disease at lower doses [168]. Multiple early phase trials for entrectinib are also in progress. Preliminary data from the phase I ALKA-372-001 trial demonstrated a partial response in an ALK-positive NSCLC patient [169]. Another phase I/II trial examining the safety and efficacy of entrectinib for advanced malignancies positive for NTRK1, NTRK2, NTRK3, ROS1 or ALK is underway (STARTK-1, NCT02097810).

Immunotherapy and the future of EGFR and ALK TKIs

The therapeutic landscape for NSCLC has recently expanded with the introduction of immunotherapeutic agents, including the programmed cell death protein (PD-1) inhibitors nivolumab and pembrolizumab. These drugs are now commonly used for second-line treatment of advanced NSCLC, as they both prolong overall survival compared to standard second-line chemotherapy [171-173]. Nivolumab also appears to have activity in first-line treatment of advanced NSCLC [174,175]. Clinical trials assessing neoadjuvant and adjuvant uses of immune checkpoint inhibitors are currently in progress (NCT02595944, NCT02504372, NCT02818920, NCT02486718, NCT02273375, NCT02572843). The future of NSCLC will be closely tied to immunotherapy, and the roles of EGFR and ALK TKIs could be called into question. However, currently, the efficacy of immunotherapy for the treatment of EGFR- and ALK-mutated NSCLC is unclear.

Subgroup analyses from Check Mate 057 and KEYNOTE-010 suggest patients with EGFR mutations may not benefit as much from nivolumab and pembrolizumab as wild-type EGFR patients. The data should be interpreted with caution as the subgroups were small and the confidence intervals on the hazard ratios were wide [171,173]. The mutational burden and neoantigen expression of tumors appear to be correlated to response to immune checkpoint blockade, which can be driven by tobacco exposure [176,177]. EGFR- and ALK-mutated NSCLCs are associated with low tobacco exposure, and these tumors can have lower mutational loads, which may partially explain lower response to immune checkpoint blockade. However, multiple studies have demonstrated that both EGFR and ALK activation can increase expression of PD-L1 [178-181]. Combination therapy with immune checkpoint blockade and EGFR TKI is under investigation. In the multi-arm phase Ib TATTON trial, 34 patients received a combination of the anti-PD-L1 monoclonal antibody durvalumab and osimertinib. At the time of the study presentation at the 2016 European Lung Cancer Conference (ELCC), 31 patients were evaluable for response. The combination had promising activity, with partial response witnessed in 64.5 % of patients, however, grade 3/4 interstitial lung disease was reported in 14.7 % of patients [182]. Much is to be learned about immunotherapy and ALK- and EGFR-mutated lung cancer, therefore, TKIs will continue to represent the core treatment of EGFR- and ALK-mutated NSCLC for the foreseeable future.

Conclusion

Discovery of the EGFR and ALK activating mutations and their respective TKIs have substantially improved outcomes for a select group of NSCLC patients. Despite these advances, challenges remain for these patients. Immunotherapy, which has recently advanced the treatment of NSCLC, does not appear to be as active in ALK and EGFR mutated patients. Acquired mutations universally lead to drug resistance to ALK and EGFR TKIs, resulting in disease progression and death. With improvement in tumor genome sequencing technology, novel resistance mutations will continue to be identified. Inevitably, new TKIs will be developed that will target the most commonly encountered resistance mutations as well as disease relapse in CNS, and indeed, many EGFR and ALK TKIs are currently in development addressing these twin issues. However, innovative treatment approaches are necessary to continue to improve survival. Combination therapies with TKIs and novel agents, including immunotherapy, may be needed to overcome the multiple molecular pathways that ultimately drive carcinogenesis.

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