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Osimertinib in the treatment of patients with epidermal growth factor receptor T790M mutation-positive metastatic non-small cell lung cancer: clinical trial evidence and experience

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***Abstract*:** Patients with advanced epidermal growth factor receptor (*EGFR*) mutant non-small cell lung cancer (NSCLC) are particularly sensitive to treatment with first- or second-generation *EGFR* tyrosine kinase inhibitors such as gefitinib, erlotinib and afatinib, which block the cell-signaling pathways that drive the growth of tumor cells. Unfortunately, the majority of patients develop resistance to them after a median duration of response of around 10 months, and in over half of these patients the emergence of the *EGFR* T790M resistance mutation is detected. Osimertinib is an oral, highly selective, irreversible inhibitor of both *EGFR*-activating mutations and the T790M-resistance mutation, while sparing the activity of wild-type *EGFR*. This article reviews clinical trial development of osimertinib in patients with NSCLC, presenting efficacy and safety evidence for its value in the *EGFR* T790M mutation-positive population and in different settings, including patients with metastatic disease. The preclinical background of clinically acquired resistance to osimertinib is presented and the combination tactics being investigated in an attempt to circumvent this are addressed.

Keywords: AZD9291, ctDNA, epidermal growth factor receptor, metastatic, non-small cell lung cancer, osimertinib, T790M

Introduction

Over the past decade, the outcomes of biomarker-selected patients with non-small cell lung cancer (NSCLC) have been improved by the *in crescendo* discovery of activating mutations, with the consequent development of targeted therapies. The first notable success in this personalized medicine era came with the identification of activating mutations in the kinase domain (exons 18–21) of the epidermal growth factor receptor (*EGFR*) gene, leading to dramatic responses to *EGFR* tyrosine kinase inhibitors (TKIs). *EGFR* mutations account for 10–17% of NSCLC cases in North America and Europe and 30–50% of NSCLCs in Asian countries [Kris *et al.* 2014; Barlesi *et al.* 2016]. The most common *EGFR* mutations are the p.Leu858Arg (L858R) point mutation in exon 21 and small in-frame deletions in the region encoded by exon 19, together accounting for approximately 85–90% of all *EGFR* mutations [Lynch *et al.* 2004; Paez *et al.* 2004; Pao *et al.* 2004]. The first-generation TKIs gefitinib (Iressa®, AstraZeneca Pharmaceuticals, London, United Kingdom) and erlotinib (Tarceva®, F. Hoffmann-La Roche, Basel, Switzerland), and the second-generation TKI afatinib (Giotrif®, Boehringer Ingelheim, Ingelheim, Germany) have shown overall response rates (ORRs) ranging from 50% to 75%, improving progression-free survival (PFS) and quality of life compared with standard platinum-based chemotherapy in patients with *EGFR*-mutant NSCLC [Mok *et al.* 2009; Rosell *et al.* 2012; Yang *et al.* 2015]. This resulted in their approval as first-line treatments for patients with advanced NSCLC harboring activating mutations in the *EGFR* kinase domain.

Despite these impressive outcomes, acquired resistance arises after a median period of 9–13

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months. Multiple mechanisms have been identified, including secondary mutations in *EGFR* (notably *EGFR* T790M), along with mutations in the *PIK3CA* and *BRAF* genes and amplifications in *ERBB2* and *MET* [Sequist *et al.* 2011; Yu *et al.* 2013; Gainor and Shaw, 2013; Stewart *et al.* 2015]. The development of a secondary mutation in *EGFR* when threonine is replaced by methionine at position 790 of exon 20, formally known as T790M (p.Thr790Met), is the most common mechanism, seen in around 50% of cases. While the *EGFR*-T790M mutation was initially reported as a secondary *EGFR* resistance mutation, several studies reported *de novo* *EGFR*-T790M mutations, sometimes concomitantly with other *EGFR*-activating mutations [Inukai *et al.* 2006; Su *et al.* 2012; Li *et al.* 2014].

First-generation TKIs compete with adenosine triphosphate (ATP) to bind to the kinase domain of *EGFR*, and *EGFR* T790M significantly increases this affinity reducing TKI efficacy [Yun *et al.* 2008]. Second-generation *EGFR* TKIs were originally developed to be irreversible *EGFR* inhibitors with the hope of being active against *EGFR*-T790M resistance mutations, but they have failed to produce meaningful disease response after resistance to gefitinib or erlotinib [Sequist *et al.* 2010; Miller *et al.* 2012; Ellis *et al.* 2014].

Osimertinib (AZD9291; AstraZeneca Pharmaceuticals), rociletinib (CO-1686; Clovis Oncology, Boulder, United States), olmutinib (BI-1482694/HM61713, Boehringer Ingelheim/Hanmi, Songpa-gu, Korea), ASP8273 (Astellas, Tokyo, Japan), EGF816 (Novartis Pharmaceuticals, Basel, Switzerland), and PF-06747775 (Pfizer, New York, United States) are third-generation *EGFR* TKIs with selectivity against *EGFR*-T790M resistance as well as *EGFR*-sensitizing mutations, all of which have progressed to clinical trials [Cross *et al.* 2014; Jänne *et al.* 2015; Sequist *et al.* 2015b; Lee *et al.* 2015; Goto et al 2015; Jia *et al.* 2016]. Table 1 presents available efficacy data from phase I and II clinical trials.

To date, osimertinib (Tagrisso™, AstraZeneca Pharmaceuticals) is the only drug to be approved by the European Medicines Agency and the US Food and Drug Administration for treatment of *EGFR*-T790M mutated NSCLC patients. This review provides an overview of preclinical and clinical data.

Biochemical and preclinical background

Osimertinib is a mono-anilino-pyrimidine compound that acts as a covalent *EGFR* TKI. In *EGFR*-recombinant enzyme assays, osimertinib showed potent activity against diverse *EGFR* mutations (L858R, L858R/T790M, exon 19 deletion, and exon 19 deletion/T790M) and exhibited nearly 200 times greater potency against L858R/T790M than wild-type *EGFR*. Subsequent murine *in vivo* studies revealed that osimertinib is metabolized to produce at least two circulating metabolites, AZ5104 and AZ7550.

In biochemical assays, AZ7550 had a comparable potency and selectivity profile to osimertinib, although AZ5104 showed greater potency against exon 19 deletions, T790M mutants (both approximately 8-fold) and wild-type (approximately 15-fold) *EGFR* [Cross *et al.* 2014]. In addition, osimertinib and its active metabolites displayed minimal off-target kinase activity for various kinases such as *ERBB2/4, ACK1, ALK, BLK, BRK, MLK1*, and *MNK2* in *in vitro* studies [Cross *et al.* 2014]. The area under the plasma concentration–time curve (AUC), maximal plasma concentration (C_{max}), and minimal concentration (C_{min}) of osimertinib increased over the 20–240 mg dose range with linear pharmacokinetics and the C_{max}/C_{min} ratio for the 80 mg osimertinib (capsule formulation) was 1.6 [Planchard *et al.* 2016]. The AUC of osimertinib metabolites AZ5104 and AZ7550 was approximately 10% of osimertinib exposure. Pharmacokinetic exposure was not significantly different between Asian *versus* non-Asian patients [Planchard *et al.* 2016]. The median time to C_{max} occurred after 6 h (range 3–24). Plasma concentrations decreased with time and the estimated mean half life was 48 h, with clearance (CL/F) of 14.2 (liter/h). Unlike erlotinib, food intake does not impact osimertinib kinetics.

The main metabolic pathways of osimertinib are oxidation (mainly by cytochrome P450, family 3, subfamily A, also known as CYP3A) and dealkylation and it is eliminated primarily in the feces (>65%) and urine (<15%). No clinically significant differences in the pharmacokinetics of osimertinib have been identified in terms of age, sex, ethnicity, body weight, smoking status, mild to moderate renal impairment, or mild hepatic dysfunction. Osimertinib is a competitive inhibitor of CYP3A but does not inhibit CYP2C8, 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, and 2E1. It is a substrate

Table 1. Efficacy of third-generation TKIs in *EGFR*-T790M-mutated NSCLC populations from phase I and II trials.

Trial	Osimertinib				Rociletinib	Olmutinib	EGF816	ASP8273
	AURA phase I (*N* = 138)	AURA phase I (*N* = 63)	AURA phase extension (*N* = 201)	AURA2 phase II (*N* = 210)	TIGER-X phase I (*N* = 51)[$]	HM-EMSI-101 phase I/II (*N* = 76) (ongoing)	NCT02108964 phase I (*N* = 132) (ongoing)	NCT02113813 phase I (*N* = 58) (ongoing)
			Pooled analysis					
Dose	20–240 mg qd	80 mg qd	80 mg qd		500–1000 mg bid	800 mg qd	75–350 mg qd	300 mg qd
ORR, % (95% CI)	61 (52–70)	71 (57–82)	66 (61–71)		45 (31–60)[$]	62	44	31
Median PFS, months (95% CI)	9.6 (8.3–NR)	9.7 (8.3–13.6)	11.0 (9.6–12.4)		6.1 (4.2–9.6)[$]	6.9*	9.7 (7.3–11.1)	6.8 (5.5–NR)
Reference	Jänne *et al.* [2015]	Yang *et al.* [2016b]			Sequist *et al.* [2016]	Lee *et al.* [2015] and Park *et al.* [2016]	Tan *et al.* [2016]	Yu *et al.* [2016]

*Updated results from 2016 ASCO Annual Meeting.
[$]Updated results from phase I TIGER-X trial. While the development of rociletinib was discontinued, the other drugs are still being developed.
bid, twice daily; CI, confidence interval; EGFR, epidermal growth factor receptor; NR, not reached; NSCLC, non-small cell lung cancer; ORR, overall response rate; PFS, progression-free survival; qd, once daily; TKI, tyrosine kinase inhibitor.

of P glycoprotein and ATP-binding cassette subfamily G member 2, but is not a substrate of organic anion-transporting polypeptide proteins. In a clinical pharmacokinetic study [ClinicalTrials.gov identifier: NCT02163733], the osimertinib exposures were not affected by concurrent administration of omeprazole [Vishwanathan *et al.* 2015]. Gastric pH modifying agents can be concomitantly used with osimertinib Tagrisso™ without any restrictions.

Clinical efficacy of osimertinib

Phase I clinical trials

The safety and efficacy of osimertinib was assessed in the phase I/II AURA trial [ClinicalTrials.gov identifier: NCT01802632] in patients with locally advanced or metastatic *EGFR*-mutated NSCLC who had radiologically documented disease progression after treatment with at least one first- or second-generation *EGFR* TKI [Jänne *et al.* 2015]. The study included 253 patients who received osimertinib at five dose levels ranging from 20 to 240 mg daily and distributed between two cohorts. Among 31 patients enrolled in the dose-escalation cohort, no dose-limiting toxic effects occurred and an additional 222 patients were treated in five expansion cohorts. All patients had received at least one prior *EGFR* TKI, and 80% had received prior cytotoxic chemotherapy. The *EGFR*-T790M mutation was detected in tumors from 138 patients (62%) in the expansion cohort. Of the 253 patients treated across all dose levels, 239 were evaluated for response. The ORR in the combined T790M-positive and T790M-negative populations was 51% [95% confidence interval (CI) 45–58], with 122 patients having a confirmed partial response (PR) and one patient a complete response (CR). Stable disease (SD) was observed in 78 patients (33%) and 34 (14%) experienced disease progression. The disease control rate (DCR; CR, PR or SD) was 84% (95% CI 79–88). The ORR was similar between the 150 Asian and 89 non-Asian patients (50% *versus* 54%). The 80 mg daily dose was adopted for future studies based on increasing toxicity at 160 and 240 mg daily combined with similar response rates across all dose levels.

Osimertinib exhibited improved efficacy in patients whose tumor harbored the *EGFR*-T790M mutation. Of 138 patients with a centrally confirmed *EGFR*-T790M mutation, 127 were evaluable for response. Outcomes were substantially better in this *EGFR* T790M-postive population compared with patients with T790M-negative tumor, with an ORR of 61% (95% CI 52–70%) *versus* 21% (95% CI 12–34%), a DCR of 95% (95% CI 90–98%) *versus* 61% (95% CI 47–73%), and median PFS of 9.6 *versus* 2.8 months, respectively [Jänne *et al.* 2015].

Updated results from this trial were recently presented. The efficacy and safety data from the 80 mg expansion cohort in patients with centrally confirmed T790M-positive NSCLC with disease progressing following either one prior therapy with an *EGFR*-TKI or both an *EGFR*-TKI and another anticancer therapy, as well as from two expansion cohorts who received osimertinib 80 mg or 160 mg once daily as first-line treatment in patients with *EGFR*-mutated advanced NSCLC. The former population included 63 patients, 61 of whom were evaluable for response with an ORR of 71% (95% CI 57–82%), a DCR of 93% (95% CI 84–98%), and median PFS of 9.7 (95% CI 8.3-13.6) months [Yang *et al.* 2016b]. The latter population included 60 patients treated with osimertinib 80 mg (n = 30) or 160 mg (n = 30) daily and all were evaluable. The confirmed ORR was 77% (95% CI 64–87%) with a DCR of 98% (95% CI 89–100%). Median PFS was 19.3 (95% CI 13.7-not calculated), supporting osimertinib use in both first-line and later settings [Ramalingam *et al.* 2016].

Phase II clinical trials

The 80 mg daily dose was evaluated in the phase II T790M-positive extension cohort of the AURA trial (described above) and in an additional phase II AURA2 study [ClinicalTrials.gov identifier: NCT02094261] designed for patients with confirmed *EGFR*-mutant T790M-positive locally advanced or metastatic NSCLC who have progressed following prior therapy with an approved *EGFR* TKI. A preplanned pooled analysis of both studies was recently presented, including a total of 411 patients: 201 patients from the extension cohort of the AURA trial and 210 patients from the AURA2 trial, 397 of whom were included in the response rate evaluation. The ORR was 66% (95% CI 61–71%) and the DCR was 91% (95% CI 88–94%). Median PFS was 11.0 (95% CI 9.6–12.4) months, with a median response duration of 12.5 months (95% CI 11.1–not reached) [Yang *et al.* 2016b].

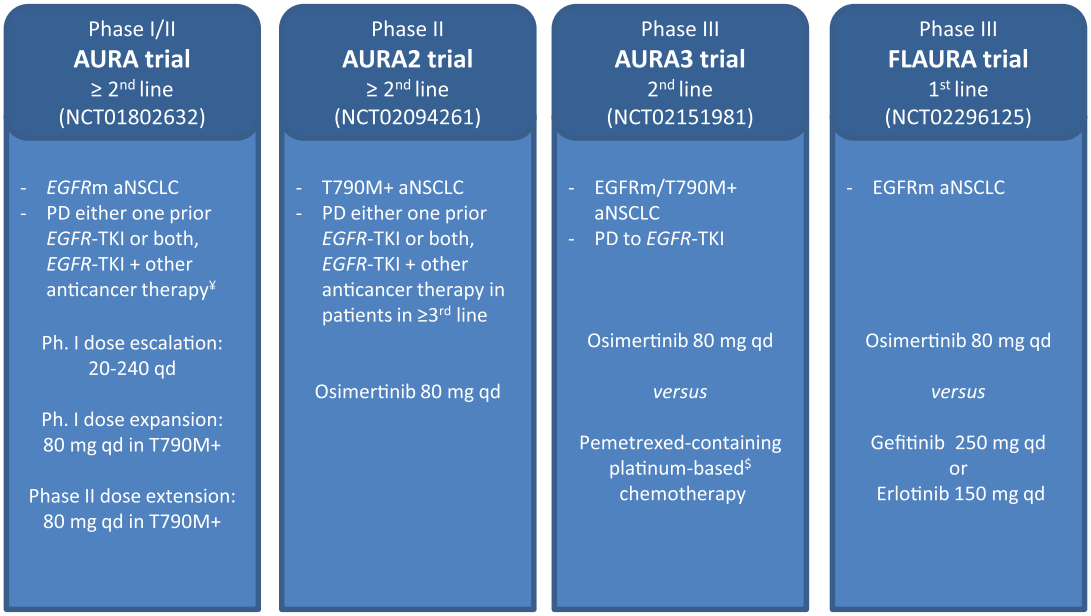

Figure 1. Osimertinib development from phase I–III trials in advanced *EGFR*-mutant NSCLC.
*No limit on prior *EGFR*-TKI or systemic regimens. $Cisplatin or carboplatin. aNSCLC, advanced non-small cell lung cancer; *EGFR*m, epidermal growth factor receptor mutant; NCT number, ClinicalTrials.gov identifier; PD, progressive disease; qd, once daily; TKI, tyrosine kinase inhibitor.

Phase III clinical trials

Additional phase III trials involving osimertinib in different settings are ongoing. The phase III First-Line-AURA (FLAURA) trial [ClinicalTrials.gov identifier: NCT02296125] in *EGFR*-mutated treatment-naïve patients with NSCLC was designed to compare osimertinib 80 mg daily *versus* current standard of care *EGFR* TKIs (gefitinib/erlotinib). The AURA3 trial [ClinicalTrials.gov identifier: NCT02151981] was an open-label, randomized study in the second-line setting of osimertinib *versus* a platinum-based doublet chemotherapy for locally advanced or metastatic NSCLC with the *EGFR*-T790M mutation. In a very recent press release (dated 18 July 2016) published on the AstraZeneca website, it was announced that the AURA3 phase III trial had met its primary endpoint, demonstrating superior PFS compared with standard platinum-based doublet chemotherapy. In this study that included over 400 patients, osimertinib demonstrated a similar safety profile as in previous trials and results for ORR, DCR, and duration of response were also clinically meaningful compared with chemotherapy. Figure 1 summarizes the development of osimertinib monotherapy from phase I through III trials in patients with advanced *EGFR*-mutant NSCLC.

In the adjuvant setting, the ongoing ADjuvant-AURA (ADAURA) trial [ClinicalTrials.gov identifier: NCT02511106] is a double-blind, randomized, placebo-controlled trial assessing the efficacy and safety of osimertinib *versus* placebo in patients with *EGFR*-mutated stage IB–IIIA NSCLC following complete tumor resection. The results are not yet available.

Osimertinib in brain and leptomeningeal metastasis

The cumulative incidence of brain metastasis (BM) and leptomeningeal metastasis (LM) in patients with NSCLC is 16–35% and 3–5%, respectively, and is associated with poor prognosis [Schouten *et al.* 2002; Chamberlain and Kormanik, 1998; Liao *et al.* 2015]. The real incidence in the *EGFR*-mutated NSCLC population is unknown, although some data are available from retrospective cohorts reporting an incidence of 24% for BM and 9% for LM [Rangachari *et al.* 2015; Kuiper *et al.* 2015]. First- and second-generation *EGFR* TKIs have limited blood brain barrier penetration [Omuro *et al.* 2005; Lee *et al.* 2010; Jamal-Hanjani and Spicer, 2012], with afatinib having the highest efficacy despite its incomplete penetration [Hoffknecht *et al.* 2015]. Osimertinib induced sustained tumor regression in an *EGFR*-mutated PC9 mouse BM model and human pharmacokinetics and mouse pharmacokinetics/pharmacodynamics models suggest

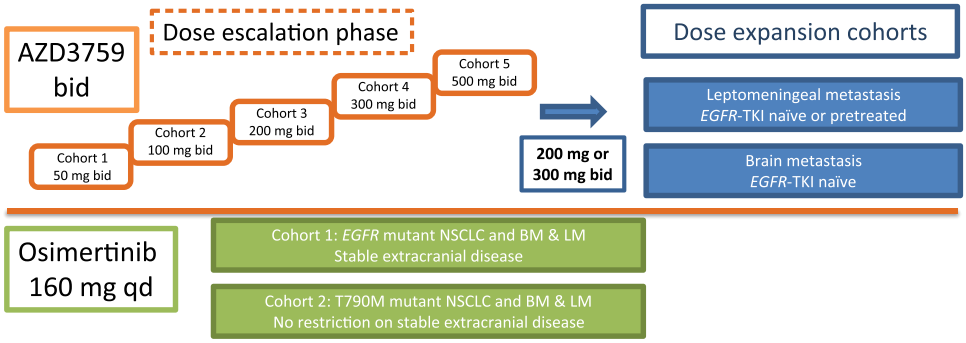

Figure 2. Phase I BLOOM trial to assess the safety, tolerability, pharmacokinetics, and antitumor activity of AZD3759 in *EGFR*-mutant NSCLC and osimertinib 160 mg daily in *EGFR*-mutant NSCLC with central nervous system disease. bid, twice daily; BM, brain metastasis; *EGFR*, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; qd, once daily; TKI, tyrosine kinase inhibitor.

that doses of 80 mg and 160 mg could be active in human central nervous system (CNS) disease [Kim *et al.* 2014]. Clinical activity of osimertinib in CNS disease was observed in the phase I AURA trial and an analysis from AURA phase II trials [Ahn *et al.* 2015] demonstrated the consistent activity of osimertinib in patients with *EGFR*-mutant T790M NSCLC with and without brain metastases, suggesting its activity in the brain. The analysis of osimertinib pharmacokinetics in cerebrospinal fluid was an exploratory objective in the AURA extension phase II trial.

The phase I BLOOM study [ClinicalTrials.gov identifier: NCT02228369] was designed to assess for the first time the safety, tolerability, pharmacokinetics, and preliminary antitumor activity of AZD3759, an oral *EGFR* TKI which has excellent CNS penetration and which induces strong regression of BM in a mouse model [Zeng *et al.* 2015]. In this study, patients with BM and LM may also be enrolled to assess the antitumor efficacy, safety, pharmacokinetics, and potential biological activity of osimertinib 160 mg daily in patients with *EGFR*-mutated NSCLC whose disease failed to respond to standard treatment and who developed CNS disease (Figure 2). The AZD3759 cohort is ongoing while an update from the *EGFR*-mutant NSCLC cohort with LM from the osimertinib arm was recently presented; 21 Asian patients with *EGFR*-mutated NSCLC and LM disease were treated with osimertinib 160 mg daily. All were evaluable for efficacy; seven (33%) had a confirmed radiological response, nine (43%) had stable disease, and neurological function improvement was seen in five (24%) patients [Yang *et al.* 2016a].

Osimertinib in the first-line setting

Considering the activity of osimertinib against *EGFR*-sensitizing as well as *EGFR*-T790M resistance mutations, added to a favorable toxicity profile, in the near future osimertinib may well be considered the option of choice to treat patients with *EGFR*-mutant NSCLC in the first-line setting. Indeed, preliminary efficacy results are encouraging in patients with *EGFR*-mutant NSCLC who are treatment naïve as reported from the two expansion cohorts from the phase I AURA trial [Ramalingam *et al.* 2016]. Results from the phase III FLAURA trial [ClinicalTrials.gov identifier: NCT02296125] are eagerly awaited; if they confirm preliminary results, changes in the current sequence strategy should be discussed.

The safety profile of osimertinib

The dose-limiting toxicity (DLT) of the currently available first- and second-generation TKIs gefitinib, erlotinib, and afatinib is dominated by inhibition of wild-type *EGFR* in the skin and gastrointestinal tract. Osimertinib exhibited around 200 times greater potency against L858R/T790M than wild-type *EGFR*, resulting in an attractive *EGFR*-selective agent in comparison with early-generation TKIs [Cross *et al.* 2014].

Osimertinib was relatively well tolerated in the phase I AURA trial [Jänne *et al.* 2015]. No DLT was observed at any dose level up to 240 mg daily. In the combined cohort of 253 patients, the most common adverse events (usually grade 1–2) were diarrhea (47%), skin toxicity (rash/acne, 40%), nausea (22%), and anorexia (21%). Diarrhea and skin toxicity increased with escalating doses of

Table 2. Summary of drug-related adverse events of osimertinib occurring in at least 15% of patients at the approved dose of 80 mg/day from the phase I AURA trial and the pooled analysis of phase II trials (AURA extension and AURA2) in patients with *EGFR*-T790M-mutant advanced NSCLC.

Adverse event, *n* (%)	Grade 1	Grade 2	Grade ⩾3	Any grade
AURA phase I *N* = 63*				
Rash	21 (33)	2 (3)	0	23 (36)
Diarrhea	16 (25)	3 (5)	1 (2)	22 (35)
Paronychia	11 (18)	6 (10)	1 (2)	18 (29)
Dry skin	11 (18)	3 (5)	0	14 (22)
Fatigue	9 (14)	0	0	10 (16)
Select AEs				
ILD	0	0	1 (2)	1 (2)
QT prolongation	0	0	1 (2)	1 (2)
Hyperglycemia	0	0	0	0
AURA pooled phase II analysis *N* = 411				
Rash	146 (36)	18 (4)	3 (<1)	167 (41)
Diarrhea	138 (34)	17 (4)	2 (<1)	157 (38)
Dry skin	116 (28)	9 (2)	0	125 (30)
Paronychia	88 (21)	30 (7)	0	118 (29)
Select AEs				
ILD	4 (1)	0	8 (2)	12 (3)
QT prolongation	7 (2)	3 (<1)	4 (1)	14 (3)
Hyperglycemia	0	1 (<1)	0	1 (<1)

*63 patients with 'centrally confirmed' T790M-positive NSCLC who have received osimertinib 80 mg/day.
AE, adverse event; EGFR, epidermal growth factor receptor; ILD, interstitial lung disease; NSCLC, non-small cell lung cancer.

osimertinib. Overall, however, osimertinib was associated with less dermatologic and gastrointestinal toxicity compared with historic data and clinical experience with other approved *EGFR* TKIs. Only 13% of patients experienced a grade 3 or higher drug-related adverse event. Serious adverse events were observed in 22% of patients (pneumonitis-like events, pulmonary embolism, and pleural effusion), with 6% of patients experiencing a serious drug-related adverse event. Adverse events prompted drug reductions in 7% of patients and drug discontinuation in 6% of patients. The frequency and severity of adverse events were similar between Asian and non-Asian patients. The six cases of potential pneumonitis-like events resolved after treatment discontinuation. Hyperglycemia and QT prolongation were reported in 6 (2%) and 11 (4%) patients, respectively. Among the seven fatal adverse events reported, only one (pneumonia) was considered as possibly drug related.

The phase II AURA extension and the AURA2 trials showed similar results regarding adverse events. The most frequent adverse events (usually grade 1–2) reported from the pooled analysis were rash (41%), diarrhea (38%), dry skin (30%), and paronychia (29%). Grade 3 or higher adverse events were seen in 36% of patients. Any grade interstitial lung disease and QT prolongation were reported in 3% of patients each and only one case of grade 2 hyperglycemia was reported [Yang *et al.* 2016b]. Unlike osimertinib, hyperglycemia was reported in 36% of patients treated with rociletinib [Sequist *et al.* 2015b]. Table 2 summarizes drug-related adverse events occurring at the approved dose of 80 mg/day from the phase I AURA trial and the pooled analysis from the AURA extension and AURA2 studies, respectively.

Osimertinib-resistant mutations

Preclinical studies and patient post-progression biopsies allowed identification of multiple resistance mechanisms to first- to third-generation *EGFR* TKIs. Following the discovery that T790M is the most common acquired resistance mutation

to gefitinib and erlotinib, several drugs were developed targeting both *EGFR*-sensitizing and T790M-resistant mutations. Although various second-generation *EGFR* TKIs such as afatinib, neratinib, and dacomitinib showed promising activity against T790M-positive cells in preclinical studies, this did not translate into the clinic, with none of them showing efficacy in patients whose disease progressed on the first-generation agents gefitinib and erlotinib [Miller *et al.* 2012; Reckamp *et al.* 2014; Sequist *et al.* 2010]. As a consequence, third-generation *EGFR* TKIs were developed to target the T790M mutation.

Despite impressive initial outcomes with these new molecules, new mutations and other mechanisms of resistance are emerging. Among these, the C797S mutation in exon 20 of *EGFR* was found to be the most common mechanism responsible for resistance to osimertinib. This point mutation was identified from circulating tumor DNA (ctDNA) of patients included in the phase I AURA trial whose disease progressed on osimertinib (6 out of 15 patients, 40%) [Thress *et al.* 2015]. The same mutation was also reported in one case that led to resistance to olmutinib, another oral, third-generation *EGFR* TKI active against mutant *EGFR* isoforms, including T790M [Song *et al.* 2016]. Preclinical *EGFR* L858R/T790M/C797S mutation cell models exhibited *in vitro* sensitivity to cetuximab, an antibody that blocks *EGFR* dimerization [Li *et al.* 2005; Ercan *et al.* 2015], but this was not confirmed in *in vivo* analyses. However, the allosteric inhibitor EAI045 in combination with cetuximab exhibited mechanistic synergy and was effective in mouse models of lung cancer driven by *EGFR* L858R/T790M and by *EGFR* L858R/T790M/C797S [Jia *et al.* 2016]. Interestingly, the allelic context in which C797S was acquired may predict responsiveness to subsequent TKI treatments. For example, if the C797S and T790M mutations are in *trans*, cells will be resistant to third-generation *EGFR* TKIs, but sensitive to a combination of first- and third-generation TKIs; and if C797S develops in T790 wild-type cells, this results in resistance to third-generation TKIs, while sensitivity to first-generation TKIs is retained [Niederst *et al.* 2015]. These data are of great clinical value in sequencing for this mutation in patients with acquired resistance to osimertinib.

The acquired resistance associated with the *EGFR* T790M mutation can occur either by selection of preexisting *EGFR* T790M-positive clones or *via* genetic evolution of initially *EGFR* T790M-negative drug-tolerant cells, suggesting that cancer cells that survive third-generation TKIs may serve as a key reservoir from which acquired resistance can emerge during treatment [Hata *et al.* 2016]. Navitoclax (ABT-263, [Ackler et al. (2012)], Abbott Laboratories, Illinois, USA) a BCL-2 family inhibitor, enhances the apoptotic response of late-resistant *EGFR* T790M cells with decreased sensitivity to *EGFR* inhibition. The combination of navitoclax with the third-generation *EGFR* TKI WZ4002 (preclinical compound) induced more apoptosis compared with WZ4002 alone in both *in vivo* and *in vitro* analyses. This approach could be an effective strategy for treating *EGFR* T790M-positive cancers that have a decreased apoptotic response to EGFR inhibition [Hata *et al.* 2016]. An ongoing phase Ib trial is evaluating the safety and tolerability of the osimertinib/navitoclax combination in patients with *EGFR*-mutant NSCLC following resistance to prior *EGFR* TKIs [ClinicalTrials.gov identifier: NCT02520778].

Additional *EGFR*-independent mechanisms of resistance to osimertinib have been reported. *NRAS* mutations, including a novel E63K mutation, and amplifications of wild-type *NRAS* or *KRAS* have been described as mechanisms of acquired resistance to osimertinib but also to gefitinib and afatinib [Eberlein *et al.* 2015]. *In vitro*, a combination of osimertinib with the *MEK* inhibitor selumetinib prevented emergence of resistance in PC9 (Ex19del) cells and delayed resistance in NCI-H1975 (L858R/T790M) cells. *In vivo*, concomitant osimertinib with selumetinib caused regression of osimertinib-resistant tumors in an *EGFR*-mutant/T790M transgenic model [Eberlein *et al.* 2015]. This association is been evaluated in the phase Ib TATTON trial [ClinicalTrials.gov identifier: NCT02143466]. In addition, the combination of trametinib, another *MEK* inhibitor, with WZ4002 prevents the development of acquired resistance in *EGFR*-mutant lung cancer models [Tricker *et al.* 2015].

Amplifications in *HER2* and *MET* genes were also described as potential mechanisms of acquired resistance to osimertinib in patients with *EGFR*-T790M-mutant NSCLC [Planchard *et al.* 2015]. Additionally, loss of T790M at the time of progression may be mediated by overgrowth of cells harboring *HER2* amplification, *BRAF* V600E or

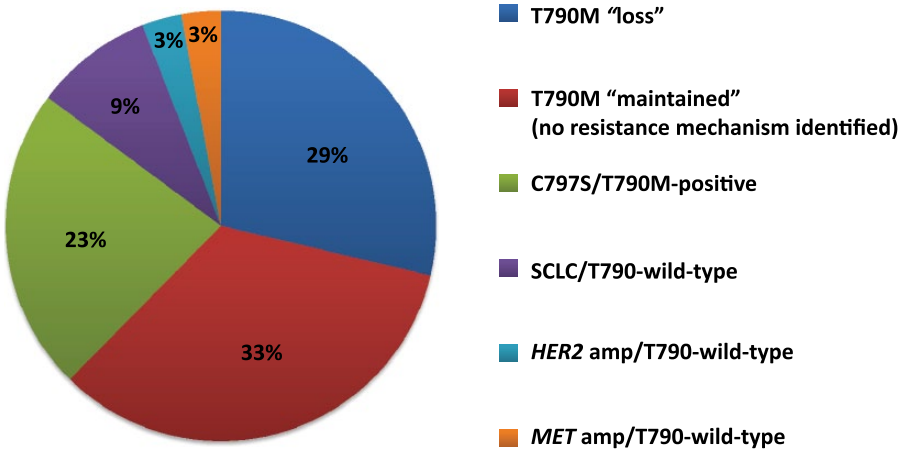

Figure 3. Mechanisms of resistance to third-generation *EGFR* TKIs osimertinib and rociletinib. Data from Piotrowska *et al.* [2015], Thress *et al.* [2015], Yu *et al.* [2015], Planchard *et al.* [2015], and Kim *et al.* [2015]. amp, amplification; EGFR, epidermal growth factor receptor; SCLC, small cell lung cancer; TKI, tyrosine kinase inhibitor.

PIK3CA mutations, as was recently reported following examination of plasma specimens from patients included in the phase I AURA trial [Oxnard *et al.* 2015].

In addition, resistant tumors have been reported to show phenotypic changes, such as small-cell lung cancer transformation or epithelial to mesenchymal transition [Sequist *et al.* 2011; Yu *et al.* 2013; Kim *et al.* 2015]. Figure 3 summarizes the known mechanisms of resistance to third-generation *EGFR* TKIs.

Overcoming osimertinib-resistant disease

The heterogeneity in the acquired resistance mechanisms to osimertinib provides the basis for investigating different inhibitory combination strategies. Therefore, osimertinib-based combinations are currently being investigated in several studies. The multiarm phase Ib TATTON trial [ClinicalTrials.gov identifier: NCT02143466] was designed to evaluate the safety, tolerability, and preliminary antitumor activity of osimertinib in combination with durvalumab (anti-PD-L1 monoclonal antibody), savolitinib (*MET* inhibitor) or selumetinib (*MEK* 1/2 inhibitor) in patients with advanced *EGFR*-mutant NSCLC whose disease has progressed on an *EGFR* TKI. Preliminary results from the osimertinib/durvalumab arm were recently presented [Ahn *et al.* 2016]. In patients with prior *EGFR* TKI therapy, investigator-assessed ORR was 67% (6/9) in those with T790M-mutant tumors compared with 21% (3/14) in T790M-negative NSCLC. Regarding safety data, interstitial lung disease was reported in 38% (13/34) of patients, higher than would be expected with either drug alone. Five events were grade 3–4 and there were no fatalities; most cases were managed using steroids [Ahn *et al.* 2016]. Based on these data, the recruitment into the osimertinib plus durvalumab arm of TATTON is currently on hold, but expansion cohorts of the *MET* and *MEK* inhibitor combinations are ongoing. In addition, the phase III Combination-AURA in Lung (CAURAL) trial [ClinicalTrials.gov identifier: NCT02454933] is being conducted in second-line metastatic *EGFR*-mutant/T790M-positive NSCLC patients testing osimertinib plus durvalumab *versus* osimertinib monotherapy for their impact on PFS. This study was also stopped prematurely due to the pulmonary toxicity observed in the TATTON trial.

On the basis of preclinical observations that afatinib (an irreversible *ErbB* family blocker) plus cetuximab (an anti-*EGFR* monoclonal antibody) overcame T790M-mediated resistance [Regales *et al.* 2009], the combination was evaluated in a phase Ib trial enrolling 126 heavily pretreated patients with advanced *EGFR*-mutant NSCLC who developed resistance to first-generation erlotinib/gefitinib. The ORR was 29%, comparable in both T790M-positive and T790M-negative tumors (32% *versus* 25%) and the median PFS was 4.7 (95% CI 4.3–6.4) months [Janjigian *et al.* 2014]. However, dual *EGFR* inhibition significantly improves toxicity,

including (all grade) rash (seen in 90% of patients), diarrhea (71%), and stomatitis (56%). Grade 3–4 adverse events were observed in 46% of patients [Janjigian *et al.* 2014]. A randomized phase II/III trial [ClinicalTrials.gov identifier: NCT02438722] of afatinib plus cetuximab *versus* afatinib alone is currently open in treatment-naïve patients with advanced *EGFR*-mutant NSCLC. The dual *EGFR* blockage is being evaluated in a phase I trial [ClinicalTrials.gov identifier: NCT02496663] combining osimertinib with the anti-*EGFR* monoclonal antibody necitumumab to assess safety and determine the optimal dose in patients with *EGFR*-mutant advanced NSCLC whose disease has progressed on a previous *EGFR* TKI.

The dual vascular endothelial growth factor receptor (VEGFR) and EGFR blockade inhibits tumor growth in *EGFR* TKI resistance xenograft models [Naumov *et al.* 2009]. Indeed, this hypothesis was confirmed in two phase II clinical trials in patients with *EGFR*-mutant NSCLC who are treatment naïve: the randomized Japanese (JO25567) trial comparing erlotinib plus bevacizumab *versus* erlotinib alone, and the single-arm (Bevacizumab and ErLotinib In EGFR Mut+ NSCLC [BELIEF]) trial in white patients. Median PFS was encouraging and similar in both studies, supporting the combination in the first-line setting [Seto *et al.* 2014; Stahel *et al.* 2015]. A phase I trial was thus designed to evaluate the safety of two osimertinib-based combination strategies, with necitumumab or ramucirumab (an anti-*VEGFR2* monoclonal antibody) in patients with advanced *EGFR*-T790M-mutant NSCLC after progression on first-line *EGFR* TKI therapy [ClinicalTrials.gov identifier: NCT02789345]. Finally, the combination of osimertinib and bevacizumab will be evaluated in another phase I/II 3+3 dose-escalation design [ClinicalTrials.gov identifier: NCT02803203] to test the safety of combining these drugs.

For patients whose tumors undergo small-cell lung cancer transformation, platinum-based plus etoposide chemotherapy is recommended. Table 3 provides information about ongoing and forthcoming osimertinib-based combination trials to treat or prevent osimertinib-acquired resistance.

Osimertinib in the era of liquid biopsies

To date, there is increasing evidence that a single tissue biopsy may not adequately represent intrinsic tumor heterogeneity, particularly in cases of disease progression. Moreover, tumor location and the risk of complications are limitations for new tissue biopsies. Emerging evidence suggests that analysis of ctDNA could more broadly capture the spectrum of resistant clones that may appear throughout the course of the disease. Performing serial ctDNA analyses could also evaluate the longitudinal response, and potentially detect resistance mutations before documented radiographic progression [Thress *et al.* 2015; Piotrowska *et al.* 2015]. For example, ctDNA was used to detect T790M in plasma in 70% (23 of 35) of patients treated with rociletinib who had a T790 wild-type tissue biopsy [Sequist *et al.* 2015a]. Notably, the efficacy of rociletinib was equivalent whether T790M was detected in tissue or in plasma, suggesting that noninvasive testing may be adequate for predicting response and could provide additional information in patients with tissue biopsies which are negative for T790M [Thress *et al.* 2015; Piotrowska *et al.* 2015]. In addition, early acquisition of *EGFR*-resistance mutations could be found by measuring ctDNA in the urine [Husain *et al.* 2015]. Recently, genotype-matched results from plasma, tissue, and urine samples from patients included in the phase I/II TIGER-X trial were reported. Considering the tissue sample as the reference, sensitivity for detecting T790M mutation in plasma and urine was 80.9% and 81.1%, respectively. Response rates were similar in the T790M-mutant population irrespective of whether the status was identified in plasma, tissue, or urine [Wakelee *et al.* 2016].

Plasma samples from 192 patients enrolled in the phase I AURA trial were collected and genotyped. Sensitivity for detecting *EGFR*-sensitive and T790M-resistant mutations was 87% and 78%, respectively. Clinical response rates were greater in T790M-positive patients, as assessed by either tissue or plasma genotyping [Thress *et al.* 2014]. Eligibility for treatment with osimertinib will be dependent on mutational status, which will be determined *via* a validated diagnostic test based on a tumor tissue sample or plasma. Availability of a blood-based test for ctDNA means that physicians and patients have multiple options to test for a T790M-resistant mutation.

Discussion

The *EGFR*-T790M mutation is the main mechanism of acquired resistance to first- and

Table 3. Ongoing and forthcoming osimertinib-based combination trials.

Trial, ClinicalTrials.gov identifier	Drug combination	Mechanism of action	Population and setting	Primary endpoint	Status
NCT02143466 'TATTON' phase Ib	Durvalumab	Anti PD-L1 antibody	Advanced *EGFR*-mutant NSCLC that has progressed to *EGFR* TKI	Part A: safety and tolerability	On hold
	Savolitinib	*MET* inhibitor		Part B: safety, tolerability and efficacy	Recruiting
	Selumetinib	*MEK* inhibitor			Recruiting
NCT02454933 'CAURAL' phase III	Osimertinib monotherapy		*EGFR* mutant/T790M-positive NSCLC that has progressed to *EGFR* TKI	PFS	On hold
	Durvalumab	Anti PD-L1 antibody			
NCT02496663 phase I	Necitumumab	Anti *EGFR* antibody	Advanced *EGFR*-mutant NSCLC that has progressed to *EGFR* TKI	Safety and tolerability	Recruiting
NCT02803203 phase I/II	Bevacizumab	Anti *VEGF* antibody	Advanced *EGFR*-mutant NSCLC in first-line setting	Phase I: MTD Phase II: PFS	Recruiting
NCT02789345 phase I	Necitumumab	Anti *EGFR* antibody	*EGFR*-mutant/T790M-positive NSCLC that has progressed on first-line *EGFR* TKI	ORR	Forthcoming
	Ramucirumab	Anti VEGFR2 antibody			
	Necitumumab + Ramucirumab				
NCT02520778 phase Ib	Navitoclax	Bcl-2 family inhibitor	Advanced *EGFR*-mutant NSCLC that has progressed to *EGFR* TKI	Safety and tolerability	Recruiting
NCT02503722 phase I/II	Sapanisertib	TOR1/2 inhibitor	Advanced *EGFR*-mutant NSCLC that has progressed to *EGFR* TKI	Safety and recommended phase II dose Safety and efficacy in T790M population	Forthcoming

MTD, maximal tolerated dose; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; ORR, overall response rate; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

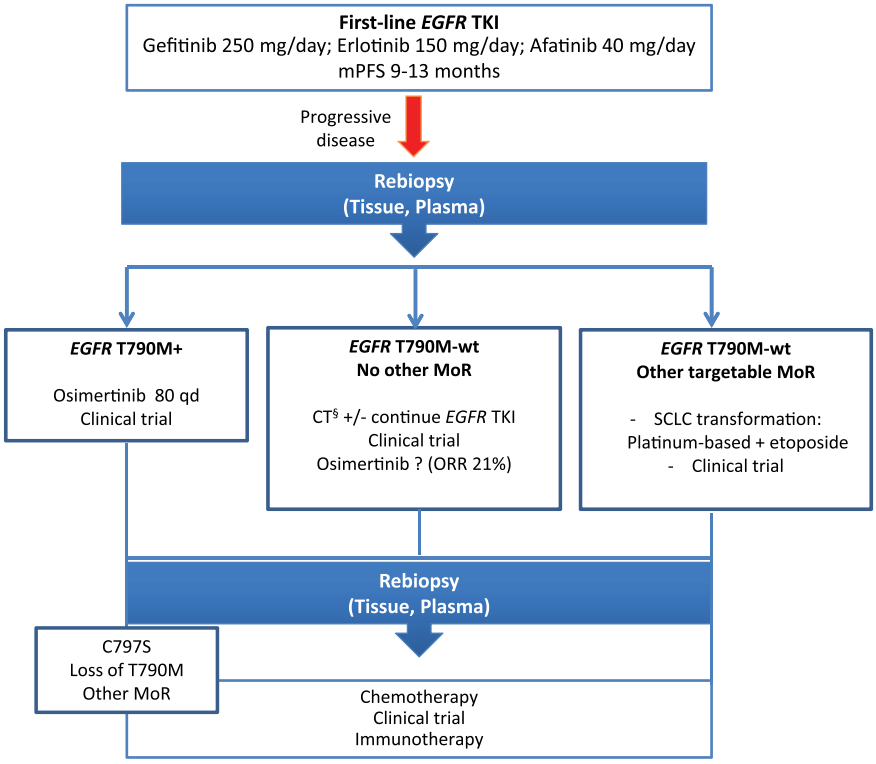

Figure 4. Potential treatment algorithm for patients with *EGFR*-mutated advanced NSCLC. CT, chemotherapy; EGFR, epidermal growth factor receptor; MoR, mechanism of resistance; mPFS, median progression-free survival; ORR, overall response rate; qd, once daily; SCLC, small cell lung cancer; TKI, tyrosine kinase inhibitor.

second-generation *EGFR* TKIs and represents a barrier in the treatment of patients with *EGFR*-mutant advanced NSCLC. Osimertinib has demonstrated strong efficacy and safety data in phase I and II trials, and has become the first *EGFR* inhibitor approved for the treatment of NSCLC with the *EGFR*-T790M mutation. Patients with advanced NSCLC with *EGFR*-activating mutations whose disease progresses on a first-line *EGFR* TKI have traditionally been offered platinum-doublet chemotherapy as second-line treatment. Platinum-doublet chemotherapy shows ORRs of approximately 30%, slightly higher than the rate observed in the T790M-negative population, but significantly lower than the 61–71% ORR reported in T790M-positive cohorts in phase I and II trials with osimertinib. The phase III AURA3 trial [ClinicalTrials.gov identifier: NCT02151981] confirms the superiority of osimertinib for treating patients with *EGFR*-T790M-mutant NSCLC in the second-line setting compared with standard pemetrexed-containing/platinum-based chemotherapy. In addition, considering the favorable safety profile of osimertinib added to its systemic and CNS efficacy, osimertinib is currently the most attractive option in the second-line setting for patients with T790M-mutant NSCLC, delaying chemotherapy to the third-line setting, as well as for patients with T790M-postive NSCLC with brain or leptomeningeal metastases. Figure 4 illustrates a potential treatment algorithm for patients with *EGFR*-mutated advanced NSCLC. If we take into consideration the encouraging response outcomes (ORR 77%, DCR 98%) and PFS (approximately 19 months in the first-line setting), osimertinib is likely to be the best option for treating patients with advanced *EGFR*-mutant NSCLC as first-line therapy. The phase III FLAURA trial [ClinicalTrials.gov identifier: NCT02296125] probably gives us the approach for better positioning osimertinib regarding current *EGFR* TKIs in order to improve sequences with the final objective of improving patient outcomes.

The role of *EGFR* TKIs in the adjuvant setting for nonmetastatic *EGFR*-mutated lung cancer is in a very early development stage and remains

controversial. Erlotinib and gefitinib were evaluated in prospective trials suggesting an improvement in disease-free survival, but none of these trials demonstrate a benefit in overall survival [Goss *et al.* 2013; Janjigian *et al.* 2011; Pennell *et al.* 2014; Kelly *et al.* 2015]. The phase III ADAURA trial [ClinicalTrials.gov identifier: NCT02511106] comparing osimertinib with placebo as adjuvant therapy in stage IB-IIIA *EGFR*-mutated NSCLC following complete tumor resection is currently recruiting patients, and the jury remains out until at least preliminary results become available. These studies have the potential to significantly expand the role of osimertinib in the treatment algorithm for *EGFR*-mutated NSCLC.

The heterogeneity of resistant cancers plays an important role, not only in terms of response and resistance to the new *EGFR* TKIs, but in allowing different combination strategies to be more effective in preventing and delaying resistance mechanisms. Due to its safety profile, osimertinib is now considered an attractive drug to combine with other targeted therapies. While combinations with *MEK* and *MET* inhibitors as well as antiangiogenic agents are promising, we must exercise precaution with respect to their toxicity profiles. Table 3 summarizes ongoing and forthcoming osimertinib-based combination trials.

Conclusion

Osimertinib, developed in less than 3 years, represents one of the fastest cancer drug development programs with respect to obtaining approval for the treatment of patients with *EGFR*-T790M NSCLC whose disease has progressed on *EGFR* TKIs. The encouraging results obtained in patients with *EGFR*-mutant NSCLC in the first-line setting place it as an established critical drug in this scenario.

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