

Epidermal growth factor receptor T790M mutation-positive metastatic non-small-cell lung cancer: focus on osimertinib (AZD9291)

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Abstract: Adenocarcinoma is the most common type of non-small-cell lung cancer (NSCLC). Adenocarcinoma with epidermal growth factor receptor (EGFR) mutations accounts for 8%–30% of all cases of NSCLC depending on the geography and ethnicity. EGFR-mutated NSCLC usually responds to first-line therapy with EGFR tyrosine kinase inhibitors (TKIs). However, there is eventual loss of efficacy to TKIs due to development of resistance. The most frequent cause for resistance is a second EGFR mutation in exon 20 (T790M), which is encountered in up to 62% of patients. Osimertinib is one of the third-generation EGFR TKIs with a high selective potency against T790M mutants. In Phase I trial of osimertinib in advanced lung cancer after progression on EGFR TKIs, the response rate and disease control rate were 61% and 95%, respectively. A subsequent Phase II (AURA2) trial demonstrated a disease control rate of 92%, a response rate of 71%, a median duration of response of 7.8 months, and a median progression-free survival of 8.6 months. Osimertinib was approved by the US Food & Drug Administration in November 2015 for patients whose tumors exhibited T790M mutation and for those with progressive disease on other EGFR TKIs. In this review, we address the role of EGFR TKIs in the management of EGFR mutation lung cancer and the mechanisms of resistance to TKIs with a focus on the role of osimertinib. Data from completed trials of osimertinib, ongoing trials, as well as novel diagnostic methods to detect *EGFR* T790M mutation are reviewed.

Keywords: osimertinib, AZD9291, third-generation EGFR TKIs, EGFR T790M, lung adenocarcinoma, NSCLC

Epidermal growth factor receptor mutation (EGFRm) and tyrosine kinase inhibitors (TKIs) in non-small-cell lung cancer (NSCLC)

Lung cancer is one of the leading causes of cancer deaths, accounting for ~27% in males and 26% in females of all cancer deaths.¹ The mortality rate from lung cancer exceeds that of breast, prostate, and colorectal cancers combined.^{1,2} In 2016, 224,390 new cases of lung cancer are estimated, with 158,080³ deaths being attributed to the disease. Only 17.7% of lung cancer patients are expected to be living 5 years after diagnosis.³

NSCLC accounts for 83% of lung cancers, and 50% of these are adenocarcinomas.² The 5-year survival rates are 55%, 27%, and 4% in localized, regional, and metastatic diseases, respectively, and the median survival is between 10 months and 12 months in advanced NSCLC.² EGFRm NSCLC accounts for 10%–15% of NSCLC patients in Europe, 30%–40% in Asia, and 7%–8% in North America.⁴ The incidence of

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EGFRm lung cancer is 22%–60% in women vs 8%–37% in men and 32%–64% in never smokers vs 6%–33% in smokers.^{4,5}

Most of the EGFRms have been found to lie between exon 18 and exon 21. The most common mutations are in-frame deletions around the LREA motif of amino acid (leucine, arginine, glutamic acid, and alanine) sequences from 747 to 750, 9–24 base pairs in exon 19, and the L858R point mutation in exon 21, which occurs in 45% and 40% of cases, respectively. Together, the axon 19 and 21 mutations account for 85%–90% of all EGFRms.⁶

Metastatic lung cancer patients with activating EGFRm receive first-line therapy with epidermal growth factor receptor (EGFR) TKIs and generally experience an objective response rate (ORR) of ~70%–80% and a median progression-free survival (PFS) of 10–12 months.^{6,7} First-line TKI therapy provides a median PFS of 14.6 months and 9.7 months in patients with exon 19 deletions⁵ and L858R mutation, respectively.^{6,8}

Till date, there exist three generations of TKIs. Erlotinib and gefitinib are the first-generation agents; afatinib, dacomitinib, and neratinib are the second-generation agents; and osimertinib, rociletinib, HM61713, and ASP8273 are the recently developed third-generation agents. The latter two are still in the development phase.

Gefitinib was the first EGFR TKI to be available. It was introduced in Japan in 2002 and then in the USA in 2003. Initial Phase II studies had shown objective response rate between 12% and 18% with median survival between 7 months and 8 months.⁹ This drug, which was restricted earlier in the USA, was reintroduced in the year 2015, after a Phase IV study demonstrating an ORR of 70%, a median PFS of 9.7 months, and a median OS of 19 months.¹⁰ Erlotinib was approved in 2004 for locally advanced and metastatic disease after the failure of at least one prior chemotherapy. In 2010, it received approval as maintenance therapy without disease progression after four cycles of platinum-based chemotherapy.^{6,8} In 2013, it was approved as first line in the metastatic setting. Based on LUX-Lung 3 and LUX-Lung 6 trials, the Food & Drug Administration approved afatinib as first-line treatment for metastatic EGFRm NSCLC in 2013 (Table 1).^{11,12}

Resistance to TKI therapy can occur as secondary mutations in the *EGFR* gene, acquired mutations in other oncogenic genes, upregulation of signaling pathways, amplification of EGFR, or histological transformation to small-cell lung cancer. KRAS and ALK rearrangements, mutation with exon 20 insertion, are among the causes for resistance to TKI.¹³ Other rare and less studied mutations

include exon 18 point mutations, exon 19 insertions, exon 21 L861Q, and exon 18 (G719X).^{5,13}

Gatekeeper mutation in the EGFR kinase domain (EGFR T790M) of exon 20 accounts for 51%–68% of cases and is the most common resistance mechanism to first- and second-generation TKIs,¹³ followed by human epidermal growth factor receptor 2 (HER2) gene amplification (12%–15%), MET gene amplification (5%–11%), transformation to small-cell carcinoma (5%), phosphatidylinositol 3-kinase A (PIK3A) gene mutation (1%), or activating mutations in RAS or BRAF.^{14–17}

T790M mutation leads to an enhanced affinity for adenosine triphosphate, thereby reducing the ability of reversible EGFR TKIs to bind to the tyrosine kinase domain of EGFR.¹⁴ Threonine amino acid replaces methionine at the T790M position of exon 20 and causes steric hindrance to bind the reversible TKIs and increases the affinity for ATP. This increases phosphorylation and reduce the potency of TKIs.^{14,18} Extracellular signal-regulated kinase (ERK) activation (via MEK1 amplification or mutation) and downstream inhibitors of this pathway are other resistant pathways detected on progression along with RET rearrangement.

Besides third-generation EGFR TKIs, several strategies are in clinical evaluation for reversal of acquired resistance to first- and second-generation EGFR TKIs. Second-generation EGFR TKIs such as afatinib, dacomitinib, and neratinib have been found to inhibit T790M in vitro, but the required doses are significantly higher in vivo, which limits their use due to unacceptable toxicity.¹⁹ Another strategy focuses on dual inhibition of EGFR.²⁰ The combination of afatinib with cetuximab in a Phase II trial resulted in a response rate of 30% and a median PFS of 4.7 months in heavily pretreated patients.²⁰ The clinical implication may be limited by severe gastrointestinal and skin toxicities. Furthermore, the combination of erlotinib and bevacizumab resulted in good outcome in the first-line treatment of patients with T790M-positive NSCLC in the BELIEF Phase II trial.²¹ The 1-year PFS rate was 72% without any unexpected toxicities.

Third-generation EGFR TKIs

Therapeutic approach to disease progressive on first- and second-generation TKIs depends on the severity of symptoms and the location of progression. National Comprehensive Cancer Network (NCCN) panel recommends to continue the same TKI with local treatment if there is local progression and to add chemotherapy to TKI or switch to third-generation TKI in case T790M mutation.²² Restarting the same TKI with or without everolimus was not beneficial and not recommended.²³

Table 1 Phase III trials of first- and second-generation EGFR TKIs

Phase III trial	Sample size/ population group	TKI	Comparator	Median OS in months (TKI vs chemotherapy)	Hazard ratio for OS, P-value	ORR	PFS in months (TKI vs chemotherapy)	Hazard ratio for PFS, P-value
LUX-Lung 3 ¹¹	345/stage IIIB or IV	Afatinib	Pemetrexed + cisplatin	16.6 vs 14.8 (OS not reached in any group)	HR 1.12, 95% CI (0.73–1.73), P=0.60	56% vs 23% (independent review) and 69% vs 44% (investigator review)	11.1 vs 6.9	HR 0.58, 95% CI (0.43–0.78), P=0.001
LUX-Lung 6 ¹²	364/stage IIIB or IV	Afatinib	Gemcitabine + cisplatin	22.1 vs 22.2	HR 0.95, 95% CI (0.68–1.33), P=0.76	66.9% vs 23% (independent review)	11.0 vs 5.6 (independent review) and 13.7 vs 5.6 (investigator assessment)	Independent review: HR 0.28, 95% CI (0.20–0.39), P<0.0001 Investigator assessment: HR 0.26, (95% CI 0.19–0.36), P<0.0001
IPASS Iressa Pan-Asia Study ²¹	1217/stage IV- All patients without prior treatment	Geftinib	Carboplatin + paclitaxel	18.8 vs 17.4	HR 0.90, 95% CI (0.79–1.02), P=0.109	43% vs 32%	5.7 vs 5.8	HR 0.74, 95% CI (0.65–0.85), P<0.0001
WJTOG3405 ²²	177/stage IIIB/IV	Geftinib	Cisplatin and docetaxel	30.9 vs not reached	HR 1.638, 95% CI (0.749–3.582), P=0.211	62.1% vs 32.2%	9.2 vs 6.3	HR 0.489, 95% CI (0.336–0.710), P<0.0001
EURTAC ⁶	174/stage IV chemo-naïve NSCLC	Erlotinib	Cisplatin + docetaxel or gemcitabine; carboplatin + docetaxel or gemcitabine	22.9 vs 19.5	HR, 95% CI (0.64–1.35), P=0.93	65% vs 16%	10.4 vs 5.2	HR 0.34, 95% CI (0.23–0.49), P<0.0001
OPTIMAL study ⁸	165/stage IIIB/IV NSCLC with EGFR mutation	Erlotinib	Carboplatin + gemcitabine	Not reported	Not reported	83% vs 36%	13.1 vs 4.6	HR 0.16, 95% CI (0.10–0.26), P<0.0001

Abbreviations: EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; TKIs, tyrosine kinase inhibitors; HR, hazard ratio.

Third-generation EGFR TKIs are more potent against T790M mutants, with higher selectivity for them over wild-type (WT) EGFR. While many such TKIs are being evaluated in preclinical and early-phase studies, such as HM61713 (BI 1482694),²⁴ ASP8273,²⁵ EGF816,²⁶ and PF-06747775,²⁷ two of these covalent EGFR inhibitors including CO-1686 (rociletinib) and AZD9291 (osimertinib) have made it through Phase I and II trials. Both drugs contain a distinctive aminopyrimidine scaffold that helps to avoid the steric interference with the mutant protein.²⁸ Of these, osimertinib is the only agent currently approved for clinical use in the USA and Europe.

Rociletinib

Rociletinib (CO-1686; Clovis Oncology, Boulder, CO, USA) is an oral, covalent inhibitor of EGFRs. Like other third-generation EGFR TKIs, rociletinib has minimal activity against WT EGFR. It does not affect exon 20 insertions but inhibits exon 19 deletions, L858R, and T790M mutants as was evident in preclinical studies that confirmed its activity against EGFRm-positive tumors.²⁹

Efficacy and dosage of rociletinib were studied in a Phase I/II study as second-line treatment in EGFR-mutated NSCLC.³⁰ Doses of 500 mg, 625 mg, and 750 mg twice daily were used, with no maximum tolerated dose (MTD) identified after enrolling 130 patients. The ORR was 59% in patients with T790M-positive disease, and the ORR was 29% in patients with T790M-negative disease. The median age was 60 years; females comprised 77% of the patients, and only 15% of the patients were Asian. However, in a subsequent pooled TIGER-X phase I/2 and TIGER-2 analysis, the ORR with rociletinib (500–750 mg twice daily) among 325 patients with EGFR T790M-positive metastatic NSCLC who progressed on at least one EGFR inhibitor was lower at 30.2% (95% CI, 25.2–35.5).³¹ The manufacturer has terminated enrollment in all ongoing rociletinib studies, including the Phase III TIGER-3 trial (a Phase III trial, patients with EGFRm NSCLC who progressed on platinum-based regimen and one previous EGFR TKI were treated with either rociletinib or single-agent chemotherapy), and has withdrawn its application for regulatory approval in the European Union.

Osimertinib

Osimertinib (monoanilinopyrimidine AZD9291) is an irreversible TKI that targets T790M-resistant mutations as well as the sensitizing forms of the EGFR tyrosine kinase with selectivity over the WT form of the receptor. Osimertinib binds covalently to cysteine-797 residue in

ATP-binding site, as confirmed by mass spectrometry of chymotrypsin digestion.³²

Preclinical data of osimertinib

EGFR recombinant enzyme assay showed that AZD9291 is 200 times more potent against L858R/T790M than WT EGFR. Products of AZD9291 metabolism are mainly two products, AZ5104 and AZ7550. Biochemical assays in murine models show that AZ7550 has similar profile to the parent molecule, while AZ5104 has more potency against both mutant and WT EGFR.²⁸ AZD9291 led to tumor shrinkage and resolution in mutant EGFR xenograft models after 14 days of 2.5 mg/kg/day dose.²⁸ AZD9291 showed activity in two mouse tumor models (EGFR^{L858R} and EGFR^{L858R/T790M}) with lung adenocarcinoma, compared to afatinib, which was active on EGFR^{L858R} only, and a control, which did not show activity in either type.²⁸

Osimertinib in Phase I/II trials

Phases I and II AURA trial tested osimertinib as second line in patients with advanced lung cancer who progressed on EGFR TKI.³³ The dose-escalating group included 31 patients using doses from 20 mg to 240 mg a day, and had no dose-limiting toxicities. An additional 222 patients were included in the expansion group. In this trial, 31% were men. Asian and Caucasian patients were 60% and 27%, respectively. The AURA2 Phase II trial abstract was presented at the 16th World Conference on Lung Cancer.³⁴ In this trial, 70% of the population were females, 63% were Asian, and 76% were nonsmokers. Osimertinib was used as second line after NSCLC progression on frontline EGFR TKI.

The objective response rate (partial response [PR] and complete response [CR]) was 51%–71%. Stable disease and disease control (which included CR, PR, or stable disease) were achieved in 33% and 84%, respectively. Disease control rate in EGFR T790M-positive group was 92%–95% with a PFS of 8.6–9.6 months, while patients with negative EGFR T790M had a PFS of 2.8 months and a response rate of 21%. Response rate was similar among Asian and non-Asian patients. Response rate and PFS from AURA I–II and AURA2 trials are summarized in Table 2.

Grade III or higher side effects were observed in 32% of patients. Gastrointestinal symptoms were the most common side effects (diarrhea 47%, nausea 22%, and decreased appetite 21%), followed by dermatologic side effects (rash 40%, dry skin, and pruritus). Dose reduction and drug discontinuation due to side effects happened in 7% and 6%, respectively. Pneumonitis-like disease was observed in 2% of patients;

Table 2 Early-phase osimertinib trial

Trial	N (number of patients)	Partial response, n (%) [*]	ORR (%)	CR/SD/PD, n (%)	Disease control, n (%) [*]	PFS (months)
AURA Phases I and II						
All patients	239	123 (51%)		1/78 (33%)/34 (14%)	84%	8.2
Positive EGFR T790M	127	61% (78)			95% (121)	9.6
Negative EGFR T790M	61	13 (21%)			37 (61%)	2.8
AURA2 Phase II		71%			92%	8.6
Phase I first line						
All patients	60		77		97%	19.3
Dose 80 mg	30		67			Not reached
Dose 160 mg	30		87			19.3

Note: ^{*}Unless indicated.

Abbreviations: CR, complete response; EGFR, epidermal growth factor receptor; ORR, overall response rate; PD, progressive disease; PFS, progression-free survival; SD, stable disease.

all except one resolved after discontinuation of the drug. Fatal events were reported in 2.7% of patients, one of which (pneumonia) was reported as being possibly drug-related. Hyperglycemia and QT prolongation were seen in 2% and 4%, respectively, but they did not result in dose reduction.

FDA approval of osimertinib

On November 13, 2015, the FDA approved osimertinib for patients with (T790M) positive NSCLC whose disease had progressed on other EGFR TKIs. The European Commission approval was received on February 2, 2016. Based on FDA approval, the presence of the EGFR T790M mutation should be confirmed before starting therapy with osimertinib. The recommended dose is 80 mg, taken daily by mouth with or without food. Missed doses of osimertinib should not be made up.³⁵ Osimertinib is available in two dosage strengths, including 40 mg and 80 mg tablets. Dose selection was based on pharmacokinetics analyses showing that the 80 mg dose ensured exposure levels greater than that observed for the 20 mg or 40 mg dose, which had also demonstrated clinical activity in the AURA Phase I study. The 80 mg dose provided substantial clinical efficacy and was associated with fewer dose reductions, lower incidence of skin disorders, nail effects, and diarrhea compared with 160 mg and 240 mg doses, which appeared unlikely to provide additional efficacy.³⁶

NCCN guidelines to use osimertinib in EGFR-positive NSCLC²²

On November 6, 2015, based upon review of the AURA and AURA2 data, NCCN panel consensus was to add osimertinib as a category 2A recommendation for the following indications: symptomatic progression with isolated systemic metastasis; symptomatic progression and multiple systemic

metastasis; progression on subsequent therapy; asymptomatic and symptomatic progression, and brain metastases.

Ongoing trials using osimertinib

Based on Phase II trial results, osimertinib is being evaluated in multiple ongoing trials. Osimertinib is being evaluated as first-line treatment for patients with locally advanced or metastatic EGFRm NSCLC in a Phase I expansion cohort trial.³⁷ Results were presented in abstract form in the *Journal of Thoracic Oncology*. A total of 80 mg and 160 mg daily doses were used. Five out of 60 patients were EGFR T790M positive. The ORR was 77% (67% at 80 mg dose and 87% at 160 mg dose). Overall PFS was 19.3 months. Dose reduction was needed in 10% and 47% of patients on 80 mg and 160 mg doses, respectively. Side effects were more prominent at 160 mg dose compared with 80 mg dose. Most common adverse events were diarrhea, stomatitis, and paronychia. Another ongoing Phase III trial compares gefitinib or erlotinib with or without osimertinib in EGFRm NSCLC.²⁷ Transgenic models with EGFRm/T790M resistant to AZD9291 showed response after adding an MEK inhibitor such as selumetinib;³⁸ thus, this combination is being tested in clinical trials as well. More combinations are under study, like AZD9291 with antibody to EGFR, such as necitumumab, navitoclax, which inhibits Bcl-2, or AZD6094, which is an MET inhibitor.²⁷ Many other trials are summarized in Table 3 with their current accrual status.

Osimertinib in EGFR-positive NSCLC with central nervous system (CNS) metastasis

Despite the fact that both third-generation EGFR TKIs target T790M mutant EGFR, the mechanism of action of rociletinib and osimertinib may be different. Sequist et al³⁹ reported a case series of nine patients who progressed on rociletinib in

Table 3 Ongoing Phase II and III osimertinib trials

National clinical trial ID number	Phase	Primary outcome measures	Clinical trial status	Official title
NCT02511106	III	DFS	Recruiting	AZD9291 vs placebo in patients with stages IB and IIIA NSCLC, following complete tumor resection with or without adjuvant chemotherapy (ADAURA)
NCT02736513	II	Intracranial overall response rate as defined by modified RECIST	Recruiting	Pilot, Phase II study assessing intracranial activity of AZD9291 (TAGRISSO) in advanced EGFRm NSCLC patients with asymptomatic brain metastases
NCT02296125	III	PFS	Active, not recruiting	A Phase III, double-blind, randomized study to assess the safety and efficacy of AZD9291 vs a standard of care EGFR TKI as first-line treatment in patients with EGFRm-positive, locally advanced or metastatic NSCLC
NCT02474355	III	Efficacy of AZD9291 by the analysis of overall survival; safety of AZD9291 by the assessment of serious adverse events; adverse events of special interest	Recruiting	Open-label, multinational, multicenter, real-world treatment study of single agent AZD9291 for patients with advanced/ metastatic EGFR T790M mutation-positive NSCLC who have received prior therapy with an EGFR TKI
NCT02151981	III	PFS	Active, not recruiting	A Phase III, open-label, randomized study of AZD9291 vs platinum-based doublet chemotherapy for patients with locally advanced or metastatic NSCLC whose disease has progressed with previous EGFR TKI therapy and whose tumors harbor a T790M mutation within the EGFR gene (AURA3)
NCT02811354	II	ORR	Not yet recruiting	Phase II study of AZD9291 in patients with advanced stage NSCLC following prior EGFR TKI therapy with EGFR and T790M mutations detected in plasma circulating tumor DNA (PLASMA)
NCT02454933	III	PFS according to RECIST 1.1	Active, not recruiting	A Phase III, multicenter, open-label, randomized study to assess the efficacy and safety of AZD9291 in combination with MEDI4736 vs AZD9291 monotherapy in patients with locally advanced or metastatic EGFR T790M mutation-positive NSCLC who have received prior EGFR TKI therapy (CAURAL)
NCT02442349	II	ORR according to RECIST 1.1	Active, not recruiting	A Phase II, open-label, single-arm study to assess the safety and efficacy of AZD9291 in Asia Pacific patients with locally advanced/ metastatic NSCLC whose disease has progressed with previous EGFR TKI therapy and whose tumors harbor a T790M mutation within the EGFR gene (AURA17)
NCT02769286	II	Efficacy	Not yet recruiting	Phase II trial of AZD9291 in first-line treatment of lung cancer harboring activating EGFRm from circulating tumor DNA and second-line treatment after acquired resistance with T790M mutation detected from circulating tumor DNA
NCT02803203	I and II	MTD (Phase I) and PFS (Phase II)	Not yet recruiting	A Phase I/II study of combination osimertinib and bevacizumab as a treatment for patients with EGFR-mutant lung cancers

NCT02094261	II	ORR	Active, not recruiting, has results	Phase II, open-label, single-arm study to assess safety and efficacy of AZD9291 in patients with locally advanced/metastatic NSCLC whose disease has progressed with previous EGFR TKI and whose tumors are EGFR and T790M mutation positive (AURA2)
NCT02179671	II	Confirmed CR rate	Active, not recruiting	A Phase IIa, open-label, multicenter, multicohort, immune-modulated study of selected small molecules (gefitinib, AZD9291, or selumetinib + docetaxel) or a first IMT (tremelimumab) with a sequential switch to a second IMT (MED4736) in patients with locally advanced or metastatic NSCLC (stages IIIB–IV)
NCT02759835	II	Determine PFS in patients with oligoprogressive disease after treatment with LAT followed by osimertinib patients who progress on their initial treatment with osimertinib and receive LAT therapy (surgery, radiation therapy, or RFA) followed by osimertinib will be evaluated for their time to second progression (PFS2)	Recruiting	A pilot study of local ablative therapy for treatment of oligoprogressive, EGFR-mutated, NSCLC after treatment with osimertinib (AZD9291, Tagrisso)
NCT02504346	II	ORR	Recruiting	AZD9291, an irreversible EGFR TKI, in relapsed EGFR-mutated NSCLC patients previously treated with an EGFR TKI, coupled to extensive translational studies

Abbreviations: CR, complete response; DFS, disease-free survival; EGFR, epidermal growth factor receptor; EGFRm, EGFR mutation; IMT, immune-mediated therapy; MTD, maximum tolerated dose; NSCLC, non-small-cell lung cancer; ORR, objective response rate; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; LAT, local ablative therapy; RFA, radiofrequency ablation.

the TIGER-X Phase I/II trial and then started on osimertinib in the AURA Phase I/II trial. Seven of them responded to osimertinib: three patients with PR and four patients with stable disease. Interestingly, three patients who developed CNS metastases on rociletinib responded to osimertinib. None of these CNS lesions underwent radiation. Two additional cases have been reported showing CNS metastasis response to osimertinib after progression on erlotinib or gefitinib.⁴⁰ Another case report showed an improvement of brain lesions on osimertinib in EGFR T790M-positive adenocarcinoma of the lung with brain metastasis, which was refractory to radiation.⁴¹

The results of BLOOM trial (Phase I) were presented at the American Society of Clinical Oncology (ASCO) annual meeting. In this study, EGFRm-positive NSCLC patients with leptomeningeal (LM) disease had an improvement in MRI signal intensity on osimertinib.⁴² A total of 21 patients were treated with 160 mg once daily osimertinib. Seven of them had objective improvement in imaging: five patients had improved symptoms and two patients cleared their cerebrospinal fluid from tumor cells. None of those patients underwent radiotherapy treatment or received intrathecal chemotherapy. There is preclinical evidence that osimertinib crosses the blood brain barrier more efficiently than other TKIs.⁴³

Diagnosis of EGFRm and liquid biopsies

Identification of mutations in the gene encoding for EGFR requires histological or cytological samples. However, it is not always feasible to get tumor samples. Frequently, tumor material is not adequate for molecular analyses⁴⁴ and decalcification procedures in bone biopsies interfere with molecular testing and results.⁴⁵ Thus, there was an urgent need to develop more accessible and less invasive methods for molecular alteration identification. A simple, validated, minimally invasive, blood/serum-based assay may also serve as a method to assess the response to EGFR TKI treatment.

Patients with EGFRm were found to have DNA fragments carrying the tumor-specific sequence (circulating cell-free tumor DNA [cftDNA]) in variable levels among their total plasma circulating DNA.⁴⁶ Detecting these cftDNA showed a high specificity to EGFR gene mutations. The analysis of cftDNA, defined as “liquid biopsy”, could be potentially repeated every time via simple blood draw. To validate cftDNA analysis for the detection of EGFRm, Kimura et al⁴⁷ compared results from cftDNA with results of tissue biopsies from the tumor. Report suggested a 92.9% concordance between serum and tissue.

Two meta-analyses of studies comparing tissues and plasma samples were published. They assessed the yield of cftDNA for EGFRms.^{48,49} Results showed a pooled sensitivity of 0.620–0.674 and a pooled specificity of 0.935–0.959. The area under the curve (AUC) resulted in high diagnostic accuracy (0.9–0.93). The results were more sensitive in poorly differentiated adenocarcinoma and in patients with advanced disease. In other words, the more aggressive the tumor and the bulkier the disease, the higher the levels of cftDNA and the higher the sensitivity to EGFRm.⁵⁰ The optimal time of blood collection and the effect of chemotherapy on these results are to be defined.⁵¹

Therapeutic management of patients with EGFRm NSCLC after the progression on first- or second-generation TKIs makes it imperative to identify the molecular mechanisms of acquired resistance. Biopsy used to be necessary to do this molecular study, which could be invasive and confounded by intra tumor heterogeneity.^{52–54} Hence, cftDNA analysis becomes more interesting and more important. Furthermore, multiple studies demonstrated that an increase in levels of EGFR activating mutation after the initiation of an EGFR TKI is a relatively early phenomenon of T790M appearance. These changes might be detected several weeks before the progression is visible radiographically.

Resistance to third-generation EGFR inhibitors

Eventually, disease progresses on third-generation EGFR TKI, representing further resistance mechanisms. C797S mutation is one of the most common mechanisms of resistance.^{55,56} The loss of the potential for covalent bond formation at position 797 by the missense mutation C797S is located within the kinase-binding site. This results in a markedly reduced cellular potency of this class of TKIs.⁵⁷ This mutation usually arises after 6–17 months of treatment in T790M-positive patients.^{55,56}

Some patients with previous T790M mutation acquire resistance without having the C797S mutation. In these cases, the mechanisms of this resistance still need to be identified.⁵⁸ Novel agents are needed to overcome the C797S tertiary EGFRm.

Disclosure

The authors report no conflicts of interest in this work.

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