Review Article

Clinical management of epidermal growth factor receptor mutation-positive non-small cell lung cancer patients after progression on previous epidermal growth factor receptor tyrosine kinase inhibitors: the necessity of repeated molecular analysis

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Abstract: One of the most important advances in the treatment of non-small cell lung cancer (NSCLC) has been the identification of molecular alterations vulnerable to targeted inhibition, such as mutations in the epidermal growth factor receptor (*EGFR*) gene. EGFR tyrosine kinase inhibitors (EGFR-TKIs) are targeted agents used to treat *EGFR* mutation-positive advanced NSCLC showing significant improvements in terms of response rate (RR) and progression-free survival (PFS) compared to conventional chemotherapy. However, all patients eventually develop resistance to first-line EGFR-TKIs. The most common mechanism of acquired resistance is the secondary acquisition of a single missense mutation within exon 20 in the EGFR gene, known as the T790M mutation (49–60%). New agents targeting the T790M mutation have undergone clinical development, and among these, osimertinib has shown significant activity in relapsing *EGFR* mutation positive patients harbouring the T790M mutation. Although precision medicine is a reality for NSCLC, obtaining relevant tissue for repeated molecular analysis from these patients remains a challenge. In this article, a group of experts from the Spanish Society of Medical Oncology (SEOM) and the Spanish Lung Cancer Group (GECP) evaluated the role of rebiopsy and the potential application of plasma-testing methodologies in advanced *EGFR* mutation patients progressing after EGFR-TKI.

Keywords: Epidermal growth factor receptor (EGFR); tyrosine kinase inhibitor (TKI); osimertinib; liquid biopsy; rebiopsy

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Introduction

Lung cancer is one of the leading causes of cancer death worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 85–90% of lung cancers. The 5-year survival rate for all stages is around 17%, while for stage IV NSCLC it is approximately 2%. In recent years, one of the most important advances has been the identification of molecular alterations vulnerable to targeted inhibition. The majority of these

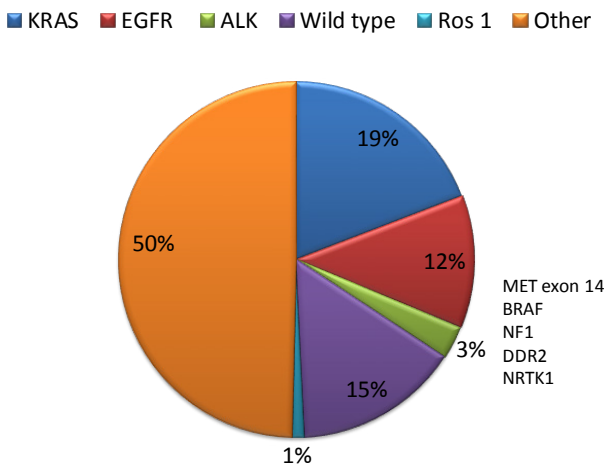

Figure 1 Mutations found in NSCLC patients. EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; NSCLC, non-small cell lung cancer. Modified from Rosell and Karachaliou (2).

alterations occur in adenocarcinomas, although potential targets in squamous cell carcinomas (SCC) are also emerging (2) (*Figure 1*). The Lung Cancer Mutation Consortium (LCMC) evaluated actionable oncogenic drivers in 10 genes from 1,102 patients with NSCLC from 14 American centers. An oncogenic driver alteration was detected in 64% of cases (3). Molecular profiling has been used to choose therapies or enroll patients into clinical trials. Those patients with oncogenic driver alterations who received a targeted therapy had a significant improvement in overall survival (OS) compared with those with genetic alterations but not treated with targeted agents, or those with no druggable target.

Activating mutations of the epidermal growth factor receptor (*EGFR*) gene have been the first molecular event that could be targeted with specific drugs in NSCLC. *EGFR* mutations are found in 10–12% of Caucasians with adenocarcinoma and are more frequent in never smokers, females, and in patients of East Asian ethnicity. The frequency of *EGFR* mutations in the Spanish population is around 10–16% of patients (4,5). The most common *EGFR* mutations are a deletion in exon 19 (Del19) and the exon 21 L858R point mutation (85–90%). *ALK* rearrangements, mainly translocations, occur in around 4% of NSCLC (6). Drugs targeting *EGFR*, *ALK* and *ROS1* genes, respectively, are currently approved. The prevalence of other molecular alterations with potentially actionable drugs, such as *MET* amplification, *HER2* mutations, *RET* fusions, and *BRAF* mutation, is low (<2%), and early clinical trials have shown the activity of targeting drugs in these small subgroups of genetically defined patient population.

However, and despite initial responses to targeted therapies, all patients will eventually show progression of disease due to both primary and secondarily acquired resistance mechanisms to targeted agents. For those *EGFR* mutation-positive patients receiving EGFR-tyrosine kinase inhibitors (EGFR-TKIs), the most common mechanism of acquired resistance is the secondary acquisition of a single missense mutation within exon 20 in the *EGFR* gene, known as the T790M mutation (49–60%) (7). New agents targeting the T790M mutation have undergone clinical development, and among these, osimertinib has shown significant activity in relapsing *EGFR* mutation positive patients harbouring the T790M mutation (8). Very recently, osimertinib has been approved for use in patients who develop this specific resistance. Although precision medicine is a reality for NSCLC, obtaining relevant tissue for repeated molecular analysis from these patients remains a challenge. In this article, a group of experts from the Spanish Society of Medical Oncology (SEOM) and the Spanish Lung Cancer Group (GECP) evaluated the role of rebiopsy and the potential application of plasma-testing methodologies in advanced *EGFR* mutation patients progressing after EGFR-TKI.

Clinical management of EGFR mutation-positive NSCLC patients

Studies comparing EGFR-TKIs with chemotherapy

There have been nine phases III studies comparing a first-generation reversible EGFR-TKI (either gefitinib or erlotinib), or a second-generation irreversible EGFR-TKI (afatinib), with platinum doublets as first-li86tt8rt8ne treatment in *EGFR* mutation-positive NSCLC patients (*Table 1*).

The first two studies, IPASS and First-SIGNAL, were conducted in a population with clinical features associated with a higher *EGFR* mutation rate. Subsequent studies were conducted exclusively in patients with *EGFR* mutations. The primary objective in these studies was progression-free survival (PFS), except in First-SIGNAL where the primary objective was overall survival (OS). All the studies showed significant differences in PFS (except First-SIGNAL, which showed a trend towards better PFS) and response rate (RR)

Table 1 Randomised trials evaluating EGFR-TKIs in EGFR-mutation positive patients with NSCLC

Study/phase	Treatment arms	No. patients; region	RR (%); P	PFS (months)	HR; P	OS (months)	HR; P
IPASS/III (9,10)	Gefitinib *vs.* carboplatin-taxol	261; Asia	71.2 *vs.* 47.3; <0.001	9.5 *vs.* 6.3	0.48; <0.001	21.6 *vs.* 21.9	1; 0.990
First-SIGNAL/III (11)	Gefitinib *vs.* cisplatin-gemcitabine	96; Korea	84.6 *vs.* 37.5; 0.002	8.5 *vs.* 6.7	0.54; 0.086	27.2 *vs.* 25.6	1.043; 0.428
WJTOC 3405/III (12,13)	Gefitinib *vs.* cisplatin-docetaxel	177; Japan	62.1 *vs.* 32.2; <0.0001	9.2 *vs.* 6.3	0.488; <0.0001	36.0 *vs.* 39.0	1.19; 0.443
NEJ002/III (14,15)	Gefitinib *vs.* carboplatin-taxol	230; Japan	73.7 *vs.* 30.7; <0.001	10.4 *vs.* 5.4	0.30; <0.001	27.7 *vs.* 26.6	0.887; 0.483
OPTIMAL/III (16,17)	Erlotinib *vs.* carboplatin-gemcitabine	154; China	83.0 *vs.* 36.0; <0.0001	13.1 *vs.* 4.6	0.16; <0.0001	22.8 *vs.* 27.2	1.19; 0.2663
EURTAC/III (18)	Erlotinib *vs.* cisplatin-docetaxel	174; Europe	58.0 *vs.* 15.0; <0.0001	9.7 *vs.* 5.2	0.37; <0.0001	19.3 *vs.* 19.5	1.04; 0.87
ENSURE/III (19)	Erlotinib *vs.* cisplatin-gemcitabine	148; Asia	62.7 *vs.* 33.6; <0.0001	11.0 *vs.* 5.3	0.34; <0.0001	26.3 *vs.* 25.5	0.91; 0.607
LUX-LUNG 3/III (20,21)	Afatinib *vs.* cisplatin-pemetrexed	345; Global	56.0 *vs.* 23.0; 0.001	11.1 *vs.* 6.9	0.58; 0.001	28.2 *vs.* 28.2	0.88; 0.39
LUX-LUNG 6/III (21,22)	Afatinib *vs.* cisplatin-gemcitabine	364; China	67.0 *vs.* 23.0; <0.0001	11.0 *vs.* 5.6	0.28; <0.001	23.1 *vs.* 23.5	0.93; 0.61
LUX-LUNG 7/II (23,24)	Afatinib *vs.* gefitinib	319; Global	70.0 *vs.* 56.0; 0.0083	11.0 *vs.* 10.9	0.73; <0.017	27.9 *vs.* 24.5	0.86; 0.258
JO25567/II (25)	Erlotinib + bevacizumab *vs.* erlotinib	154; Japan	69.3 *vs.* 63.6; 0.4951	16.0 *vs.* 9.7	0.54; 0.0015	NR	NR

EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; HR, hazard ratio; NR, not reached; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; RR, response rate.

in favour of EGFR-TKI therapy. Even so, no significant differences in OS were seen in any of the studies, probably because of treatment crossover after progression. All the studies showed a better toxicity profile with EGFR-TKIs, although this treatment was associated with higher rates of skin rash and diarrhoea. The studies also showed improved improvement in the quality of life in for EGFR-TKI-treated patients.

Gefitinib

The IPASS study was conducted in Asian adenocarcinoma patients who were non-smokers or former smokers who had smoked less than 10 pack-years. Patients were randomised to receive gefitinib or carboplatin combined with paclitaxel (9). The study met its primary objective of non-inferior PFS (5.7 *vs.* 5.8 months; P<0.0001). Regarding retrospective *EGFR* mutation analysis, histological specimens were only available in 36% of patients, and a significant benefit in PFS (9.5 *vs.* 6.3 months; P<0.001) and RR (71.2% *vs.* 47.3%, P=0.0001) was seen in favour of gefitinib in the *EGFR* mutation-positive subgroup. In terms of OS, there were no significant differences either in the overall study population (P=0.10) or in the *EGFR* mutation-positive subgroup (21.6 *vs.* 21.9 months; P=0.990) (10).

The First-SIGNAL study, conducted in Korean non-smokers with adenocarcinomas, compared gefitinib with combination cisplatin and gemcitabine (11). The general population did not meet either the primary objective of OS (22.3 *vs.* 22.9 months; P=0.604) or the PFS objective (5.8 *vs.* 6.4 months; P=0.128). About *EGFR* mutation analysis, material was only available from 31% of patients. A favourable trend was seen in PFS (8.5 *vs.* 6.7 months; P=0.086), with a significantly higher RR for gefitinib (84.6% *vs.* 37.5%; P=0.002) but no significant differences in OS (27.2 *vs.* 25.6 months; P=0.428), in the *EGFR* mutation-positive patient subgroup.

Study WJTOG3405 compared gefitinib with combined cisplatin and docetaxel in Japanese patients harbouring *EGFR* mutations (12,13). The study showed greater PFS (9.2 *vs.* 6.3 months; P<0.0001) and RR (62.1% *vs.* 32.2%; P=0.0001) for gefitinib, with no differences in OS (36 *vs.* 39 months; P=0.443).

Study NEJ002 also evaluated the efficacy of gefitinib versus combination carboplatin and paclitaxel, in Japanese patients (14,15). A significant increase was observed in PFS (10.8 *vs.* 5.4 months; P<0.001) and RR (73.7% *vs.* 30.7%; P<0.001) in favour of gefitinib. No significant differences were found in OS (27.7 *vs.* 26.6 months; P=0.483).

Erlotinib

Three phases III studies have compared erlotinib with a platinum doublet in patients with *EGFR* mutations. The OPTIMAL study, conducted in a Chinese population, compared erlotinib therapy with combination carboplatin and gemcitabine. A significant benefit was seen in PFS (13.1 *vs.* 4.6 months; P<0.0001) and RR (83% *vs.* 36%; P<0.0001) for erlotinib (16,17). There was no evidence of any differences in OS (22.8 *vs.* 27.2 months; P=0.2663).

The EURTAC study, conducted in European patients, showed a significant benefit in favour of erlotinib in PFS (9.7 *vs.* 5.2 months; P<0.0001) and RR (58% *vs.* 15%; P<0.0001) (18). No differences were found in OS (19.3 *vs.* 19.5 months; P=0.87).

The ENSURE study compared erlotinib therapy with combination cisplatin and gemcitabine in the Asian population. Significant differences were observed in favour of erlotinib in PFS (11.0 *vs.* 5.5 months; P<0.0001) and RR (62.7% *vs.* 33.6%; P<0.0001) (19). Again, there were no differences in OS (26.3 *vs.* 25.5 months; P=0.607).

Afatinib

Two studies have compared afatinib with a platinum doublet in *EGFR* mutation-positive patients. The LUX-Lung 3 study compared afatinib with the combination cisplatin-pemetrexed in *EGFR* mutation-positive patients. It showed a significant benefit in favour of afatinib in PFS (11.0 *vs.* 6.9 months; P=0.001) and RR (56% *vs.* 23%; P=0.001), with no differences in OS (28.2 *vs.* 28.2 months; P=0.39) (20,21). On the other hand, the LUX-Lung 6 study compared afatinib with the combination cisplatin-gemcitabine, obtaining benefits in PFS (11.0 *vs.* 5.6 months; P<0.001) and RR (67% *vs.* 23%, P<0.0001) for afatinib, with no differences in OS (23.1 *vs.* 23.5 months; P=0.61) (21,22).

Randomised studies comparing two EGFR-TKIs

Currently, only the results of the LUX-Lung 7 study are available (23). This randomised phase IIb trial compared afatinib with gefitinib. The study showed a small but significant benefit in favour of afatinib in PFS (11.0 *vs.* 10.9 months; P=0.017) and RR (70% *vs.* 56%; P=0.0083), but no impact on OS (27.9 *vs.* 24.5 months; P=0.258) (24).

Randomised studies evaluating erlotinib in combination with bevacizumab

A Japanese randomised phase II study compared the efficacy of the combination erlotinib plus bevacizumab

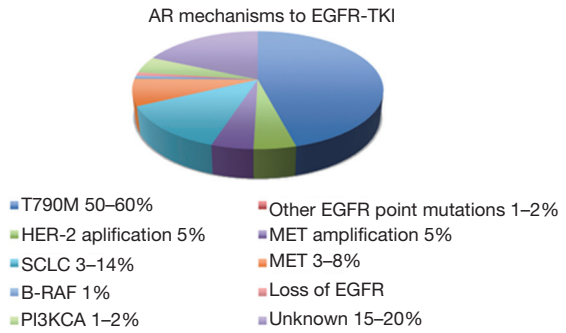

Figure 2 Mechanisms of AR to EGFR-TKI therapy. AR, acquired resistance; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; SCLC, small cell lung cancer.

against erlotinib monotherapy (25). Its primary objective was PFS, and a significant benefit was observed in favour of the combination (16.0 *vs.* 9.7 months; P=0.0015). No differences were seen in RR (69.3% *vs.* 63.6%; P=0.4951). At the time of publication, survival data is not yet available. As far as adverse effects are concerned, the combination showed statistically significantly higher rates of hypertension (60% *vs.* 10%) and proteinuria of grade 3 or above (8% *vs.* 0%).

Clinical and molecular features of EGFR mutation-positive NSCLC patients who progress on EGFR-TKIs

Three models of progression on EGFR-TKI therapy have been described. These may have implications for treatment management in these patients (26):

(I) Dramatic progression: patients who, after 3 months or more of disease control on EGFR-TKIs, show rapid progression with a significant, usually symptomatic, increase in tumour burden of the disease;

(II) Gradual progression: patients who, after 6 months or more of disease control on EGFR-TKIs, show slow disease progression, with no significant increase in tumour burden and usually few symptoms;

(III) Local progression: patients who, after 3 months or more of disease control on EGFR-TKIs, show a solitary extracranial lesion or limited progression in the central nervous system, with few associated symptoms.

Although *EGFR* mutation-positive patients derive benefit from treatment with reversible and irreversible TKIs, studies indicate that most of them will nevertheless suffer disease progression within 9 to 12 months (10-22).

Several mechanisms of acquired resistance to EGFR-TKI therapy have been described. They can be divided into three main categories: the presence of a secondary mutation in *EGFR*; the presence of bypass track activation; and phenotypic transformation. In almost 30% of cases, the mechanism of resistance is unknown (*Figure 2*).

Second-site mutations in EGFR

Approximately 50–60% of cases of acquired EGFR-TKI resistance have the T790M mutation. It is located in the EGFR tyrosine kinase domain and coexists with the activating mutation in *EGFR* (27,28). The T790M mutation produces EGFR-TKI resistance by increasing the binding affinity between ATP and EGFR, causing decreased binding by EGFR-TKIs (29).

The T790M mutation can also be detected in the tumours of EGFR-TKI naïve patients, and is associated with a worse prognosis (30). However, patients with an acquired T790M mutation tend to show slower progression, with greater prevalence of pleuropulmonary and lymphatic spread, progression at existing metastatic sites, and better functional status than patients without the T790M mutation who also progress (31,32).

In patients with acquired EGFR-TKI resistance, clones with and without the T790M mutation can coexist. This might explain the "flare" phenomenon sometimes seen when this treatment is stopped, and also why patients can respond again to an EGFR-TKI after previous treatment discontinuation (33,34).

As well as the T790M mutation, other mutations associated with acquired EGFR-TKI resistance have also been described: T854A in exon 21, and L747S and D761Y in exon 19, albeit at a lower frequency than T790M (35-37).

Bypass track activation

Other mechanisms of acquired resistance to EGFR-TKIs exist, based on the activation of parallel signalling pathways, in which the EGFR pathway is activated independently. The first mechanism to be described was amplification of the MET receptor tyrosine kinase gene and over-expression of its ligand hepatocyte growth factor (HGF)

(38-41). MET amplification leads to activation by ERBB3 phosphorylation, which maintains activation of the PI3K/AKT signalling cascade, providing an alternative signalling pathway even in the presence of an EGFR-TKI. MET amplification has been detected in 5–22% of samples from patients with acquired EGFR-TKI resistance (7), can be detected together with the T790M mutation in 40% of cases (38,42), and may be found in 3% of treatment-naïve EGFR mutation-positive patients (38).

Another bypass mechanism of acquired EGFR-TKI resistance, found in preclinical models, consists of MET activation by over-expression of its ligand HGF (43). Other mutations that may activate alternative pathways as mechanisms of acquired EGFR-TKI resistance are PI3KCA mutation (7), HER2 amplification (44) or BRAF mutation (45). Loss of the activating EGFR mutation has also been described as a mechanism of acquired resistance to EGFR-TKIs (46).

Phenotypic transformation

It has also been observed that histological transformation can act as a mechanism of acquired EGFR-TKI resistance. This includes tumour transformation to small-cell lung carcinoma (SCLC) (3–14%) and epithelial to mesenchymal transition (EMT) (8%) (7,47). The mechanism by which these histological transformations take place is unknown, as is the rate at which they occur.

EMT is the mechanism by which tumour cells lose their epithelial phenotype and develop mesenchymal-like morphology. This transition is accompanied by the loss of binding proteins, such as E-cadherin, and the acquisition of mesenchymal markers, such as vimentin or fibronectin (48). EMT has been linked to AXL activation (49), increased NOTCH-1 expression (50), or aberrant expression of transforming growth factor beta (TGF-β) (51,52).

Therapeutic management in EGFR mutation-positive NSCLC patients who progress on EGFR-TKI therapy

Acquired resistance to EGFR-TKIs has been widely investigated, and several therapeutic strategies to counter this have been explored.

Benefit of maintaining EGFR-TKI despite progression

The historical algorithm for cancer treatment has been to discontinue a therapy at the time of progression and switch to another drug. However, with oncogene-addicted cancers, this paradigm may require revision. Treatment selection considers type (slow *vs.* rapid) and location (single versus multiple sites) of progression, and the presence of cancer-related symptoms. For patients with *EGFR* mutation-positive who develop localised disease progression, several studies suggest that local therapy to these sites, with surgery or radiation, in combination with ongoing use of the same EGFR-TKI might also be clinically beneficial (53).

In one retrospective study of patients with *EGFR*-mutant NSCLC and acquired resistance, near 25% experienced a clinically significant rapid flare of disease within days of stopping an EGFR-TKI (34). One explanation for this phenomenon is that tumors are comprised of heterogeneous population of cell clones. Likely, a large proportion of such cell clones are still sensitive to the original EGFR-TKI, but latent (G0 cell-cycle arrest), and they could grow rapidly without EGFR-TKI. Repeated biopsies have documented that if chemotherapy is given instead of erlotinib after the development of erlotinib-acquired resistance, a previously documented T790M mutation can "disappear" and patients can then re-respond to erlotinib (7).

Evidence suggests that in case of slow progression, continuation of treatment with EGFR-TKIs may be an option for selected patients, in particular for those who have benefitted from EGFR-TKIs and lack of cancer-related symptoms. In case of progression in a single site, local radiotherapy or surgery may be added to continued treatment with EGFR-TKIs (54). There is no evidence that the switch to other types of EGFR-TKIs improves OS. However, switch to afatinib or dacomitinib after progression to first generation EGFR-TKIs have shown to improve PFS and RR, but not OS (55,56).

Benefit of chemotherapy with or without EGFR-TKI

Most patients are chemotherapy-naive at the time of acquired resistance. One current major clinical question is whether patients with widespread acquired resistance should stop their initial EGFR-TKI (with potential flare risk) and switch to chemotherapy, or continue EGFR-TKI beyond progression with the addition of chemotherapy to the regimen (57). Several prospective trials had focus in this question.

The results of a phase II study suggested that the addition of standard chemotherapy to 33 *EGFR*-mutant patients treated with gefitinib and three cycles of cisplatin plus docetaxel might prevent the development of acquired resistance to EGFR-TKIs (58). In the LUX-Lung 5 trial,

202 patients with progressive disease following clinical benefit with afatinib were randomized to afatinib plus paclitaxel, or investigator's single-agent chemotherapy. PFS (5.6 *vs.* 2.8 months; P=0.003) and RR (32.1% *vs.* 13.2%; P=0.005) significantly improved with afatinib plus paclitaxel, but there was no difference in OS (59). However, the phase III IMPRESS study showed no significant improvement in PFS and OS with continued use of gefitinib plus pemetrexed/cisplatin doublet chemotherapy compared with chemotherapy alone in 265 *EGFR* mutation-positive patients who progressed on first-line treatment with EGFR-TKIs (60).

There is a lack of data regarding the efficacy of chemotherapy plus EGFR-TKIs in advanced NSCLC with intrinsic resistance due to the presence of *de novo* T790M mutations.

Therapeutic management of patients EGFR T790M-positive

Second-generation EGFR-TKIs, such as afatinib, dacomitinib, or neratinib, inhibit T790M *in vitro*, but with insufficient efficacy in clinical studies. The combination of afatinib with cetuximab may overcome T790M-mediated resistance in preclinical studies. In a phase II study with 126 *EGFR* mutant-positive patients who progressed to EGFR-TKI, the RR was 29% and PFS 4.7 months, without differences according T790M status (61). In the BELIEF study, the treatment of patients T790M-positive with the combination of erlotinib plus bevacizumab resulted in 1-year PFS of 72% (62).

Third-generation EGFR-TKIs target EGFR-activating mutations and the T790M resistance mutation. On the other hand, they less effectively inhibit wild-type EGFR. Thus, these EGFR-TKIs should have greater efficacy and less toxicity in comparison to first- and second-generation EGFR-TKIs. Third-generation EGFR-TKIs in clinical development include osimertinib, rociletinib, HM61713, and others (63).

Osimertinib is the only drug approved through several clinical trials (64). In the phase I part of the AURA trial (NCT01802632), patients received osimertinib 20, 40, 80, 160 or 240 mg/day (n=31), or five expansion cohorts at different doses (n=222). The *EGFR* T790M mutation was detected in the tumor samples from 138 of the 222 patients (62%) of the expansion cohorts, not detected in 62 patients (28%), and unknown in 22 patients (10%). Of 239 patients evaluated for response, 123 (51%) had a confirmed response. The disease control rate (DCR) was 84%. Of the 138 patients with confirmed EGFR T790M mutation, 127 could be evaluated for response. The RR was observed in 78 patients (61%), and DCR in 121 patients (95%). Regarding duration of response (DR) and PFS, in the subgroup of patients with EGFR T790M mutation, 88% of patients had DR of ≥6 months, with a median PFS of 9.6 months (8).

The AURA 2 trial (NCT02094261) was a single-arm phase II study of AZD9291 80 mg/day in patients with T790M-positive NSCLC after failure of first-line EGFR-TKI. A total of 210 patients were included. The RR was 71% and the DCR was 92%. The median DR was 7.8 months and the median PFS was 8.6 months. Rate of patients who are alive without progression at 6 months were 70% (65).

In the pooled data from the two AURA studies (the AURA phase II extension study of cohorts and the AURA 2 included a total of 411 patients, of which 14 patients had no measurable disease and patients with negative T790M were excluded), the RR was 66% (263/398) and the DCR was 91% (360/397). The median DR was not reached and median PFS was 9.7 months (*Table 2*) (66).

The AURA 3 trial (NCT02151981) is a phase 3 trial including 419 advanced NSCLC patients with T790M-positive who had disease progression after first-line EGFR-TKI therapy, and were randomized to receive oral osimertinib versus chemotherapy based on platinum and pemetrexed. The RR and PFS were significantly superior with osimertinib in comparison with chemotherapy (71% *vs.* 31% and 10.1 *vs.* 4.4 months, respectively; HR: 0.30; 95% CI: 0.23–0.41; P<0.001). In 144 patients with metastases to the central nervous system (CNS), PFS was 8.5 *vs.* 4.2 months, respectively (HR: 0.32; 95% CI: 0.21–0.49). The proportion of patients with adverse events of grade 3 or higher was lower with osimertinib (23%) than with chemotherapy (47%) (67).

Additionally, the FLAURA trial (NCT02296125) is an ongoing phase III trial that compare osimertinib *versus* gefitinib or erlotinib as first-line therapy in advanced EGFR mutation-positive NSCLC.

Therapeutic management of patients with other molecular alterations

Intrinsic resistance to EGFR-TKIs can be developed through upregulation or amplification of *MET*. In a phase II trial investigating the MET inhibitor INC280 plus gefitinib in *EGFR*-mutated and *MET*-positive NSCLC patients who progressed after prior EGFR-TKI treatment, 6 of 41 patients (15%) reached a response (68). The dual MET-VEGF inhibitor cabozantinib plus erlotinib showed

Table 2 Efficacy data of osimertinib in EGFR mutant patients with NSCLC after progression on EGFR-TKI

Efficacy (95% CI)	AURA (expansion phase I) (n=63) (8)	AURA (expansion phase II) (n=201) (8)	AURA 2 phase II (n=210) (65)	Pooled AURA I–II (n=411) (66)	AURA 3 (n=279) (67)
RR (%)	61 [48–74]	61 [54–68]	71 [64–67]	66 [61–71]	71 [65–76]
DR (months)	9.7 [8.3–NR]	NR	7.8 [7.1–NR]	NR [8.3–NR]	9.7 [8.3–11.6]
DR up to 6 months (%)	72 [54–84]	83 [74–89]	75 [65–82]	78 [72–84]	49
DCR (%)	95 [86–99]	90 [85–94]	91 [87–95]	91 [88–94]	93 [90–96]
PFS (months)	11 [7–15]	NR [8.1–NR]	8.6 [8.2–9.7]	9.7 [8.3–NR]	10.1 [8.3–12.3]

DCR, disease control rate; DR, duration of response; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; NR, not reached; NSCLC, non-small cell lung cancer; PFS, progression-free survival; RR, response rate.

a RR of 11% in *EGFR*-mutated NSCLC patients following progression on EGFR-TKI therapy (69). Targeting the PI3K pathway could be a novel strategy to overcome TKI resistance. The dual inhibitor of PI3K/mTOR, NVP-BEZ235, was found to inhibit the growth of gefitinib-resistant NSCLC cells *in vivo* as well as *in vitro* (70). A phase II study of the AKT inhibitor MK-2206 plus erlotinib showed a RR of 9% in advanced mutant *EGFR*-positive NSCLC previously treated with erlotinib, with a PFS of 4.4 months (71). Therefore, specific inhibitors targeting MET, PI3K, or other pathways may be promising treatments for NSCLC patients with mutations associated with intrinsic resistance to EGFR-TKIs.

Indications for rebiopsy in EGFR mutation-positive NSCLC patients who progress on EGFR-TKIs

Considering the efficacy of drugs such as osimertinib for treating patients with the T790M resistance mutation (8,65), it is important to determine whether this mutation is present at the time of progression (72-75).

Whenever possible, tissue should be obtained from the most accessible part of a lesion that has progressed, be it a new lesion, a metastasis of the primary tumour, or lymphadenopathies. In a retrospective study led by Kawamura, in which the role of rebiopsy was analyzed in 120 patients, no differences in mutation rate were found between rebiopsies of the primary lesion or of metastases (76). In another study analysing 88 tumour specimens collected synchronously or metachronously from the same or different sites, Quéré *et al.* found no discordance in the detection of *EGFR* mutations between the various biopsy sites (77). Likewise, virtually no differences were found between specimens of primary tumour and metastases using next-generation sequencing techniques (78).

There will be times when obtaining a tissue biopsy is difficult or impossible. In these cases, other techniques can be used, such as liquid biopsy. Studies indicate that peripheral blood contains circulating free DNA (cfDNA), including from circulating tumour cells (CTCs), as well as small amounts of circulating tumour DNA (ctDNA). Such DNA can be detected by various techniques (cobas®, therascreen®, BEAMing, ddPCR). A retrospective study that conducted BEAMing analysis on over 200 samples of cfDNA from patients treated with osimertinib from the AURA study found 70% sensitivity for detecting the T790M mutation (79). Outcomes in patients whose plasma proved positive for T790M were equivalent to those seen in patients with positive tissue tests (PFS: 9.7 months). A recent study compared T790M mutation detection rates in cfDNA and CTCs against biopsies in 40 patients. The T790M mutation was found in 75% of biopsies, 70% of CTC samples and 80% of ctDNA samples. It was concluded that the various ways of detecting cfDNA are similar (80).

Plasma samples were also analyzed by BEAMing and cobas® and compared against tissue in the Phase I rociletinib study. Positive percent agreement was found to be 73% for BEAMing and 64% for cobas® (81). Thress *et al.* compared the *EGFR* mutations present in 38 ctDNA samples using two non-digital platforms (cobas® EGFR mutation test and therascreen® EGFR ARMS-PCR) and two digital platforms (Droplet Digital™ and BEAMing dPCR), using tissue for test comparisons (82). They found that both cobas® and BEAMing possessed greater sensitivity for detecting the T790M mutation. They also found that 30% of patients whose biopsies were previously negative or inconclusive tested positive for the T790M mutation with cobas® and BEAMing (*Table 3*). In another study, reported at ASCO 2016, samples from over

Table 3 Comparison of the different techniques used to detect T790M in circulating DNA (adapted from Thress *et al.*) (82)

Technique	cobas® (%)	therascreen® (%)	ddPCR (%)	BEAMing (%)
Sensitivity	41	29	71	71
Specificity	100	100	83	67
Concordance	57	48	74	79

It can therefore be concluded that the gold standard is to detect the mutation in rebiopsy tissue, whereas liquid biopsy is useful in cases in which rebiopsy would be difficult or impossible (*Figures 3,4*).

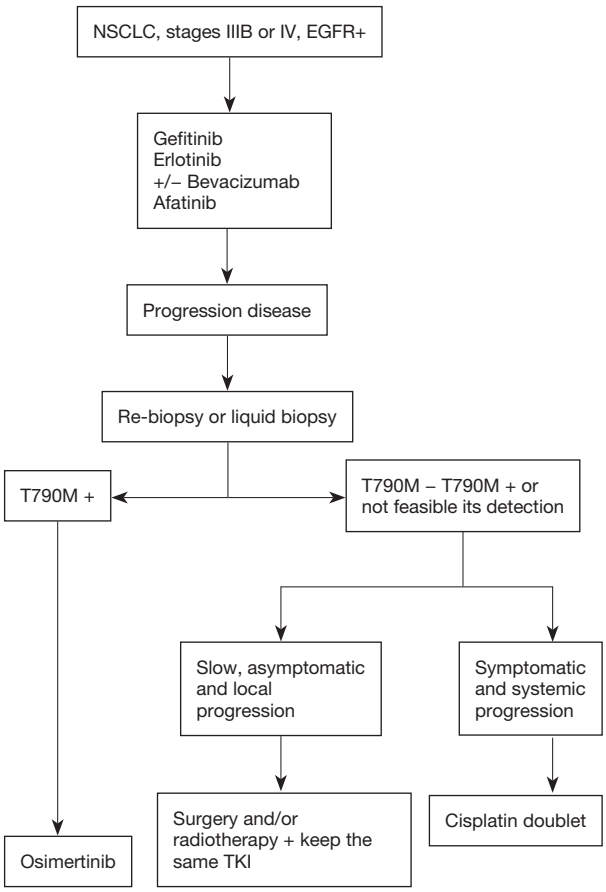

Figure 3 Treatment algorithm for managing EGFR mutation-positive NSCLC patients. EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; NSCLC, non-small cell lung cancer. Modified from Novello *et al.* (84).

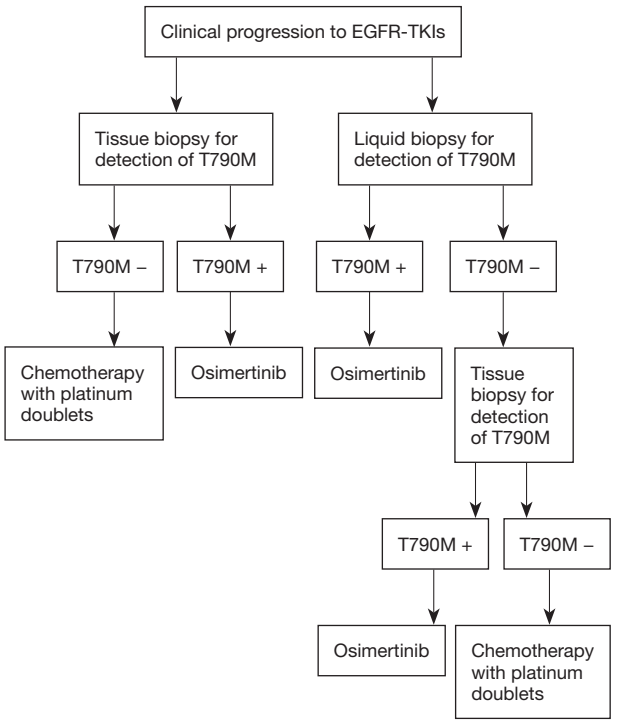

Figure 4 Diagnostic protocol for tissue and liquid biopsies for the T790M mutation. EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; NSCLC, non-small cell lung cancer. Modified from Oxnard *et al.* (79).

400 patients treated with rociletinib were analyzed. The T790M mutation was detected in tissue (therascreen®), plasma (BEAMing) and urine (Trovagene), in 417, 189 and 136 patients, respectively. Good correlation of RR and duration of response was also observed between the different assay procedures (83). It can therefore be concluded that the gold standard is to detect the mutation in rebiopsy tissue, whereas liquid biopsy is useful in cases in which rebiopsy would be difficult or impossible (*Figures 3*,*4*).

Conclusions

The identification of actionable oncogenic driver mutations in NSCLC patients has changed their treatment, greatly affecting the OS of patients administered targeted agents compared with those administered other therapies or whose

tumours have no known genetic dependency to date.

EGFR mutations (deletions in exon 19 or mutations in exon 21) are present in 10–12% of the Caucasian population with NSCLC. Several reversible and irreversible EGFR-TKIs exist, however, that are effective against these tumours, as confirmed by nine superiority trials versus platinum doublet chemotherapy as first-line treatment. Unfortunately, EGFR-TKI activity lasts for 9 to 12 months, after which resistance develops by various mechanisms (acquired resistance). The most common resistance mechanism (50–60%) is acquisition of the missense mutation in codon 790 of *EGFR* exon 20 (T790M). Other mechanisms of TKI resistance, such as *MET* amplification, *PI3KCA* mutations, *HER2* amplification, *BRAF* mutation, loss of the activating *EGFR* mutation, phenotypic transformation of the tumour to SCLC and EMT, are less common.

The development of several agents active against the T790M mutation, such as osimertinib, has changed the approach to progression after initial EGFR-TKIs, not just in terms of treatment but also in respect of determining the mechanisms by which the tumour escaped from the first EGFR-TKI, and the various forms of NSCLC progression.

Clinical progression on the first EGFR-TKI may be manifested locally, gradually or dramatically, entailing a different approach to treatment in each case. In cases of slow progression, where symptoms are mild or absent, treatment with the same initial EGFR-TKI can be maintained, together with surgery or radiotherapy for the progressing lesion. In other cases, it will be necessary to identify which resistance mechanism has arisen, discontinue EGFR-TKIs, and administer a specific anti-T790M agent if tests for this mutation prove positive.

Osimertinib is the only approved EGFR-TKI that is active against the EGFR T790M mutation. It has shown a RR of over 70% in progression after an initial EGFR-TKI, at least 90% disease control rate, and PFS of at least 10 months. These results make it essential to identify the molecular alteration causing resistance to initial EGFR-TKIs, both for the therapeutic consequences and for the patient's benefit. If accessible and feasible, relapsed tissue should be taken from the primary lesion or lymphadenopathies, resorting to analogue or digital analyses of liquid biopsies when specimen collection is difficult or impossible. Several recent studies have confirmed the similarity of results obtained from tissue or cfDNA, in terms of sensitivity, specificity and concordance. Not only do these methods provide similar treatment benefits, but they also enable the mutation to be monitored and quantified when the patient progresses on EGFR-TKIs.

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Footnote

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