CURRENT ONCOLOGY
A Canadian Cancer Research Journal

REVIEW ARTICLE

Management of *EGFR*-mutated non-small-cell lung cancer: practical implications from a clinical and pathology perspective

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ABSTRACT

Starting in the early 2000s, non-small-cell lung cancer (NSCLC) subtypes have evolved from being histologically described to molecularly defined. Management of lung adenocarcinomas now generally requires multiple molecular tests at baseline to define the optimal treatment strategy. More recently, second biopsies performed at progression in patients treated with tyrosine kinase inhibitors (TKIs) have further defined the continued use of molecularly targeted therapy.

In the present article, we focus on one molecular subtype: *EGFR*-mutated NSCLC. For that patient population, multiple lines of TKI therapy are now available either clinically or in clinical trials. Each line of treatment is guided by the specific mutations (for example, L858R, T790M, C797S) identified in *EGFR*. We first describe the various mechanisms of acquired resistance to *EGFR* TKI treatment. We then focus on strategies that clinicians and pathologists can both use during tissue acquisition and handling to optimize patient results. We also discuss future directions for the molecular characterization of lung cancers with driver mutations, including liquid biopsies. Finally, we provide an algorithm to guide treating physicians managing patients with *EGFR*-mutated NSCLC. The same framework can also be applied to other molecularly defined NSCLC subgroups as resistance patterns are elucidated and additional lines of treatment are developed.

Key Words Lung adenocarcinoma, *EGFR*, resistant mutations, re-biopsies, liquid biopsies

Curr Oncol. **2017 Apr;24(2):111-119** **www.current-oncology.com**

INTRODUCTION

Discovery of mutations within the kinase domain of the epidermal growth factor receptor gene (*EGFR*) has ushered in an era of targeted therapy in lung cancer[1,2]. First-generation (gefitinib and erlotinib) or second-generation (afatinib) EGFR tyrosine kinase inhibitors (TKIs) are now standard for the first-line management of patients with advanced *EGFR*-mutated non-small-cell lung cancer (NSCLC)[3,4]. Although those agents improve progression-free survival, resistance usually develops within 12 months. Studies of tumours biopsied at the time of progression while the patient was on an EGFR TKI[5,6] have elucidated the common mechanisms of acquired resistance. That work has led to the development of third-generation TKIs targeting the most common resistance mechanism, T790M mutation. In patients with advanced *EGFR*-mutated NSCLC tumours progressing on first-line EGFR TKI whose tumours have developed T790M mutations, osimertinib was shown to be superior to standard platinum–pemetrexed chemotherapy[7]. That finding has led to the approval of osimertinib in the United States and Europe, a Notice of Compliance with Conditions in Canada, and an expansion of the lines of therapeutic options for *EGFR* mutation-positive NSCLC. It also underscores the importance of re-biopsy at the time of progressive disease.

Although this evolving molecular paradigm for the management of *EGFR* mutation-driven NSCLC has resulted in significant clinical benefit, it presents a number of

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clinical challenges for the multidisciplinary health care team managing affected patients. Here, we review the common mechanisms of acquired resistance to EGFR TKIs and discuss the challenges of treating beyond progression, the management of oligo-progressive disease, the appropriate timing and site for re-biopsy, tissue processing, and the potential for plasma-based molecular testing.

THE CLINICAL PICTURE

Mechanisms of Acquired Resistance to EGFR TKI Therapy

Mechanisms of acquired resistance to EGFR TKIs can be classified into four broad categories: secondary resistant *EGFR* mutations, activation of bypass pathways, activation of downstream pathways, and histologic transformation. All of those mechanisms can potentially result in continued signalling in the presence of first- or second-generation EGFR TKIs (Figure 1).

The most common acquired mechanism of resistance by far is the *EGFR* "gatekeeper" mutation T790M in exon 20, which accounts for approximately 60% of all resistance mechanisms. This mutation occurs in *cis* with the original sensitizing *EGFR* mutation, and it restores the binding pocket's affinity for adenosine triphosphate[8], rendering the competitive inhibition of adenosine triphosphate by the first- and second-generation TKIs ineffective[5,6,9,10]. The high rate of T790M resistance after first- and second-generation TKI therapy has prompted the development of third-generation EGFR TKIs.

Rare *EGFR* kinase domain mutations—including L747S, D761Y, and T854A—can occasionally occur *de novo* as primary resistance mutations[11,12]. Other target alteration mechanisms of resistance include *EGFR* amplification (10%), which is usually found with T790M and is thought to "outcompete" TKI inhibition by providing stronger kinase activation[13].

Alterations that lead to activation of bypass pathways are able to restore the signalling inhibition by EGFR TKI therapy through compensative utilization of other related receptors or circumvention of the EGFR receptor, or both[14]. The *EGFR*/*ErbB* family of receptor tyrosine kinases relies on the Ras/Raf/MEK/ERK and PI3K/Akt/mTOR signal transduction pathways to affect proliferation and survival. *MET* amplification (5%) that results in high-level Met receptor expression can enhance heterodimerization with HER3 (*ErbB3*) to activate downstream PI3K–Akt signalling despite inhibition of EGFR activation[15]. This bypass mechanism can also result from increased production of hepatocyte growth factor, the ligand for Met[16]. As with *MET*, HER2 (*ErbB2*) amplification (8%–13%) leads to parallel signalling that bypasses the effects of EGFR TKIs[17].

Cancer signalling rewiring and rebound effects of inhibition have also been implicated as adaptive mechanisms of resistance[18]. As a result of ERK or PI3K pathway inhibition, *EGFR*-mutant lung cancers increase the expression of several receptor tyrosine kinases, including HER3 (*ErbB3*). Upregulation of insulin-like growth factor 1 receptor has been shown to confer resistance to EGFR TKIs[19] despite combination therapy with receptor blockade, and EGFR TKIs have not been effective[20]. Overexpression of the receptor tyrosine kinase AXL or its ligand GAS6 has also been shown to confer resistance to TKIs[21].

Inhibition of EGFR signalling by a TKI suppresses proliferation and increases proapoptotic signalling. Alterations involving downstream effector molecules are able to reconstitute the proliferation signals and inhibit apoptosis without relying on EGFR activation. *BRAF* mutations (1%),

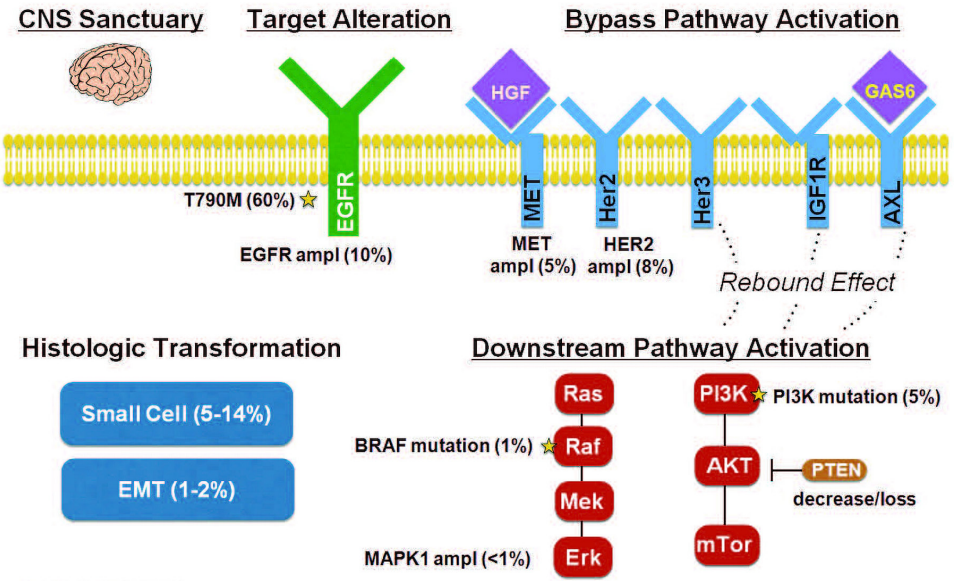

FIGURE 1 Mechanisms of resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) can be broadly categorized into central nervous system (CNS) sanctuary, target alteration, bypass pathway activation, histologic transformation, and downstream pathway activation. Ampl = amplification; gold star = mutation; rebound effect = upregulation of tyrosine kinases after ERK or PI3K pathway inhibition; EMT = epithelial-to-mesenchymal transition.

and also *MAPK1* amplifications (<1%), drive continual downstream signalling of the MEK/ERK pathway[18]. Similarly, *PIK3CA* activating mutations (5%) and loss or decrease of *PTEN* have been implicated in TKI resistance through activation of PI3K/Akt/mTOR pathways[19].

Initially described in 2006, observations of small-cell lung cancer (SCLC) transformation after EGFR TKI treatment represents a consistent acquired resistance mechanism, with a prevalence of 5%–14% in multiple case series[22]. The absence of response to EGFR TKIs is explained by decreased levels of EGFR protein expression, a well-known feature of SCLC[23–25]. Epithelial-to-mesenchymal transition is characterized by a phenotypic transformation from an epithelial to a spindle-like morphology, as well as molecular transformation with loss of epithelial markers (E-cadherin) and gain of mesenchymal markers (vimentin)[14]. Signalling tracks associated with epithelial-to-mesenchymal transition, such as AXL activation, might mediate this mechanism of resistance[17].

Although not classically considered an evolutionary mechanism of acquired resistance, the high prevalence of central nervous system (CNS) progression after systemic therapy deserves acknowledgment. Concentrations of TKI are dramatically reduced in the CNS, and common TKI resistance mechanisms are not found in CNS-metastatic tumours, suggesting that the CNS is a pharmacologic respite for *EGFR*-sensitizing mutant tumours, without the pressure to evolve a secondary resistance mutation[17].

Molecular Testing of *EGFR* Mutations in NSCLC: Clinical Perspectives

The approval of osimertinib, a third-generation EGFR TKI targeting *EGFR* activating mutations and the resistance T790M mutation, is linked to a companion diagnostic test for detection of the T790M mutation. Although companion diagnostics for molecular mechanisms of acquired resistance guide precision medicine in NSCLC, they create new challenges when introduced as standard clinical care. Previously, serial biopsies were used predominantly in research contexts to determine the dynamic changes in *EGFR*-mutated tumour characteristics when treated with first-line EGFR TKI therapy. The efficacy of third-generation EGFR TKIs in those who harbour a T790M resistance mutation has ensured that repeat tissue biopsies will become standard to determine which patients should receive those agents.

Determining the optimal time to test for T790M mutation will become paramount. Conceivably, molecular testing for T790M mutation could occur at the initial biopsy before any EGFR TKI treatment, at progressive disease while receiving a first- or second-generation EGFR TKI, or at discontinuation of an EGFR TKI. The latter distinction is important, because patients with advanced *EGFR*-mutated NSCLC are often treated beyond radiologic progression until symptomatic or until multi-site progressive disease becomes evident. The ASPIRATION trial, an open-label phase II single-arm study conducted in Asian centres, demonstrated that treatment beyond radiographic progression is feasible and could delay salvage therapy in selected patients[25]. Furthermore, single-institution studies have shown the benefit of local ablative therapy for oligo-progressive disease while continuing with initial EGFR TKI therapy[26,27].

De novo T790M mutations were originally considered to be sporadic events captured rarely (<1%) through conventional assays before treatment with an EGFR TKI. However, with the use of more sensitive techniques, the T790M mutation might be detected in up to 79% of tumour biopsies before EGFR TKI therapy[28,29]. That hypothesis is consistent with the general belief that most T790M mutation exists as a minor subclone before EGFR TKI therapy and becomes the prevalent tumour cell population during therapy. However, recent carefully conducted *in vitro* studies showed that T790M mutation can occur *de novo* in a subpopulation of tumour cells that persist during EGFR TKI therapy[30]. Clinically, when a T790M resistance mutation is detected together with a sensitizing *EGFR* mutation on initial tissue biopsy, first- and second-generation EGFR TKI therapy should be avoided because of their limited efficacy against cells harbouring T790M[31]. Further study using third-generation EGFR TKIs with activity in the presence of T790M mutation is ongoing for this subpopulation, and encouraging data have been seen with the combinations of erlotinib–bevacizumab and afatinib–cetuximab[32,33].

Most molecular testing for T790M mutation will occur at the time of documented progressive disease during therapy with an EGFR TKI. Few data have compared a repeat biopsy at radiologic Response Evaluation Criteria in Solid Tumors progression and at time of treatment failure when EGFR TKI therapy is discontinued. Regardless, the latter clinical distinction might not be relevant because of tumour heterogeneity in the development of acquired resistance to EGFR-targeted therapies[29]. Spatial and temporal heterogeneity has been retrospectively described for the T790M resistance mutation[34].

Detection of the T790M mutation is not consistent over time on serial biopsy. Tumours that are T790M mutation-positive on first post–EGFR TKI biopsy can become T790M-negative in later biopsies, and the reverse also occurs[26]. Intriguingly, activity of third-generation EGFR TKIs is still seen in patients with T790M mutation-negative tumours; the reported overall response rate is 21%–29%, and the disease control rate is approximately 60%. Tumour heterogeneity could be one plausible hypothesis to explain that activity[35,36]. Indeed, defining an optimal time to re-biopsy and test for the T790M mutation is not clear because of the dynamic nature of the resistance mutation; inherently, however, it should occur at some point after progressive disease is documented. Practical considerations that could affect the appropriate time to re-biopsy include accessibility and suitability of the progressing site (for example, bone or brain), local expertise with re-biopsy, availability of T790M testing, and the potential to treat beyond progression with a first-line EGFR TKI.

Issues Relating to Tumour Re-biopsy at Disease Progression

"Re-biopsy" refers to performing a biopsy after the initial diagnostic biopsy. Re-biopsy should be considered in patients when progression or relapse of disease occurs during treatment with a first-line EGFR TKI, or when tumour behaviour deviates from its expected course. Re-biopsy permits histologic and molecular characterization of a tissue sample to determine the mechanism of resistance.

Re-biopsy in advanced-stage patients who are progressing while on treatment poses several challenges. The chosen biopsy technique should be robust enough to provide an adequate diagnostic sample while reducing the risk of complications. Tumour heterogeneity arising from drug-induced necrosis and fibrosis interspersed with viable tumour tissue can lead to sampling bias. Heterogeneity within the tumour tissue could make it difficult to target areas containing viable tissue based on imaging characteristics alone. The sampling method should be such that a sufficient amount of tissue is obtained to facilitate extended molecular testing beyond histologic diagnosis. Concerns have been raised about the risks of a re-biopsy, and particularly a higher risk of pneumothorax and hemorrhage because of the presence of fibrosis and increased vascularity within and around the tumour; complication rates are low, however. In one large series, 1 among 162 patients who underwent re-biopsy developed pneumothorax necessitating chest drain insertion[6]. In another study involving in 124 patients, pneumothorax and intra-alveolar hemorrhage were encountered in 6% and 7% of patients respectively[33].

Several approaches and techniques are available for tumour re-biopsy, including washing and brushing during bronchoscopy, fluid aspiration from pleural effusion, fine-needle aspiration (FNA) supported by rapid on-site evaluation (ROSE) by cytology, core-needle biopsy, and excisional biopsy.

Arcila *et al.*[37] reported the success rate of tumour re-biopsy in advanced NSCLC patients who progressed on EGFR TKI therapy. Of 130 patients who met the study criteria, 121 underwent re-biopsy, yielding 153 samples. Samples were obtained by surgical excision, image-guided core biopsy, or FNA, and by aspiration of fluids. Of the 121 patients sampled, 106 (87.6%) had pathologic confirmation of tumour and histologic classification. Morphologic confirmation failed in the remaining 15 patients primarily because of the absence of tumour cells[9] or insufficient[5] or degenerated[2] tumour cells precluding molecular analysis. Molecular analysis for *EGFR* mutations was successful in 104 (86%) of the 121 patients. Unsuccessful tumour sampling was related to low tumour content or poor quality of extracted DNA, especially in bone samples that had been decalcified. The success rate for obtaining a diagnostic specimen improved with ROSE, with the highest success rates being obtained with core biopsy (89%), FNA cytology (79%), and pleural fluid aspirate (77%).

In another single-institution study involving patients with *EGFR*-mutant lung cancer and acquired resistance to afatinib[38], only 14 of 23 patients (60.9%) who progressed were able to complete re-biopsy, with 11 patient samples being sufficient for molecular analysis. The challenge of re-biopsy is also reflected in an 18-centre study involving advanced NSCLC patients who had progressed on first-line targeted therapy or chemotherapy[39]. Re-biopsy could not be performed in 18% of the 100 patients enrolled in the study. Of samples from 82 patients who underwent re-biopsy, 94% were histologically evaluable, but 18.3% and 7.3% of samples contained no or too few tumour cells for molecular analysis. Thus, the overall re-biopsy success rate for obtaining the intended molecular analysis was only 54%.

Yoon *et al.*[40] reported that, with careful patient selection, a 100% technical success rate could be achieved. Of 124 patients with resistance to conventional treatment, one quarter were excluded on the basis of selection criteria such as proximity of the lesion to bronchovascular structures or basally situated lesions in the presence of severe respiratory compromise. In the remaining patients, 80% of the tissue samples were adequate for mutation analysis. The same study also proposed positron-emission tomography in patients with tumours larger than 3 cm to differentiate viable from necrotic tissue.

Compared with FNA, core biopsy provides more tumour tissue and DNA. Fine-needle aspiration cytology provides 100 mg of tissue compared with 100–200 mg of tissue from a core biopsy[41]. Fresh-frozen core biopsies provide 3.9 μg DNA, formalin-fixed core biopsies provide 1.69 μg, and FNA provides 0.23 μg. Compared with core biopsy, FNA provides a lower amount of DNA; however, the tumour population might be purer because of less aspiration of non-tumour cells. For bone sampling, FNA might be preferable, because decalcification of bone can destroy DNA[37].

Molecular Testing: Pathology Perspectives

The pathologist's role in lung cancer has been ever-changing since the introduction of targeted therapy; pathology assessment of a new tumour tissue sample upon progression is no different. The expertise of a pathologist is required for the proper management of tissue samples obtained specifically for *EGFR* testing upon progression.

Tissue, body fluids, and aspiration specimens, which represent the most common samples in anatomic pathology, are processed in the laboratory using several common steps that assure their ideal preparation and preservation for interpretation and for prolonged storage. Archival tissue serves as excellent material for further tumour testing, and such samples are currently used for almost all predictive and prognostic biomarker testing across tumour sites[42].

Processing protocols and the various fixatives and preservatives actively affect the quality of nucleic acids that can be obtained from tissue samples. In the era of targeted therapy, it should be assumed that most cancer specimens will be used for complex testing. Efforts to process and preserve the tissue properly should therefore start in the procedure suite or operating room. Two basic fixative types are commonly used in anatomic pathology laboratories: cross-linking fixatives (formaldehyde, glutaraldehyde, paraformaldehyde) and precipitation fixatives (alcohol, methanol, acetone)[43]. The most popular fixative is 10% buffered neutral formalin, which has been extensively studied with respect to molecular testing[44]. All fixatives damage nucleic acids to some degree through chemical interactions. Some fixatives—for example, picric acid, mercury-containing solutions, and acid decalcifiers—might not be compatible with molecular testing[45,46]. Several processing factors might also play a role in molecular testing. For example, the optimal time of fixation is between 12 and 24 hours, after which time the DNA obtained is of much lower quality[47].

Based on a higher risk of complications from more-aggressive procedures to obtain tissue samples, FNA samples from lung and metastatic sites, as well as cytology

samples from effusions, might preferentially be obtained. Communication with the pathology department should be initiated to assure the proper handling of samples obtained for the sole purpose of assessment of resistance mechanisms. Pathologists can significantly improve the results of such biopsies by the following means:

- Provide ROSE service, which is often requested but rarely available in most hospitals because of the lack of proper funding of laboratories. To circumvent the economic constraint, the pathology department could provide ROSE by skilled cytotechnologists or, during the initial learning curve, with the introduction of a new procedure to one or several radiologists, respirologists, or surgeons.
- Include pertinent negatives in the pathology report for lung cancer diagnosis, which adds important educational value to the report. Synoptic reports are helpful in this respect. Communication is key, and clinical pathology rounds, with correlation of tissue samples, even for a limited number of cases, is extremely beneficial.
- Design and implement specific requisitions to assure proper communication of clinical history and to specify the clinical intent for re-biopsy.
- Perform histologic examination of all tissue samples obtained at progression to exclude the 5%–14% of cases with small-cell carcinoma as the mechanism of resistance.

Pathologists remain the gatekeepers of tissue at diagnosis and at progression by providing critical assurance of obtaining adequate tissue for testing using the most economically feasible algorithm and the best turnaround time.

Testing Plasma Circulating Tumour DNA for Resistant Mechanisms

Given the improved efficacy of third-generation EGFR TKIs in tumours harbouring the T790M mutation, determining the presence of this predictive biomarker is of significant clinical utility. As already discussed, repeat tissue biopsies are occasionally not feasible because of tumour accessibility or patient preference. A circulating tumour DNA (ctDNA) analysis for T790M could overcome those challenges. The concept of ctDNA was first described more than 50 years ago[48] and has previously been exploited in prenatal diagnostics (circulating fetal DNA)[49]. With recent technological advances, ctDNA can now be isolated and sequenced in advanced cancer patients[50]. This method, often called "liquid biopsy," continues to be refined and could preclude the need for repeat tissue biopsies.

Tumour-derived DNA can be found as cell-free fragments in the circulating plasma[50], in circulating tumour cells (entire tumour cells within the circulation)[51], or within circulating exosomes[52]. Many laboratory techniques are available, and more are emerging, that can isolate ctDNA. Because the molecular compartments each arise from a different biologic mechanism and contain unique information, their clinical application to lung cancer diagnosis and treatment and their relevance to patient care are likely to be different—and remain to be elucidated[53]. An expanded role for the liquid biopsy technique is on the horizon in lung cancer treatment, not only for detecting T790M mutation, but also for detecting the newly discovered mutation resistant to third-generation EGFR TKIs, C797S[54], and the *ALK* mutation L1198F[50]. The ctDNA assay could also potentially be applied to primary *EGFR* mutation detection in patients who are lacking suitable tissue samples and who are clinically unsuitable for further tumour tissue biopsy[54].

Given the current state of liquid biopsy technologies, assessment methods depend largely—but not exclusively—on polymerase chain reaction (PCR) and are best suited for identifying known hotspot mutations. The two most sensitive and quantitative techniques are targeted next-generation sequencing (NGS) and emulsion PCR ["droplet digital PCR" (ddPCR)][55].

In the case of NGS, assay sensitivity increases, and limit of detection improves, with increasing depth of coverage. Although whole-genome and whole-exome analyses using ctDNA have previously been reported for other tumour sites[56,57], targeted assessment of known mutational hotspots in several genes provides the coverage depth required for low-level variant detection[58]. Targeted variant assessment by NGS can be carried out using both amplicon-based—for example, TruSight Tumor 15 (Illumina, San Diego, CA, U.S.A.)—and hybridization capture-based library preparation methods such as CAPP-Seq12 ("cancer personalized profiling by deep sequencing"). Custom NGS panels, inclusive of variants of interest with application to ctDNA analysis, can be designed for either approach. A major limitation of both methods is sample availability; sufficient blood volumes are required to enable adequate sampling of low-concentration ctDNA. Specifically, a minimum of 65 ng template DNA is required to be able to detect, with 99.9% probability, 1 tumour DNA fragment among 2000 normal fragments (Pugh TJ. Personal communication).

The assay sensitivity of ddPCR is technology-dependent—that is, the number of partitions, or "droplets," in the emulsion. For example, the Bio-Rad (Hercules, CA, U.S.A.) ddPCR system creates 20,000 reaction partitions and has a theoretical detection limit of 0.01% mutant alleles; the RainDrop ddPCR system (RainDance, Billerica, MA, U.S.A.) creates 1×10^6 partitions, for a theoretical detection limit of 0.00001% mutant alleles. By contrast, NGS has a reported limit of detection of 0.02%, although a 1% limit is more conventionally accepted.

One challenge with high-sensitivity genotyping assays such as NGS and ddPCR is the potential to detect low-variant-allele-frequency alleles with uncertain clinical significance. Such a situation has been reported for the detection of *EGFR*-mutant alleles in lung cancer that did not always correlate with expected benefit in response to EGFR TKIs[59]. That lack of correlation is potentially a result of the identification of false-positive mutant alleles or the presence of minor clones within the tumour, and thus challenges the diagnostic requirement to maximize the positive predictive value of a clinical test for mutations such as *EGFR* T790M in ctDNA. In high-sensitivity genotyping assays such as NGS and ddPCR, maximization of the positive predictive value is achieved by sacrificing assay sensitivity

for assay specificity. Accurate, reproducible quantification of the variant allele frequency is therefore essential because it enables the establishment of the appropriate diagnostic sensitivity thresholds and, ultimately, better assessment of the test's positive predictive value in situations in which the prevalence of the tumour-associated mutation in the disease population is low[60]. Recent efforts have been focused on maximizing the positive predictive value of noninvasive assays by ameliorating the problems posed by low quantities of ctDNA in blood and the presence of sequencing artifacts in NGS[61].

One recent report quantified the positive predictive value for a ddPCR-based *EGFR* T790M assay in plasma ctDNA at 79% because of a relatively high false-positive detection rate[62]. One potential explanation advanced in the literature is that a tissue biopsy at a single site might not be fully reflective of the heterogeneity of the lung tumour, but that ctDNA is representative of DNA from dead cells being released into the periphery across the entire tumour burden[54,62,63].

Liquid biopsy is currently not optimized for comprehensive tumour sequencing and characterization because it has become clinically relevant only in the past few years. Furthermore, other non-T790M mechanisms of EGFR TKI resistance (for example, *MET* amplifications) cannot yet be reliably detected using the procedure[64]. Detection of ctDNA is also variable between patients, contributing to low overall sensitivity (albeit in the setting of high specificity), potentially mitigating the role of the technique. At the moment, overall tumour burden, and *EGFR* driver status appear to be the best predictors for successful ctDNA analysis[62].

Notably, sensitive quantification of T790M allele burden in ctDNA allows for temporal monitoring of mutation prevalence during therapy or disease progression (or both), enabling better modelling of the evolution of a patient's lung cancer and more refined approaches to patient management[60]. Temporal monitoring of T790M allele burden in ctDNA is possible using both the NGS[65] and ddPCR[62] approaches.

As sequencing and molecular technology continue to improve, the role of liquid biopsy methods in lung cancer treatment, specifically in EGFR-targeted therapy, will continue to grow. Although the current role of liquid biopsy is yet fully defined, the potential benefits are high and must be exploited to maximize clinical utility for patients with cancer.

SUMMARY

The identification of actionable mutations, particularly *EGFR* mutations, in some patients with NSCLC has forever changed the approach to management of this disease (Figure 2). First-line EGFR TKI therapy results in high response rates and prolonged disease control; however, resistant disease or clones eventually develop. Serial biopsies and plasma-based assays have identified the common mechanisms of resistance. Therapies active against the most common resistance mutation, T790M, are now available. The mechanisms of resistance to third-generation EGFR TKIs (including identification of the novel C797S mutation) are being elucidated[54,66]. Given that third-generation TKIs also have activity against classical sensitizing *EGFR* activating mutations (exons 19 and 21), they may eventually have value in the first-line setting. The FLAURA trial (NCT022916125) is comparing first-line osimertinib with standard TKI (gefitinib or erlotinib). That trial has completed accrual, and results are expected in the near future. The importance of re-biopsy at progression on osimertinib will become increasingly important. Given the complexities involved in managing patients with *EFGR*-mutated NSCLC, it is critical that the multidisciplinary team—including pathology—be involved to ensure that diagnostic, laboratory, and treatment paradigms evolve quickly to meet those challenges.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare the following interests: MST declares research grants from AstraZeneca, Pfizer, and Merck, and honoraria from AstraZeneca, Pfizer, Merck, Hoffman–La Roche, Bristol–Myers Squibb, and Boehringer Ingelheim; RS declares honoraria from AstraZeneca, Boehringer Ingelheim, Pfizer, Merck, Bristol–Myers Squibb, Roche, Eli Lilly, and Novartis; SKR declares a research grant from Pfizer and honoraria from AstraZeneca, Pfizer, Bristol–Myers Squibb, Roche, and Novartis; AK declares honoraria from AstraZeneca, Pfizer, Merck, Hoffman–La Roche, and Bristol–Myers Squibb; MP declare honoraria from AstraZeneca; DI declares honoraria from AstraZeneca, Pfizer, Merck, Hoffman–La Roche, and Bristol–Myers Squibb; RAJ declares a research grant from AstraZeneca and honoraria from AstraZeneca, Boehringer Ingelheim, Bristol–Myers Squibb, Eli Lilly, Merck, Novartis, Pfizer, and Roche; CB declares honoraria from AstraZeneca, Pfizer, Merck, Bristol–Myers Squibb, and Boehringer Ingelheim; MC, BSS, and MS have no declarations to make.

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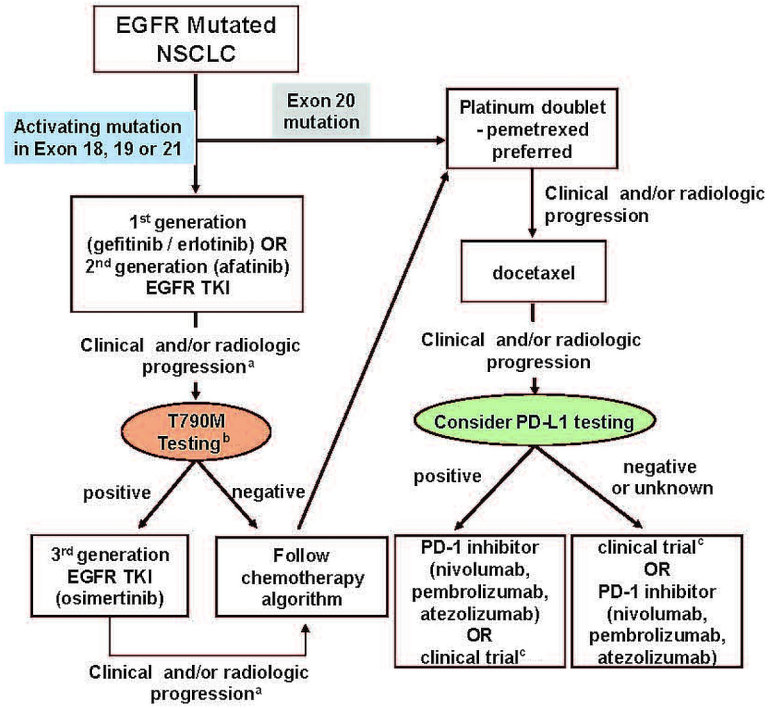

FIGURE 2 Algorithm for the management of *EGFR*-mutated non-small-cell lung cancer (NSCLC). Molecular testing is recommended at multiple points along the treatment pathway for this patient population. Additional challenges arise because of the known benefits of treating oligometastatic disease, especially in the brain, and treating beyond classical radiologic progression. Clinical trials should be considered at all steps along the treatment path. [a]If oligo-progression (such as isolated brain metastasis) occurs, consider local therapy and continuation of tyrosine kinase inhibitor. [b]Re-biopsy currently required. Biopsy of growing lesion is recommended if possible. Testing can be performed on histology or cytology cell block. Consider testing plasma free DNA as an alternative if clinically available. [c]Clinical trials are preferred at all treatment steps, if available. EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor.

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