Review Article

Resistances to *EGFR* tyrosine kinase inhibitors in lung cancer—how to routinely track them in a molecular pathology laboratory?

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Abstract: Patients with advanced or metastatic forms of lung cancer with an activating mutation in *epidermal growth factor receptor* (*EGFR*) are given tyrosine kinase inhibitors (TKIs) targeted therapies that are more efficient than chemotherapy. These patients are excluded from first-line immunotherapy. After a phase of regression these tumors develop systematically resistance requiring a rapid change in therapy. At present two strategies are being discussed. The first strategy, so called "historical' sequential treatment strategy, is based on the administration of first- or second-generation TKIs until the emergence of therapeutic resistance and, in the case of a *EGFR* T790M mutation, on the administration of third-generation TKIs. The recently proposed second strategy, so called the "next-generation" TKIs strategy, concerns initial treatment with third-generation TKIs. This latter strategy appears to be promising but needs to be confirmed by data comparing survival curves of patients treated in a sequential manner. Several criteria influence the choice of these strategies, in particular the presence of brain metastases, the potential toxicity and the economic model. The selected therapeutic algorithm has certainly an impact on the activity of laboratories. The sequential approach requires investigation into *EGFR* T790M resistance mutations, using blood and then possibly a tissue biopsy, or into other mechanisms of resistance in the absence of this mutation. In the case of tumor progression under treatment with third-generation *TKIs* the *EGFR C797S* mutation in the *cis* or *trans* positions is looked for. In the absence of this latter mutation other mechanisms of resistance are then investigated. We describe here the different methodological approaches used to identify resistance mechanisms linked to treatment with TKIs targeting mutations in *EGFR*.

Keywords: Epidermal growth factor receptor (*EGFR*); lung carcinoma; targeted therapy; T790M; C797S; resistance mechanisms

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Introduction

Approximately 10% to 15% of Caucasian patients and 50% of Asian patients develop metastatic or advanced lung adenocarcinomas with activating mutation in the *epidermal growth factor receptor* (*EGFR*) gene (1). For several years, patients with an activating mutation in *EGFR* have been treated with first- or second-generation tyrosine kinase inhibitors (TKIs). In about 50% of cases this results in the *s*ystematic emergence of the *EGFR* T790M mutation resistance to this therapy after a few months (2). Detection of this latter mutation leads to treatment of the patient with third-generation TKIs (3,4). However, this third-generation inhibitor has also shown efficient targeting of activating

mutations in *EGFR*. Consequently, a second therapeutic strategy with its initial administration has been proposed recently (4,5). Despite initial efficacy, the latter strategy does not avoid emergence of one or several mechanisms of resistance (6). So, these two strategies, so called "historical sequential" and "next generation" TKIs treatment strategies highlight that the discovery of novel biological data, progress in therapy and the development and improvement in methods of detection of genomic alteration (both with blood and/or tumor tissue) will drive clinicians and biologists to quickly modify their working algorithms.

This review will provide an update on the mechanisms of resistance of non-small cell lung carcinomas (NSCLC) to TKI targeting *EGFR* mutations and on the advantages and limits of methods for their detection.

Mutations in non-small cell lung cancer: which therapeutic strategy?

The detection of activating mutations in *EGFR* has given rise to two different algorithms of treatment. Either sequential treatment with first- or second-generation TKIs and then with third-generation TKIs on emergence of tumor progression and on detection of the *EGFR* T790M mutation or initial treatment with third-generation TKIs (7). The therapeutic choice is presently under discussion and it is difficult at the moment to systematically opt for initial administration of a third-generation TKI. Comparative results concerning the overall survival of patients depending on the therapeutic option are in waiting. However, the efficacy of third-generation TKI in treatment of brain metastases of NSCLC with an *EGFR* activating mutation has shown initial promise (8).

Mechanisms of resistance and first- and second-generation TKIs

After a more or less extensive period, in general a few months, lung cancer patients presenting with an *EGFR* mutation and treated with first- and second-generation TKIs relapse and the tumor progresses (6). The most frequent resistance mechanism concerns the emergence of the *EGFR* T790M mutation occurring in at least 50% of cases (6,9). This mutation appears to arise *de novo* but may emerge from a minor clone of resistance present in the initial tumor. The detection of this mutation results in treatment with a third-generation TKI (6). More than one out of two patients develops other mechanisms of resistance to first- and second-generation TKIs. Thus, a number of genomic alterations can emerge, including those in the *MET* (amplification or mutation), *HER2* (amplification or mutation) or *RET* (rearrangements) genes (6,9). In a certain percentage of cases, resistance is associated with histological transformation into small cell lung carcinoma (9). In some cases the mechanism of resistance is uncertain or unknown and quite difficult to identify, in particular when linked to the phenomena of epithelial to mesenchymal transition (10,11). Following the development of immunotherapy new mechanisms of resistance have been revealed more recently. Thus, a strong expression of PD-L1 in tumor cells has been found to be associated with primary resistance to first- and second-generation TKIs in patients with an *EGFR* activating mutation (12). It is recognized that patients with tumors with a high tumor mutational burden (TMB), in particular in the absence of *EGFR* mutations, have the best response to treatment with anti-PD1/PD-L1 and the TMB may soon be proposed as a routine clinical test (13). Interestingly, it has been reported recently that patients with tumors with *EGFR* activating mutations (del 19 or the L858R mutation) and a high TMB (constituting a relatively small percentage of patients with an *EGFR* mutation) do not respond to first- and second-generation TKI as well as patients with an *EGFR* mutation and a low TMB (14).

How to detect mechanisms of resistance associated with first- and second-generation TKIs and which approach to adopt?

Detection of the *EGFR* T790M mutation is done with blood and/or tumor tissue and/or cytological samples (7,15). To date the approach consists in looking for this mutation in circulating free DNA (cfDNA) in blood sample first, and, if negative, to use tissue or cytological material (7,15,16). The sensitivity and specificity of the methods of detection have evolved in recent years and several parameters need to be taken into consideration when choosing a method (15). Two approaches are possible, either targeted investigation of *EGFR* or investigation into panels of genes including *EGFR* [using next-generation sequencing (NGS)] (15). The composition of these panels is more or less large and some of the genes included (*RET*, *HER2*, *MET*), which can show genetic alterations that emerge on first- and second-generation TKI treatment, can be accessible to targeted therapies associated with clinical trials (17-19). These different approaches are possible with liquid biopsies, tissue or cell samples. The targeted approaches hold

certain advantages (15). Thus, the methods used are very accessible for all molecular pathology laboratories since the equipments are not costly and the techniques are quite easy to perform. These targeted investigations use a number of approaches, in particular the techniques of COBAS, Therascreen, Idylla, Beaming or digital PCR (dPCR) (15,16,20,21). Interpretation of the results is relatively simple, standardized and can be performed by most investigators. The results are obtained very rapidly, most techniques give results in a few hours. The amount of tumor DNA required is probably lower than for methods such as NGS, which is particularly important when investigation into mutations is done with cfDNA or from very few tumor cells. A certain number of these targeted tests are considered in the USA as companion diagnostics to treatment (15). The NGS approach allows detection in a single timeframe of the different causes of resistance observed in patients, when a *EGFR* T790M mutation is absent, and provides additional information for administration of an alternative therapy. However, the sensitivity of the different analytical methods and the threshold of detection that defines a negative result for a *EGFR* T790M mutation of a patient with a tumor that progresses rapidly still needs to be discussed (15,16,22). However, the presently used NGS and PCR methods (in particular COBAS) are quite sensitive and must give relatively identical results for detection (22). The question is open as to whether the dPCR techniques should be used first for initial detection or if this ultra-sensitive technique should be reserved for tumors with *EGFR* activating mutations that progress very rapidly, for which no other method identifies a resistance mutation (15,16,23). The dPCR techniques are more sensitive and the threshold of detection of the *EGFR* T790M mutation is extremely low, questioning the possibility of sometime getting a false positive result (24,25). In addition, the possibility of germline circulating DNA with a *EGFR* T790M mutation must not be excluded in this situation (24). When the threshold of detection is very low the question of treating or not treating the patient with a third-generation TKI, a costly, sometimes toxic and ineffective therapy, while still not having excluded other mechanisms of resistance overlooked by the targeted method, can be raised.

Mechanisms of resistance and third-generation TKIs

Third-generation TKIs are administered sequentially as second-line treatment after the emergence of the *EGFR* T790M mutation but can also be proposed as initial treatment (7). In the first therapeutic option the mechanisms of resistance depend on the association of two pathways resulting in the loss of expression of the *EGFR* T790M mutation and the emergence of mutations in the kinase (6,7). The mechanisms of resistance associated to the loss of the *EGFR* T790M mutation can result from histological transformation into a small cell lung carcinoma, a mechanism that involves epithelial to mesenchymal transition or from the emergence of genomic alterations in genes other than *EGFR* (7,26). As research studies progress the length of this list of genomic alterations increases, including mutation (*BRAF*, *PI3KCA*, *KRAS*), fusion (*RET*, *FGFR3*, *BRAF*) and amplification (*MET*) (6). If the *EGFR* T790M mutation persists tumor progression is associated with the emergence of the *EGFR* C797S mutation. In the case of initial treatment with third-generation TKI the mechanisms of resistance are the same except that the *EGFR* T790M mutation is not detected (7). The *EGFR* C797S mutation occurs in the *cis* or *trans* position (6,7). Guided by the mechanism of resistance, treatment with a third-generation TKI can be proposed. Chemotherapy can be given in the case of transformation into a small cell lung carcinoma, or if the mechanism is not identified or if no clinical trial can be proposed depending on the mutation identified. A treatment from a clinical trial targeting a genomic alteration can be sometimes proposed (7). A *trans* allelic conformation of the C797S can result in the association of a first- or second- (erlotinib or gefitinib) generation TKI with a third-generation (osimertinib) TKI. A *cis* allelic conformation of this mutation results in chemotherapy or a treatment associated with a clinical trial (7).

How to detect mechanisms of resistance associated with third-generation TKIs and which approach to adopt?

The detection of mechanisms of resistance associated with third-generation TKI as well as the *cis* or *trans* allelic configurations uses blood and/or tumor tissue (7,15,27). Detection of the C797S mutation is more often performed with cf-DNA. A negative result leads to analysis of tumor tissue. The marketed tests for the detection of mutations in *EGFR* have not yet integrated the possible detection of C797S in *EGFR*. Thus, the two companion tests approved by the FDA (the COBAS and Therascreen tests) are currently not able to detect it. The commercial NGS

panels do not detect all mutations too. One of the recent approaches uses the dPCR technique to detect mutation in C797S in *cis* or *trans* positions (7,15). The algorithm that can be proposed involves investigation into the loss or maintenance of the *EGFR* T790M mutation if the patient has received sequential TKIs and, in the case of persistence of this mutation, investigation into the C797S mutation and its *cis* or *trans* allelic configuration (7). The latter mutation is looked for when a tumor progresses when the patient received first-line treatment with third-generation TKI. The absence of the C797S mutation leads to investigation into other mechanisms of resistance. The NGS technique can use blood samples (16). However, a negative result obtained with blood sample suggests either that the method is not sensitive enough or that circulating somatic DNA is absent. Thus, a noncontributory result from blood leads to a tissue biopsy with which the *EGFR* T790M mutation may be then detected. However, a negative result with the tissue biopsy does not exclude the emergence and detection of the *EGFR* T790M mutation with a second tissue biopsy (27). Additionally, a histological, immunochemical or molecular analysis of the tissue biopsy can show evidence of histological transformation into a small cell lung carcinoma or of different genomic alterations when using NGS. Examination of the tissue with immunohistochemical markers may identify also the phenomena of epithelial to mesenchymal transformation (6).

Conclusions

The treatments administered to patients with lung tumors carrying mutations in *EGFR* orientate the choice of approach developed by the laboratories for the detection of resistance mutations to TKIs. Thus, if first- or second generation TKI are administered investigation into the *EGFR* T790M mutation in blood need to be systematic if the tumor progresses. Administration of a third-generation TKI followed by tumor progression leads to investigation into the C797S mutation, if the *EGFR* T790M mutation is maintained. Initial administration of third-generation TKI calls for investigation into the C797S mutation if the tumor progresses. It is then important to distinguish between a mutation in the *cis* or *trans* position since this information allows continuation or not of treatment with third-generation TKI in association with erlotinib or gefitinib.

The organization of the care of patients, the proximity of a molecular pathology laboratory which can perform analyses with liquid biopsies particularly, the economic model and the budget of the institution as well as the turnaround time in obtaining the results, all influence in fact more or less directly the choice of therapy. Systematic administration of a third-generation TKI as a first option may be possible if the benefit to overall survival is better than for sequential administration with different TKI. Then the superior cost of third-generation TKI and the possibility of toxicity must be discussed.

Liquid biopsies for investigation of resistance mutations in *EGFR* are strongly recommended for patients with advanced stage or metastatic lung cancer (15,28). For this, the methods of extraction of nucleic acids and the ability to detect different mutations are becoming more accessible to the majority of laboratories. Apart from tumor tissue and blood, other biological samples such as urine or exhaled air may allow in the future detection of mutations in *EGFR*, in particular mutations of resistance (29,30). However, these novel non-invasive approaches are not performed in the routine practice and require strong validation in different clinical trials.

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