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Review

Targeting EGFR T790M mutation in NSCLC: From biology to evaluation and treatment

Antonio Passaro[a,*], Elena Guerini-Rocco[b], Alessia Pochesci[a], Davide Vacirca[b], Gianluca Spitaleri[a], Chiara Matilde Catania[a], Alessandra Rappa[b], Massimo Barberis[b], Filippo de Marinis[a]

[a] Division of Thoracic Oncology, European Institute of Oncology, Milan, Italy
[b] Clinic Unit of Histopathology and Molecular Diagnostics, Division of Pathology, European Institute of Oncology, Milan, Italy

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ABSTRACT

The identification of EGFR mutations and their respectively tyrosine kinase inhibitors (TKIs), changed dramatically treatment and survival of patients with EGFR-positive lung cancer. Nowadays, different EGFR TKIs as afatinib, erlotinib and gefitinib are approved worldwide for the treatment of NSCLC harbouring EGFR mutations, in particular exon 19 deletions or exon 21 (Leu858Arg) substitution EGFR mutations. In first-line setting, when comparing with platinum-based chemotherapy, these target drugs improves progression-free survival, response rate and quality of life. Unfortunately, the development of different mechanism of resistance, limits the long term efficacy of these agents. The most clear mechanism of resistance is the development of EGFR Thr790Met mutation. Against this new target, different third-generation EGFR-mutant-selective TKIs, such as osimertinib, rociletinib and olmutinib, showed a great activity.

In this review, we summarize the scientific evidences about biology, evaluation and treatment on NSCLC with EGFR T790M mutation.

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1. Introduction

Despite a significant increase in terms of survival of patients harbouring EGFR mutations observed in the last years, a new plateau seems to have been reached. Indeed, all patients with EGFR-mutant NSCLC will inevitably develop acquired resistance following treatment with EGFR tyrosine kinase inhibitors (EGFR-TKI). The time to disease progression slightly varies among the different drugs, ranging from 11 to 13 months. In addition, among patients with EGFR-mutant tumors, it has been calculated a 75% response rate to TKI, indicating that approximately 25% of cases display primary resistance. Since EGFR mutant tumors represent unique entities, even after development of acquired resistance most patients have a good performance status and are expected to require further lines of treatment. Therefore, the choice of the best treatment to administer in this setting is becoming a topic of primary importance in the field. At the same time, a common consensus about the guide-

* Corresponding author at: Division of Thoracic Oncology, European Institute of Oncology—IEO, Via G. Ripamonti, 435, 20141 Milan, Italy.
E-mail address: antonio.passaro@ieo.it (A. Passaro).

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lines to follow beyond EGFR-TKI progression is still missing. Some consistent evidences have emerged in the last years that must be considered in the clinical practice. The aim of this review is to summarize these data in order to provide some indications about how to manage patients who progressed to EGFR-inhibitors.

2. Biology of EGFR T790M positive lung tumors

The epidermal growth factor receptor (EGFR) belongs to ErbB family of tyrosine kinases receptor. Upon ligand binding, the increase kinase activity of the receptor leads to autophosphorylation of the intracellular domain and the activation of downstream pathways involved in cellular growth and proliferation [1,2] (Supplementary Fig. 1). Activating EGFR mutations cluster in the gene region encoding for the adenosine triphosphate (ATP)-binding pocket of the kinase domain (exons 18–21), inducing ligand-independent receptor activation [3,4]. Tyrosine kinase inhibitors (TKI) are small molecules that target the ATP binding site of tyrosine kinase domain. Gefitinib and erlotinib are first-generation EGFR TKIs that yield dramatic antitumor activity in patients with advanced NSCLC harboring activating EGFR mutations [5–7]. Therefore, these drugs represent the first-line standard of care for this group of patients [8]. Unfortunately, acquired resistance eventually develops in most cases (Fig. 1).

In 2003 an in vitro-induced mutation of residue 790 (766 in alternative codon numbering system) of EGFR gene was described as a potential determinant of resistance to first generation EGFR inhibitors [9]. This mutation was the equivalent to T315 substitution found in BCR-ABL fusion gene from CML patients with chronic myelogenous leukemia relapsed on imatinib therapy [10]. Two years later, the presence of T790M mutation was identified in patients with NSCLC harboring primary activating EGFR mutations that progressed on TKI therapies [11,12]. Nowadays, the secondary EGFR mutation at the position 790 represents the most common and well-characterized resistance mechanism of acquired resistance to gefitinib and erlotinib.

The missense T790M variant (c.2369C>T) in exon 20 of EGFR gene entails the substitution of the non-polar hydrophobic and larger amino acid methionine (M) for the hydrophilic threonine (T) residue in the ATP binding cleft of EGFR kinase domain [13]. As for gatekeeper residue T315 mutation of BCR-ABL1, the aberrant presence of bulkier amino acid at 790 position of EGFR was supposed to interfere with the binding of first generation TKIs through steric hindrance effect [11,12]. However, T790-mutant EGFR has been shown to retain affinity to gefitinib, albeit lower [13]. Gefitinib and erlotinib are reversible ATP-competitive inhibitors of EGFR. The most frequent somatic activating mutations of EGFR, such as small in-frame deletions of exon 19 and the single nucleotide variant of exon 21 (L858R), have been shown to reduce the ATP affinity increasing the sensitivity of mutant receptors to competitive inhibitors [14,15]. Indeed, the activating and sensitizing effects of these mutations is the grounds of the clinical efficacy of gefitinib and erlotinib in patients with EGFR exon 19 or exon 21-mutated NSCLCs. The introduction of T790M substitution in cis with the primary activating mutation of EGFR allele markedly increases the affinity of mutant receptor for ATP [13]. In particular, kinetic analyses revealed a Michaelis-Menten constant (Km) for ATP of $8.4 \pm 0.3\ \mu M$, $5.2 \pm 0.02\ \mu M$ and $148 \pm 4\ \mu M$ in the L858R/T790M mutant, wild-type and single L858R mutant receptor, respectively [13]. This kinetic effect of the T790M mutation reduces the potency of competitive inhibitors and represents the primary mechanism of T790M-mediated first generation TKI resistance.

Irreversible TKIs covalently bind the cysteine residue 797 of the EGFR-kinase domain increasing the occupancy of ATP binding site and providing the molecular substrate to overcome T790M resistance [13,16]. Second generation irreversible TKIs are non-selective EGFR inhibitors that do not discriminate between wild-type and mutant receptor [17,18]. Despite the promising pre-clinical results [19,20], the clinical efficacy of these compounds in patients with first generation TKI-resistant tumors has been disappointing. In fact, the nondiscriminatory nature of the blockade with consequent high rate of on-target toxicities (rash and diarrhea) limited the clinical dosage of these drugs to level insufficient to overcome T790M mutant EGFR [18,21]. Conversely, third-generation irreversible inhibitors have been designed to target mutant EGFR – including T790M – more selectively than wild-type receptor [22,23]. These compounds have demonstrated clinical efficacy in patients with T790M-mediated resistance to gefitinib and erlotinib (as detailed in next sections). Based on this evidence osimertinib has been approved by FDA and EMA for the treatment of patients with advanced EGFR T790M mutated NSCLC who have progressed on or after EGFR TKI therapy.

In addition to the role in acquired resistance, in vitro and in vivo studies highlighted the oncogenic function of T790M EGFR mutation. The presence of this substitution alone has been shown to increase the catalytic activity of receptor conferring a growth advantage to mutated cells [24]. Mice models with lung-specific expression of T790M-mutant EGFR develop lung tumors [25]. Moreover, germ line T790M mutations have been reported in individuals with NSCLC (~0.5% of never smokers with lung cancer), implying a role of this variant in the predisposition to lung cancer [26,27]. All these patients developed tumor with long latency and in most cases a concurrent mutation of exon 18, 19 or 21 was detected in tumor samples, suggesting that the oncogenic role of T790M is enhanced by the presence of additional activating EGFR mutations [26,28]. Indeed, somatic T790M mutations reported in TKI therapy-naïve NSCLC are mostly associated with other in cis activating mutations, in particular exon 21 L858R variant [29,30]. Among 1144 NSCLC analyzed in the last published TCGA Pan-Lung cancer study, 3 samples harbored EGFR T790M substitution (0.2%) and all were coupled with L858R mutation [31–33]. However, the prevalence of T790M in untreated tumors is largely affected by the sensitivity of the detection method, ranging from 0 to 6% with low sensitivity assays up to 80% using ultrasensitive tests (reviewed in [30]). Given the wide range of frequencies reported and the great variability of methods used, it is still challenging to assess the actual clinical significance of pre-treatment T790M mutation. Su et al. have reported similar rate of TKI response but shorter progression free survival in patients with primary double EGFR T790M-activating mutation compared to patients with activating mutations alone [34]. Conversely, in a recent retrospective study, patients with baseline T790M mutation and concurrent sensitizing EGFR mutation showed a low response rate to erlotinib and an overall survival similar to that of EGFR wild-type patients [35]. Although the detection of a T790M mutation in TKI-naïve tumors has no clinical implication so far, standardization of methods and definition of clinically significant T790M mutant allelic frequencies (MAF) are essential to drive proper first-line treatment strategies of these patients.

3. Methods for detecting EGFR T790M mutation

Assessment of the EGFR T790M mutation in NSCLC is a pivotal tool to identify patients that can potentially benefit from therapy with the approved T790M-targeted TKI osimertinib. However, there is still a “grey zone” regarding timing, type of specimen, and detection methods to be adopted for this test. Importantly, the T790M mutation can be acquired during disease progression, under the selective pressure of TKI therapy. On the other hand, a mutant TKI-resistant clone can be already present at baseline