

# EGFR T790M Mutation

## A Double Role in Lung Cancer Cell Survival?

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**Abstract:** Even though lung cancer patients harboring a mutation in the epidermal growth factor receptor (*EGFR*) gene exhibit an initial dramatic response to EGFR tyrosine kinase inhibitors (EGFR-TKIs), acquired resistance is almost inevitable after a progression-free period of approximately 10 months. A secondary point mutation that substitutes methionine for threonine at amino acid position 790 (T790M) is a molecular mechanism that produces a drug-resistant variant of the targeted kinase. The T790M mutation is present in about half of the lung cancer patients with acquired resistance, and reported to act by increasing the affinity of the receptor to adenosine triphosphate, relative to its affinity to TKIs. Nevertheless, several lines of evidence indicate that the T790M mutation confers growth advantage to cancer cells, and it was shown that mice expressing tetracycline-inducible EGFR transgenes harboring the T790M mutation develop lung tumors. Thus, T790M mutation seems to play a double role in the survival of lung cancer cells. Several second-generation EGFR-TKIs are currently being developed to overcome the acquired resistance caused by the T790M mutation. *MET* (met proto-oncogene) amplification or activation of IGF1R are reported as alternative mechanisms for acquired resistance to EGFR-TKIs. Clarification of the pathways leading to acquired resistance is essential to maximize the efficacy of EGFR-TKI therapy for patients with lung cancer.

**Key Words:** Molecular target therapy, *EGFR* mutation, Acquired resistance, EGFR tyrosine kinase inhibitor.

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Gefitinib and erlotinib are low molecular-weight epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) that mimic adenosine triphosphate (ATP). Both drugs reversibly and specifically inhibit EGFR in a competitive fashion. About 70 to 80% of non-small cell lung

cancers harboring a somatic mutation in the tyrosine kinase domain of the *EGFR* gene respond to gefitinib/erlotinib, whereas only 10% of tumors without this mutation are responsive to these drugs;<sup>1–3</sup> however, acquired resistance to EGFR-TKI therapy almost always develops after a median of approximately 10 months from the onset of treatment, even in patients who exhibited initial dramatic responses.<sup>4</sup>

A secondary point mutation in the *EGFR* tyrosine kinase domain that substitutes methionine for threonine at amino acid position 790 (T790M) has been described as a mechanism to explain the acquired resistance to TKIs.<sup>5,6</sup> In this review, we discuss the role of the T790M mutation in the mechanism of resistance to EGFR-TKI therapy and in oncogenesis.

### EGFR and Downstream Signaling Cascades

Upon binding to its ligands, EGFR forms homo- or heterodimers with other ERBB (erythroblastic leukemia viral oncogene homolog avian) receptors; in addition, tyrosine residues within the cytoplasmic domain are phosphorylated and downstream signaling cascades are activated. These include the phosphatidylinositol-3-kinase (PI3K)-Akt pathway (Figure 1A) or the STAT pathway, which are mainly associated with cell survival, and the RAS-RAF-MAPK pathway, which is mainly associated with cell cycle progression.<sup>7,8</sup>

Somatic activating mutations in the *EGFR* genes were described in 2004, mainly in patients with lung adenocarcinoma who were female, never-smokers, and of Asian ethnicity.<sup>1,2</sup> *EGFR* mutation usually occurs in the first 4 exons of the tyrosine kinase domain, and a deletion involving 5 amino acids (codons 746–750) together with a point mutation at codon 858 (L858R) account for 90% of all *EGFR* mutations.<sup>3</sup> EGFRs harboring these mutations are constitutively activated without ligand binding and cancer cells harboring this mutation become highly dependent on the EGFR pathway, a state often referred to as “oncogene addiction.”<sup>9,10</sup> It is reported that the PI3K-Akt signaling pathway is mainly activated in EGFR-mutant cells where ERBB3 acts as a dimer partner of EGFR, and the down-regulation of this pathway is required for the process of gefitinib-induced apoptosis in these cells.<sup>11</sup> In addition, mutant EGFR kinases have a higher affinity to EGFR-TKIs (Figure 1B)<sup>12</sup>; therefore, patients with lung cancer harboring *EGFR* mutations often exhibit a dramatic response to EGFR-TKIs.

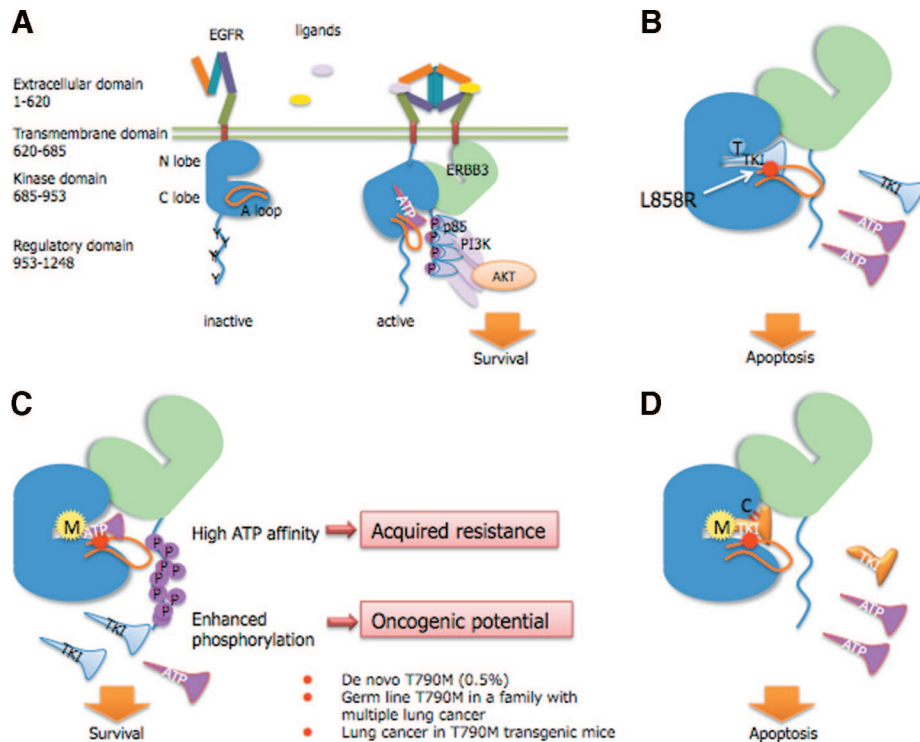
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**FIGURE 1.** The T790M mutation plays a double role in lung cancer cell survival. *A*, In the inactive conformation of the epidermal growth factor receptor (EGFR), the activation loop (A-loop) precludes the binding of peptide substrate. When the specific ligands bind the extracellular domain, EGFR dimerizes with other members of the ERBB family (ERBB3 in this scheme) in a tail-to-head fashion.<sup>35</sup> The C lobe of the kinase domain plays a role analogous to that of cyclin in activated cyclin-dependent kinase (CDK)/cyclin complexes.<sup>35</sup> The A-loop becomes extended to allow peptide substrate binding (active conformation), resulting in phosphorylation of tyrosine residues in regulatory domains. Phosphorylated tyrosine residues serve as docking sites for adaptor molecules that facilitate downstream signaling pathways, the most significant of which is the phosphatidylinositol-3-kinase (PI3K)-Akt pathway. *B*, EGFR mutation (L858R in this case) also promotes formation of active conformation. Gefitinib or erlotinib competitively inhibit binding of ATP to the EGFR kinase, resulting in inhibition of phosphorylation and downstream signaling. Gefitinib binds 20-fold more tightly to the L858R mutant than to the wild-type enzyme.<sup>36C</sup> When threonine 790 is substituted by methionine (T790M), the ATP affinity of the oncogenic L858R mutant is increased by more than an order of magnitude, leading to resistance to gefitinib/erlotinib.<sup>18</sup> The T790M mutation also possesses enhanced phosphorylating activity, especially in combination with the L858R mutation. Several lines of evidence indicate that the T790M mutant is actually an oncogene. *D*, An irreversible EGFR-tyrosine kinase inhibitor (TKI) forms a covalent bond at cysteine 797 (C) even when the T790M mutation is present, and thus is able to inhibit the T790M mutant kinase.

### T790M Mutation as a Cause of Acquired Resistance to TKI Treatment

Patients with lung cancer who show an initial dramatic response to EGFR-TKI almost always acquire resistance to the drug after a progression-free period of approximately 10 months.<sup>4</sup> A 2002 report described a secondary mutation in chronic myeloid leukemia patients that substitutes isoleucine for threonine at codon 315 of the *ABL* gene and causes acquired resistance to imatinib.<sup>13</sup> Since the T315 of *ABL* corresponds to the T790 of EGFR, based on amino acid homology, researchers investigated whether an artificial T790M mutation conferred resistance to gefitinib and showed that this was the case.<sup>14</sup> Based on this report, 2 groups of investigators confirmed that the T790M mutation is present in patients who develop acquired resistance to EGFR-TKI treatment in 2005.<sup>5,6</sup> We and others showed that the EGFR T790M mutation, in *cis* with the primary activating mutation, occurs in approximately 50% of patients with acquired resis-

tance to EGFR-TKI treatment.<sup>15,16</sup> Nomura et al.<sup>17</sup> showed that the EGFR mutations were found to favor the shorter allele of polymorphic CA dinucleotide repeat in intron one of the EGFR gene, and this or another genetic factor can be the reason that the acquired T790M mutation occurs in *cis*, in addition, secondary somatic activating mutation occurs in *cis* with the inherited T790M mutation discussed below.

The T790 in EGFR is located at a key position in the ATP binding cleft, often referred to as the “gatekeeper residue.” Initially, it was thought that the larger methionine residue caused steric hindrance to the binding of EGFR-TKI<sup>5,6</sup>; however, it is difficult to explain why structurally similar, irreversible EGFR-TKI is able to overcome the T790M mutation, as discussed later. A recent analysis showed that the T790M mutant retains affinity to gefitinib; i.e., the T790M-mutant EGFR kinase binds gefitinib with a Kd of 4.6 nM, nearly as tightly as the L858R mutant (Kd = 2.4 nM).<sup>18</sup> In contrast, introduction of the T790M mutation

increases the ATP affinity of the oncogenic L858R mutant by more than an order of magnitude.<sup>18</sup> The authors of this report claim that increased ATP affinity is the primary mechanism by which the T790M mutation confers drug resistance (Figure 1C).<sup>18</sup>

### The T790M Mutation as an Oncogenic Agent

We previously reported two examples of surgically-treated patients who carry both the T790M and L858R mutations, among 397 patients with *EGFR* mutations (0.5%) who had never been exposed to EGFR-TKI treatment<sup>19</sup>; one of the patients showed inherent resistance to gefitinib when she was treated with this drug after tumor recurrence.<sup>19</sup> Others reported a family with multiple cases of lung cancer associated with germ line transmission of the T790M mutation, and four of the six tumors analyzed showed a secondary somatic activating *EGFR* mutation (either L858R, del L747-T751, or G719A) occurring in *cis* with the germ line T790M mutation<sup>20</sup>; however, the T790M mutation was never found among 237 lung cancer family probands.<sup>21</sup> These results suggest that the T790M mutation not only confers resistance to EGFR-TKIs, but also grants growth advantage to cancer cells (Figure 1C).

Although it was initially reported that the kinase activity of the EGFR T790M mutant was indistinguishable from wild-type EGFR,<sup>5,6,14</sup> Mulloy et al. showed that the T790M mutant exhibits tyrosine phosphorylation levels comparable to wild-type EGFR, whereas the T790M/L858R double mutant exhibits a substantial increase in phosphorylation, compared with the L858R mutant alone. Thus, the T790M resistance mutation, when combined with activating EGFR kinase domain mutations, confers a significant enhancement of its catalytic phosphorylating activity, which suggests that these mutations cooperate to produce a more potent kinase. This may potentially explain the additional role of the T790M mutation in predisposing to tumorigenesis.<sup>22</sup> Vikis et al.<sup>21</sup> further indicated that the T790M mutation alone leads to increased phosphorylation levels. A human bronchial epithelial cell line overexpressing EGFR carrying the T790M mutation displayed a growth advantage over wild-type EGFR.<sup>21</sup>

Animal models were generated to inducibly express the T790M mutation, alone or together with the L858R mutation, in type II pneumocytes that develop lung adenocarcinomas. Mice expressing the T790M mutation alone develop tumors with longer latency than those expressing both the T790M and L858R mutations.<sup>23</sup> In contrast to what is observed in tumors of patients carrying human germ line T790M mutations (discussed above), no additional kinase domain mutations were detected in the tumors of these mice.<sup>23</sup> These results indicate that the T790M mutation is not only a cause of resistance to gefitinib/erlotinib but is also an oncogenic mutation that confers growth advantage to cancer cells. Its oncogenic potential is maximized when the mutation arises in combination with other common *EGFR* activating mutations (Figure 1C).

### Strategies to Overcome the Resistance Conferred by the T790M Mutation

Since the T790M mutation confers resistance to gefitinib/erlotinib by increasing the affinity of EGFR to ATP, relative to that of EGFR to TKIs,<sup>18</sup> it is possible to overcome the resistance caused by this mechanism by developing a novel class of EGFR-TKIs that have a higher affinity for the T790M kinase, when compared with the affinity of ATP for the mutant kinase. Several kinds of so-called second generation TKIs are currently in various stages of development. BIBW2992,<sup>24</sup> PF00299804,<sup>25</sup> and HKI-272<sup>26</sup> are examples of this new type of EGFR-TKI and belong to the class of irreversible TKIs that covalently bind the sulfhydryl group of cysteine 797 at the catalytic pocket of EGFR (Figure 1D); however, Yun et al.<sup>18</sup> indicate that irreversible binding is not required for effective inhibition of the T790M mutant: a reversible inhibitor that binds EGFR-T790M with an affinity sufficient to compete with ATP should be as effective. Accordingly, the XL647 is reported to inhibit the T790M EGFR mutant, even though this compound is a reversible TKI.<sup>27</sup> Nevertheless, it should be noted that one of the acquired resistance mechanisms was also the T790M mutation in the cell culture model of acquired resistance to an irreversible EGFR-TKI, HKI-272.<sup>28</sup> This observation seems to be somewhat puzzling, but it reflects the fact that HKI-272 can overcome T790M only at high doses (approximately 1  $\mu$ M), but not at clinically achievable concentrations (approximately 0.2  $\mu$ M).<sup>28</sup>

Inhibition of the heat shock protein 90 (HSP90) is also effective in inhibiting the T790M mutant.<sup>29</sup> Addition of rapamycin (an inhibitor of mammalian target of rapamycin [mTOR]) to irreversible TKI potentiates the antitumor effect in the L858R/T790M mouse model.<sup>26</sup>

### *MET* Amplification and Other Mechanisms for Acquired Resistance to TKI Treatment

Over 50 secondary mutations of the *ABL* gene are reported in acquired resistance to imatinib in chronic myeloid leukemia<sup>30</sup>; however, the *EGFR* D761Y and L747S mutations are the only two other rare examples of secondary mutations associated with acquired resistance to gefitinib (other than T790M).<sup>15,31</sup>

In 2007, Engelman et al.<sup>32</sup> reported amplification of *MET*, a receptor tyrosine kinase for hepatocyte growth factor, as another mechanism of resistance to EGFR-TKIs. The authors isolated gefitinib-resistant clones from HCC827 lung cancer cells (*EGFR* exon 19 deletion and amplified) by exposing the cells to increasing concentration of gefitinib. The resistant cells maintained activation of the ERBB3/PI3K/Akt antiapoptotic pathway in the presence of gefitinib. The resistant cells but not parental cells harbor *MET* amplification by a factor of 5 to 10, and inhibition of *MET* by specific TKI restores gefitinib sensitivity. They concluded that *MET* amplification activates the PI3K/Akt pathway through ERBB3 activation.<sup>32</sup> *MET* amplification is present in about 20% of patients with acquired resistance but only in 3% of untreated patients.<sup>32,33</sup> Interestingly, *MET* amplification sometimes coexists with the T790M mutation,<sup>32,33</sup> and one patient is reported to have two independent resistant tumors;

one of which had the T790M mutation, whereas the other had *MET* amplification.<sup>32</sup> More recently, it was shown that hyperphosphorylation of the insulin-like growth factor I (IGF-I) receptor (IGF-IR) and constitutive association of IRS-1 with PI3K through loss of expression of IGF binding protein in gefitinib-treated cells is another mechanism of acquired resistance.<sup>34</sup>

The mechanisms underlying acquired resistance to gefitinib/erlotinib treatment are still unknown in more than 30% of cases. Further clarification of the pathways leading to acquired resistance is essential to maximize the efficacy of EGFR-TKI therapy in patients with non-small cell lung cancer.

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