



# Resistance to Tyrosine Kinase Inhibitors in EGFR-mutant of Non-Small Cell Lung Cancer

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Fariz Nurwidya,\* Akiko Murakami,\* Fumiyuki Takahashi,\*  
Faisal Yunus,\*\* Kazuhisa Takahashi\*

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\*Department of Respiratory Medicine, Juntendo University, School of Medicine, Tokyo

\*\*Department of Pulmonology and Respiratory Medicine, Faculty of Medicine  
University of Indonesia, Jakarta

**Abstract:** The epidermal growth factor receptor (EGFR) pathway plays a crucial role in non-small cell lung cancer (NSCLC), contributing to a number of highly relevant processes in tumor development and progression, including cell proliferation, regulation of apoptotic cell death and angiogenesis that leads to tumor growth and metastatic spread. Accordingly, targeting EGFR has been intensely pursued, with the development of series of promising molecular inhibitors for use in clinical oncology. In recent years, tyrosine kinase (TK) domain of the EGFR was discovered in patients with NSCLC and has given a dramatic clinical response to treatment with TK inhibitors (TKIs) such as gefitinib. However, molecular studies and clinical trials have demonstrated the occurrence of primary and/or secondary resistance to these drugs. A greater understanding of these mechanisms that lead to EGFR resistance may provide valuable insights to help design new strategies that will enhance the impact of this promising class of inhibitors for the treatment of patients with NSCLC. **J Indon Med Assoc. 2011;61:493-7.**

**Keywords:** epidermal growth factor receptor (EGFR), tyrosine kinase inhibitors (TKIs), non-small cell lung cancer (NSCLC), drug-resistance

## Resistensi Kanker Paru Kategori Bukan Sel Kecil dengan Mutasi EGFR terhadap Tyrosine Kinase Inhibitor

Fariz Nurwidya,\* Akiko Murakami,\* Fumiyuki Takahashi,\*  
Faisal Yunus,\*\* Kazuhisa Takahashi\*

\*Department of Respiratory Medicine, Juntendo University, School of Medicine, Tokyo

\*\*Departemen Pulmonologi dan Kedokteran Respirasi,  
Fakultas Kedokteran Universitas Indonesia, Jakarta

**Abstrak:** Jalur epidermal growth factor receptor (EGFR) memegang peranan penting pada kanker paru kategori bukan sel kecil (KPKBSK). EGFR berkontribusi pada beberapa proses utama perkembangan dan progresivitas tumor; antara lain proliferasi sel, regulasi apoptosis sel yang mengakibatkan pertumbuhan tumor dan metastasis. Penelitian yang menarget EGFR secara intensif banyak dilakukan, berfokus pada pengembangan molekul penghambat yang menjanjikan di bidang onkologi. Pada beberapa tahun terakhir, ditemukan domain tirosin kinase (TK) pada EGFR yang memberi respon klinis dramatis terhadap terapi inhibitor TK (TKI) seperti gefitinib. Namun berbagai penelitian molekular dan studi klinis menunjukkan munculnya resistensi, baik primer dan sekunder, terhadap obat-obat golongan ini. Pemahaman mendalam terhadap mekanisme resistensi akan membantu peneliti dan klinisi dalam menyusun strategi baru yang akan meningkatkan efek terapi golongan inhibitor ini pada pasien-pasien dengan KPKBSK. *J Indon Med Assoc.* 2011;61:493-7.

**Kata kunci:** epidermal growth factor receptor (EGFR), inhibitor tirosin kinase (TKIs), kanker paru kategori bukan sel kecil (KPKBSK), resistensi obat

### Introduction

Lung cancer, the leading cause of cancer-related death, accounts for one third of all cancer deaths globally. In the United States, lung and bronchus cancer were the highest ranked among all cancer mortality in both male and female.<sup>1</sup> Lung cancer is classified according to its histopathology characteristic into small-cell lung cancer (SCLC, about 20%) and non-small cell lung cancer (NSCLC, up to 80%). NSCLC is further classified as adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma, and large cell carcinoma.<sup>2</sup> Because of remarkable advances over the last 6 years in the understanding of lung adenocarcinoma, particularly in area of medical oncology, molecular biology and radiology, there is a pressing need for a revised classification, based not on histopathology alone, but rather on an integrated multidisciplinary platform. Nevertheless, in the absence of molecular, immunohistochemical, or histochemical testing, the diagnosis and subclassification of lung adenocarcinoma are based purely on light microscopic evaluation of pathologic material.

One of the most important findings in the field of medical oncology was the discovery of epithelial growth factor receptor (EGFR), along with targeted therapy and emerging resistance that will be addressed in this review.

### Epithelial Growth Factor Receptor

The beginning of growth-factor research can be traced back to 1952, when Rita Levi-Montalcini in the laboratory of Viktor Hamburger discovered nerve growth factor (NGF).<sup>3</sup> This growth factor was purified from snake venom and mouse salivary-gland extracts by Levi-Montalcini and Stanley Cohen in 1957.<sup>3</sup> Five years later, following their work on NGF, Cohen found epidermal growth factor (EGF), as it stimulated the proliferation of epithelial cells.<sup>3</sup> In 1986, these important discoveries of NGF and EGF earned Levi-Montalcini and Cohen the Nobel Prize in Physiology or Medicine.

EGFR is expressed in 50% of NSCLC and its expression is correlated with poor prognosis.<sup>4</sup> EGFR belong to a family of receptor tyrosine kinases (TKs) that includes EGFR, ERBB<sup>2</sup> (also known as HER2), ERBB3 (also known as HER3) and ERBB4 (also known as HER<sup>4</sup>). Structurally, each receptor is composed of an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain.<sup>5</sup>

### Tyrosine Kinase Inhibitor

Tyrosine Kinase Inhibitors are small molecules that specifically inhibit the tyrosine kinase activity of the epidermal growth factor receptor (EGFR). Examples for this class of drug are gefitinib and erlotinib. Those two drugs were the

first drugs to become clinically available in the treatment of non-small cell lung cancer (NSCLC). Gefitinib was approved in Japan for the first time in 2002.<sup>6</sup> Later on, the U.S. Food and Drug Administration (FDA) approved gefitinib in 2003 and erlotinib in 2004 for the treatment of NSCLC.<sup>7</sup> Clinical responses to these agents were more common in women than men; in patients from Japan than from Europe and the U.S.; patients with adenocarcinoma than other histologic subtypes, and patients who had never smoked cigarettes.<sup>8</sup> Response Evaluation Criteria in Solid Tumors (RECIST) is being used to assess clinical evaluation in terms of objective responses. Several means of diagnostic radiology procedures are incorporated in RECIST such as chest X-ray, CT-scan and MRI. These procedures eventually categorize solid cancer patients if they are improving ('respond'), stay the same ('stable') or worsen ('progression') during treatments.<sup>9</sup>

### EGFR Mutation

Kinase domain mutations in EGFR are generally referred as activating mutations because they seem to result in the increase kinase activity of the receptor.<sup>4</sup> The kinase domain located in exon 18 to 21.<sup>4</sup> There are types of EGFR mutation that confer NSCLC to become sensitive to gefitinib. The types of EGFR mutations in patients responded to gefitinib and erlotinib have been similar around the world.<sup>8</sup> The mutations involve multiple overlapping deletions of exon 19 (45% of patients), point mutations in exon 21 ([40% of patients] predominately L858R), and point mutations or insertions in exons 18 to 21 in the rest of patients.<sup>8</sup>

Morita *et al.*<sup>10</sup> analyzed clinical trials that prospectively evaluated the efficacy of gefitinib for advanced NSCLC with *EGFR* mutations in Japan. Seven eligible trials were identified for a total of 148 NSCLC patients with *EGFR* mutations. The overall response rate to gefitinib was 76.4% (95% CI, 69.5-83.2). The median progression-free survival and overall survival were 9.7 months (95% CI, 8.2-11.1) and 24.3 months (95% CI, 19.8-28.2), respectively. Among these patients, 87 patients received gefitinib as first-line therapy and 61 patients received systemic chemotherapy as first line treatment followed by gefitinib. The response rate was significantly higher for the first-line gefitinib group than for the first-line chemotherapy group (79.3% versus 24.6%;  $P < 0.001$ ). Good performance status and chemotherapy-naïve status were significantly associated with a longer progression-free survival or overall survival. The median progression free-survival after the start of first-line therapy was significantly longer in the gefitinib-first group than in the chemotherapy-first group (10.7 vs 6.0 months;  $P < 0.001$ ), whereas no significant difference in median overall survival was apparent between the two groups (27.7 vs 25.7 months;  $P = 0.782$ ).<sup>10</sup>

### Resistance to Tyrosine Kinase Inhibitor

Although information of EGFR mutation may enable us

to identify the subgroup of patients with NSCLC who will respond to gefitinib and erlotinib, not all EGFR mutations are correlated with sensitivity to gefitinib and erlotinib. There are two types of resistance in this regard: primary resistance and secondary resistance.

### Primary Resistance to Tyrosine Kinase Inhibitor

Primary resistance is defined as drug-resistance occur in a tumor that initially refractory to EGFR tyrosine kinase inhibitor treatment.<sup>11</sup> Certain molecular mechanisms have been identified to take part in this primary resistance. In the following part, we will discuss the mechanism of exon 20 mutations<sup>12</sup>, KRAS mutation<sup>13</sup> and PTEN downregulation<sup>14</sup> in the occurrence of primary resistance to TKIs.

Wu *et al.*<sup>12</sup> investigated the clinical features of lung cancer with exon 20 mutations. They found that EGFR exon 20 mutations of NSCLC patients result in poorer responsiveness to gefitinib treatment, but variability exists between different individuals.<sup>12</sup> The gefitinib response rate of NSCLC with exon 20 mutations was 25%, far lower than those with deletions in exon 19 and L858R mutations.<sup>12</sup> The response rate in patients harboring exon 19 mutation was 73%. Meanwhile, the response rate in L858R mutations (exon 21 point mutation) was 50%.<sup>15</sup> However, exon 20 mutations are relatively rare, suggesting that other mechanisms probably contribute to EGFR TKI primary resistance of NSCLC.<sup>16</sup>

Somatic mutations in exon 2 (codon 12-13) of *KRAS*, encoding a GTPase downstream of EGFR, have been associated with primary resistance to EGFR inhibitors.<sup>13</sup> Pao *et al.*<sup>17</sup> conducted a study to determine whether mutations in *KRAS* could be used to further enhance prediction of response to gefitinib or erlotinib. His team screened 60 lung adenocarcinomas defined as sensitive or refractory to gefitinib or erlotinib for mutations in *EGFR* and *KRAS*. It is shown that mutations in *KRAS* are associated with lack of sensitivity to either drug.<sup>17</sup>

Knockdown of PTEN expression using small interfering RNA specific for PTEN in PC-9 (human lung adenocarcinoma cells) resulted in drug resistance to gefitinib and erlotinib.<sup>14</sup> Reduced PTEN expression was also seen in tumor samples from a patient with gefitinib-refractory NSCLC. These findings reinforce the therapeutic importance of PTEN expression in the treatment of NSCLC with EGFR-targeted drugs.<sup>14</sup> In other study, PTEN expression sensitizes C4-2 cells, a prostate cancer cell lines, to EGF and serum stimulation.<sup>18</sup> This hypersensitivity to EGF stimulation correlated with the amount of PTEN expressed. Furthermore, restoration of PTEN expression alters the sensitivity of prostate cancer cells to EGFR inhibitors.<sup>18</sup> In terms of clinical evidence, PTEN is associated with prolonged survival after gefitinib treatment in EGFR-mutated lung cancer patients.<sup>19</sup>

### Secondary Resistance to Tyrosine Kinase Inhibitor

Clinically, secondary (acquired) resistance criterias are

patient: (1) previously treated with a single-agent EGFR TKI (eg, gefitinib or erlotinib); (2) having either or both of the following: a tumor that harbors an EGFR mutation known to be associated with drug sensitivity or objective clinical benefit from treatment with an EGFR TKI; (3) suffer from systemic progression of disease (Response Evaluation Criteria in Solid Tumors [RECIST] or WHO) while on continuous treatment with gefitinib or erlotinib within the last 30 days; (4) and no intervening systemic therapy between cessation of gefitinib or erlotinib and initiation of new therapy.<sup>20</sup> Simply stated, secondary resistance affects patients who initially respond to treatment but subsequently experience loss of response.<sup>11</sup>

One of the established mechanisms for secondary resistance is second mutation, T790M, that account for half of all resistance to gefitinib and erlotinib.<sup>21</sup> This second mutation has been thought to cause resistance by sterically blocking binding of TKIs such as gefitinib and erlotinib.<sup>21</sup> T790M mutation might exist in a small fraction of tumor cells before drug treatment, and the tumor cells harboring this mutation might be enriched over time during treatment with gefitinib or erlotinib.<sup>22</sup> T790M mutation is substitution of threonine 790 with methionine.<sup>21</sup> Threonine 790 is the “gatekeeper” residue, an important determinant of inhibitor specificity in the ATP binding pocket. The increased ATP affinity is the primary mechanism by which the T790M mutation confers drug resistance. The increased ATP affinity will reduce the affinity of any ATP-competitive kinase inhibitor such as gefitinib.<sup>21</sup>

*MET* amplification has been detected in up to 20% of NSCLC with EGFR mutations progressing after an initial response to TKI therapy.<sup>23</sup> *MET* amplification activates ERBB3/PI3K/AKT signaling in EGFR mutant lung cancers and causes resistance to EGFR kinase inhibitors.<sup>24</sup> Engelman *et al.*<sup>25</sup> examined whether *MET* inhibition suppressed growth of the gefitinib resistant lung cancer cells. HCC827-GR (gefitinib resistant NSCLC cells) were exposed to PHA-665752, a *MET* tyrosine kinase inhibitor, alone or in combination with gefitinib. Combined treatment resulted in substantial growth inhibition and induced apoptosis.<sup>25</sup> In clinical settings, the development of anti-*MET* therapeutic strategies should be focused on patients with acquired EGFR-TKI resistance.<sup>23</sup>

Several studies have been conducted to identify the existence of drug efflux from cancer cytoplasm. ATP-binding cassette (ABC) transporters, including breast cancer resistance protein (BCRP), also known as ABCG2, capable of pumping out various structurally unrelated agents using ATP hydrolysis energy.<sup>26</sup> It was shown that ABCG2-transduced human lung cancer PC9 (PC9/ABCG2) cells show gefitinib resistance.<sup>26</sup> Furthermore, PC9/ABCG2 cells show lower accumulation and higher efflux of gefitinib than their parental cells.<sup>26</sup> However, the correlation between gefitinib and ABCG2 transporter is unique because low concentration of gefitinib also inhibited ABCG2-dependent active drug extru-

sion and significantly reduce drug resistance patterns in cells expressing ABCG2.<sup>27</sup>

## Conclusion

In conclusion, our understanding about the biology of EGFR activating mutation has helped us to identify which NSCLC patient will benefit most from tyrosine kinase targeted therapy, such as patients harboring exon deletion mutation,<sup>19</sup> and exon 21 point mutation (L858R). But increasing number of drug resistance requires further basic molecular studies followed by clinical trials to overcome this threatening problem. The most important initial steps remain clear: (1) widespread implementation of EGFR genotyping for lung adenocarcinoma; (2) development of a distinct management paradigm for these oncogene-addicted cancers; (3) improved utilization of rebiopsy tissue for molecular typing of resistance; and (4) genotype-driven trials of rationally targeted therapies for patients with acquired TKI resistance.<sup>28</sup>

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